Molecular characterization of antibiotic resistant bacteria in newly HIV diagnosed adults in a community setting in Tanzania. Implications for infection prevention and control in HIV

Antibiotic resistant bacteria, HIV, Community, Sub-Saharan Africa

Joel Manyahi

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2022

UNIVERSITY OF BERGEN

Molecular characterization of antibiotic resistant bacteria in newly HIV diagnosed adults in a community setting in Tanzania. Implications for infection prevention and control in HIV

Antibiotic resistant bacteria, HIV, Community, Sub-Saharan Africa

Joel Manyahi

Thesis for the degree of Philosophiae Doctor (PhD)
at the University of Bergen

Date of defense: 26.08.2022

Year: 2022

Title: Molecular characterization of antibiotic resistant bacteria in newly HIV diagnosed adults in a community setting in Tanzania. Implications for infection prevention and control in HIV

Name: Joel Manyahi

Print: Skipnes Kommunikasjon / University of Bergen

Table of Contents

Abbreviations

AMR	Antimicrobial resistance
CA-MRSA	Community acquired methicillin resistant *S. aureus*
CAP	Community acquired pneumonia
CC	Clonal complex
CLSI	Clinical and Laboratory Standard Institute
CPE	Carbapenemase producing Enterobacterales
DHFR	Dihydrofolate reductase
ESBL-PE	Extended spectrum β-lactamase producing Enterobacterales
HA-MRSA	Hospital acquired methicillin resistant *S. aureus*
HIV	Human immunodeficiency virus
MDR	Multi-drug resistance
MLST	Multi-locus sequence typing
MUHAS	Muhimbili University of Health and Allied Sciences
MVLA	Multilocus variable number tandem repeat analysis
NDM	New Delhi metallo-β-lactamase
NGS	Next generation sequencing
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PFGE	Pulsed field gel electrophoresis
PVL	Panton valentine leucocidin
QRDR	Quinolone resistance-determining region
SCC	Staphylococcal cassette chromosome
SSTI	Skin and soft tissue infection
ST	Sequence typing
STGG	Skim milk, tryptone, glucose, and glycerin
VRE	Vancomycin resistant Enterococcus
WHO	World Health Organization

Scientific environment

During my PhD program, I was affiliated with the National Centre for Tropical Infectious Diseases, Haukeland University Hospital in Bergen, Norway, and Muhimbili University of Health and Allied Sciences (MUHAS) in Tanzania, in addition to the University of Bergen (UiB).

Acknowledgments

First and foremost, I would like to express my gratitude to the Western Norway Regional Health Authority (Helse Vest Regionalt Helseforetak), which provided my PhD scholarship and funded the project through PhD grant no. 912132 and project grant no 911971. On the other hand, I'd like to thank the National Advisory Unit for Tropical Infectious Diseases at Haukeland University Hospital in Bergen, Norway, for providing financial support for the project's implementation and publication of articles.

To my main supervisor, Bjørn Blomberg, thank you for being not only a supervisor but also a friend. You and Chiku have been very welcoming during my time in Bergen; your house was like a second home. Thank you for the mentorship, encouragement, and inspiration. You were instrumental in securing the scholarship that supported this PhD work. I am very appreciative of the trust you bestowed on me and of your availability to meet for discussion, even at the last minute.!

Sabrina, my co-supervisor, made all this possible. First for introducing me to the team, secondly, for your support on the PhD research journey. You have been tough on me since I met you during my master's training in Microbiology and Immunology at MUHAS. Thank you for your dedication, guidance, and support throughout the journey.

Nina Langeland, my co-supervisor, has helped me all the way to the end of this project. Thank you for your words of encouragement and faith in me; I recall your words of encouragement when you paid a visit at the start of data collection, when we had a false start.

I'd like to thank Prof Said Aboud, my co-supervisor, for his inspiration and help with the project. Dr. Mtebe Majigo, Prof. Eligius Lyamuya, Prof. Mecky Matee, Dr. Joachim Agricola, Dr. Frank Masoud, Dr. Salim Masoud, Dr. Doren Kamori, Ms. Upendo Kibwana, and Ms. Lilian Nkinda from the Department of Microbiology and Immunology at MUHAS, thank you all for your help throughout this project.

My heartfelt gratitude goes to Helene Heitmann Sandnes and Marit Gjerde Tellevik for their invaluable assistance during the laboratory experimentation of my specimens in Bergen. I would like to thank our laboratory staff at the MUHAS Bacteriology Research Laboratory, Saniru Bia and Mathius Lushona, for their technical assistance. I'd like to thank Kristine Morch for her assistance in getting our work published.

Brianna-Malaika and Karen Joel, my beautiful girls, you have my undying love. Your perseverance, patience, and love have always been an inspiration to me. You never complained when Dad worked late or was away from home pursuing his PhD.

Mary, my lovely wife, thank you for your unwavering support and for caring for our girls while I was away. Thank you for your motivation, and you were always able to perk me up when things were slow.

Last but not least, Samuel Mateo, you were born while your father was finishing up his thesis. Thank you, God, for giving you this life, and the entire family admires your fighting spirit. We hope you grow into a strong man with a strong desire to help those in need. This work is also dedicated to you.

Summary

In developing countries human immunodeficiency virus infected individuals are at increased risk of acquiring antibiotic resistant bacteria. High frequency of antibiotic use associated with HIV related illness drives emergence of bacteria with antimicrobial resistance (AMR) due to selection pressure. The carriage of antibiotic resistant bacteria coupled with virulence genes increases the risk of severe infections in this population. The aim of this thesis was detection and characterization of antibiotic-resistant bacteria colonizing newly HIV diagnosed adults in a community setting in Tanzania. The findings could help in developing strategies for preventing and controlling the spread and infection of antibiotic resistant bacteria in HIV-positive individuals. Rectal and nasal/nasopharyngeal swabs collected from newly HIV diagnosed adults from the community were used for detection of antibiotic resistant bacteria including extended spectrum β-lactamase producing Enterobacterales (ESBL-PE), carbapenemase producing Enterobacterales (CPE), methicillin-resistant *Staphylococcus aureus* (MRSA), and antibiotic-resistant *Streptococcus pneumoniae*.

The study found that only 4% (22/537) of the HIV-positive participants carried MRSA in the nose/nasopharynx. These MRSA isolates were frequently resistant to gentamicin (95%), ciprofloxacin (91%), and erythromycin (82%), but less often to trimethoprim-sulfamethoxazole (9%). We found that the phenotypic susceptibility patterns of all MRSA isolates were highly concordant with genotypic findings (resistance genes and mutations in genome). All MRSA isolates belonged to the CC8 and ST8-SCCmecIV MRSA clone and negative for panton-valentine leukocidin (PVL) and arginine catabolic mobile element (ACME) type 1. All ST8-SCCmecIV-spa-t1476 MRSA clones from Tanzania were unrelated to the globally successful USA300 clone.

The study showed a high prevalence (32.6%, 194/595) of fecal carriage of ESBL-PE in newly HIV diagnosed adults in the community setting, and confirmed the predominance of the *bla*$_{\text{CTX-M-15}}$ genotype. Antibiotic use in the last 4 weeks and CD4 count <350 cells/μL were independent risk factors for fecal carriage of ESBL-PE in this HIV-infected population.

In this study, we detected *bla*$_{NDM-5}$ carried on an IncX3 type plasmid in one *E. coli* ST2083 isolate obtained from an HIV-infected adult from the community setting in Tanzania. In addition, *E. coli* from the HIV-infected adult carried three more plasmid types; IncFIA, IncFIB and Col(BS512). The IncFA type plasmid was found to carry several genes conferring resistance against fluoroquinolone, aminoglycosides, sulfamethoxazole, trimethoprim, macrolides and tetracycline.
The study found that *Streptococcus pneumoniae* colonizing the nasopharynx of HIV-infected adults displayed a high rate of resistance to penicillin (74%) and cotrimoxazole (71%), antibiotics commonly used as first line treatment in suspected bacterial pneumonia in Tanzania. Furthermore, 26.3% were multidrug-resistance (MDR) and cotrimoxazole-resistant *Streptococcus pneumoniae* had multiple mutations in the dihydrofolate reductase gene.

In conclusion, we found that carriage of antibiotic resistant bacteria was common among newly diagnosed HIV-infected adults from community settings. This highlights the large-scale spread of these resistant bacteria in Tanzania, not only in hospitals, but also in the community as well. The detection of a *bla*$_{NDM-5}$ producing *E. coli* carried on a plasmid with clusters of other resistance determinant genes calls for urgent implementation of intervention strategies to curb the spread of antimicrobial resistant bacteria.

List of publications

Paper I

Joel Manyahi, Sabrina J Moyo, Said Aboud, Nina Langeland, Bjørn Blomberg **Predominance of PVL-negative community associated methicillin resistant *Staphylococcus aureus* sequence type 8 in newly diagnosed HIV-infected adults, Tanzania.** European Journal of Clinical Microbiology & Infectious Diseases. 2021 Jul;40(7):1477–1485

Paper II

Joel Manyahi, Sabrina J Moyo, Said Aboud, Nina Langeland, Bjorn Blomberg **High Prevalence of Fecal Carriage of Extended Spectrum β-Lactamase-Producing Enterobacteriaceae Among Newly HIV-Diagnosed Adults in a Community Setting in Tanzania.** Microb Drug Resist. 2020 Dec; **26**(12)

Paper III

Joel Manyahi, Sabrina J. Moyo, Upendo Kibwana, Richard N. Goodman, Ellie Allman, Alasdair T.M. Hubbard, Bjørn Blomberg, Nina Langeland and Adam P. Roberts. **First identification of *bla*$_{\text{NDM-5}}$ producing *Escherichia coli* from neonates and a HIV infected adult in Tanzania. Journal of Medical Microbiology 2022; 71(2)**

Paper IV

Joel Manyahi, Sabrina J Moyo, Said Aboud, Nina Langeland, Bjorn Blomberg **High rate of antimicrobial resistance and multiple mutations in the dihydrofolate reductase gene among *Streptococcus pneumoniae* isolated from HIV-infected adults in a community setting in Tanzania**

J Global Antimicrob Resist. 2020, Sept; 22:749-753

1. Introduction

1.1 Background on HIV infection

1.1.1. Virology of HIV

Human immunodeficiency virus is the cause of acquired immunodeficiency syndrome (AIDS), which has killed millions in Sub-Saharan Africa [1]. HIV is a retrovirus virus belonging to the family Lentivirinae. It is an enveloped virus containing two identical copies of a positive-strand RNA genome. The virion contains reverse transcriptase (RNA dependent RNA polymerase), integrase, and two copies of cellular transfer RNA (tRNA) [2]. The envelope is a lipid bilayer surrounding the nucleocapsid, and the envelope contains a glycoprotein (gp) complex. The glycoprotein consists of trimer gp 120, which is responsible for viral attachment to the cell, and transmembrane spanning gp 41, which is a membrane fusion protein [2]. Beneath the lipid bilayer envelope is the matrix protein p17, capsid protein p24, and nucleocapsid protein p7 (bound to RNA). Figure 1.

Image Courtesy from Stephen Cusack *et al*. The EMBOL journal. 1999

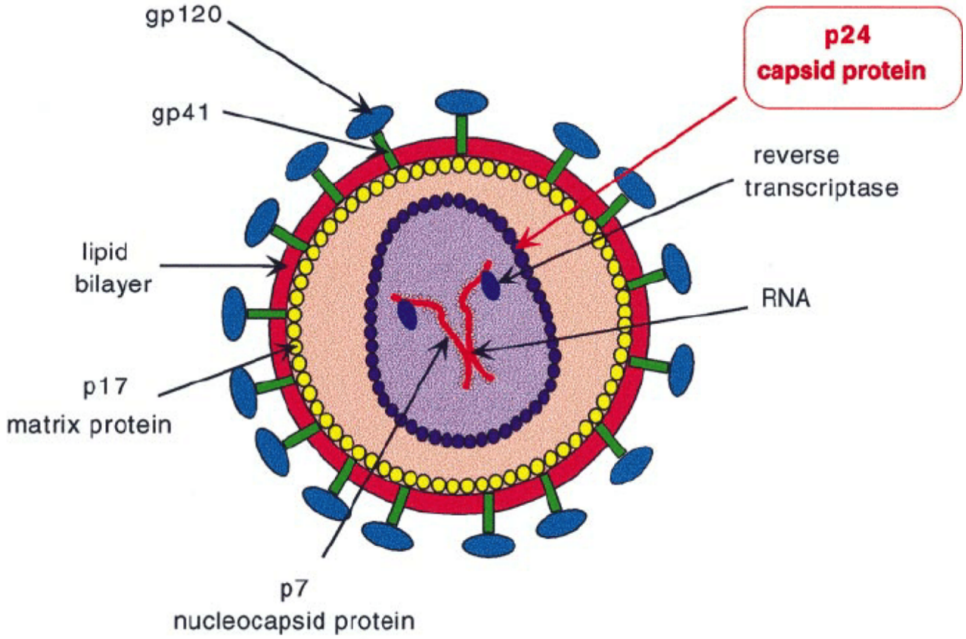


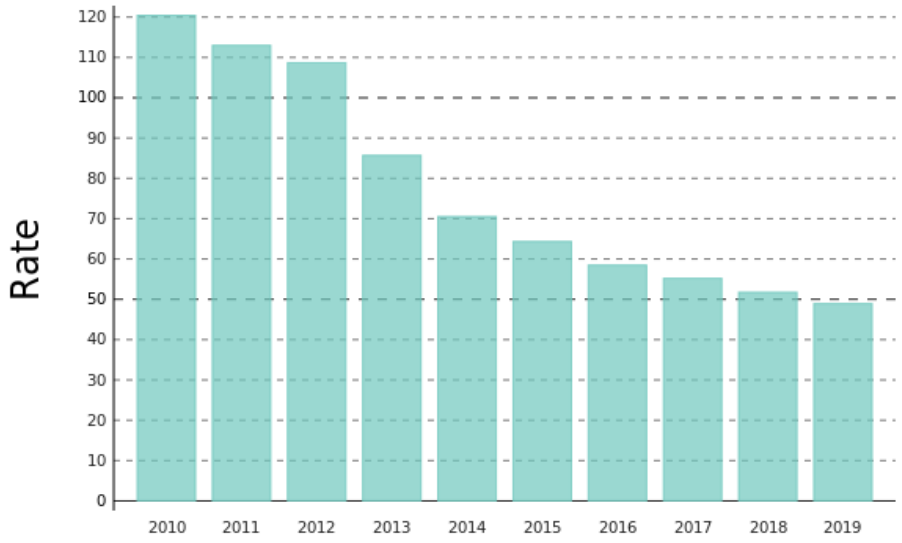
Figure 1: Structure of HIV 1 Virus

1.1.2. Epidemiology of HIV

In 2020, there were 37.7 million (30.2 million–45.1 million) people living with HIV globally, with 1.5 million (1.0 million–2.0 million) people becoming newly infected [1]. Eastern and Southern Africa account for more than half of the global HIV cases, with an estimated 20.6 million (16.8 million–24.4 million) adults and children living with HIV [1]. The prevalence of HIV in Tanzanian adults aged 15–49 years has steadily declined over the last decade, from 7% in 2003 to 4.6% in 2018 [3]. The number of people newly infected with HIV has been decreasing over the past decade (Figure 2), and in 2019 there were 58,000 new HIV infections [4]. In the last decade, there has been a significant decrease in the total number of people who have died from HIV/AIDS-related illnesses (Figure 3) [3]. The country has also reported an increase in the number of people living with HIV who are receiving antiretroviral therapy Figure 4.

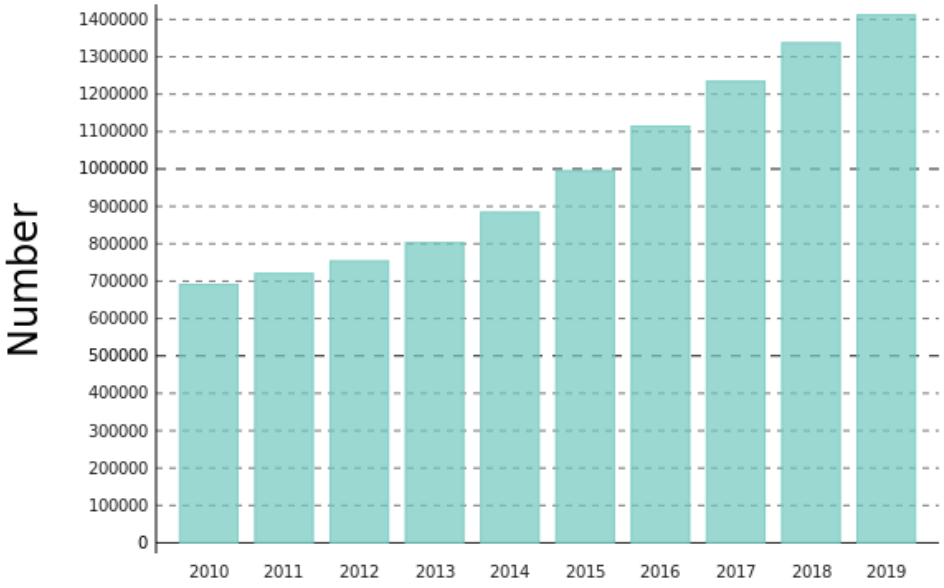
Source: Global AIDS monitoring report 2020-United republic of Tanzania

Figure 2: HIV incidence rate per 1000, United Republic of Tanzania (2010-2019)



Source: Global AIDS monitoring report 2020-United republic of Tanzania

Figure 3: AIDS mortality per 100 000, United Republic of Tanzania (2010-2019)



Source: Global AIDS monitoring report 2020-United republic of Tanzania

Figure 4: People living with HIV who know their HIV status, United Republic of Tanzania (2010-2019)

1.1.3. Immune-pathogenesis of HIV

The immunological hallmark of HIV infection is destruction and depletion of CD+ T lymphocytes. Several potential mechanisms have been suggested in HIV-induced loss of CD+ lymphocytes including; 1) Direct cytopathic effects of HIV and its proteins on $CD4^{+}$cells and progenitor cells 2) Effect of HIV on cell membrane permeability; enhanced fragility of CD4+ cells 3) Induction of apoptosis via immune activation 4) Anti-$CD4^{+}$ cell cytotoxic activity ($CD8^{+}$ and $CD4^{+}$ cells; NK cells) and 5) Anti-$CD4^{+}$ cell autoantibodies [5].

HIV infection also has a direct effect on cells involved in innate cellular response, cytokines, lymphocytes, and the complement system, which causes increased susceptibility to bacterial infection [6]. HIV mediates defects in phagocytosis, cell signaling, and cytokine production produced by monocytes and macrophages [7].

Th17 cells, a subset of CD4+ cells that predominate in the gastrointestinal tract and are important in antibacterial and antifungal defense, are significantly depleted in HIV infection, increasing susceptibility to bacterial infection [8, 9].

Neutropenia is very common in HIV patients, increasing susceptibility to bacterial infections [10, 11]. Possible mechanisms include increased apoptosis caused by autoantibodies, activation in the absence of secondary infection, and decreased production caused by low CSF3 levels [12, 13].

1.2. Antibiotic resistance in HIV-infected populations

1.2.1. Antibiotic resistance bacteria in an era of HIV

In developing countries HIV-infected individuals are at increased risk of acquiring antibiotic resistant bacteria. High frequency of antibiotic use associated with HIV related illness drives emergence of antimicrobial resistance (AMR) due to selection pressure [14]. On the other hand, frequent exposure to hospital environments and their immune suppression status predispose HIV-infected individuals to a high risk of acquiring AMR bacteria [14]. Furthermore, HIV by itself is an independent risk factor for colonization with antibiotic resistant bacteria and subsequent infections [15]. Antibiotic resistant bacterial infections account for a large proportion of morbidity and mortality in the general population, worldwide. In HIV positive populations, the epidemic renders infected individuals drastically more susceptible to bacterial infections compared to HIV non-infected [16-19].

Antiretroviral therapy has been shown to reduce the prevalence of opportunistic infections [20], and bacterial infections have now emerged as the leading cause of hospitalization and mortality among HIV patients [16, 21, 22].

HIV-infected individuals are at higher risk of invasive AMR bacterial infections compared to HIV-uninfected [18, 19, 23] and these infections tend to be more invasive, severe, disseminated and associated with poor outcomes. The mortality of invasive bacterial infections is much higher in HIV-infected individuals compared to the HIV uninfected population [24, 25]. While antibiotics have helped save millions from the consequences of these infections, emerging AMR now threatens to reverse to pre-antibiotic era where simple infections resulted in death. Prevention and control of

antibiotic resistant bacterial infections in the HIV-infected population remains critical for their wellbeing.

Bacterial pneumonia, bloodstream infection as well as enteric infections are common infections in HIV-infected individuals usually associated with hospitalization, prolonged hospital stay and increased mortality [24, 25]. Worldwide, nontyphoid Salmonella, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* are main pathogens in community-acquired bloodstream infections (CA-BSI) in HIV-infected persons [17, 19, 23, 24, 26-28]. Methicillin resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamase (ESBL) producing Enterobacterales and penicillin non-susceptible *Streptococcus pneumoniae* have also been increasingly associated with CA-BSI in HIV-infected populations worldwide [19, 26, 27, 29]. Causative agents of bacterial community acquired pneumonia (CAP) [30] in HIV-infected individuals are often similar to those in the HIV noninfected population. However, bacterial CAP remains one of the most common causes of morbidity in the HIV-infected population [31-34], with inpatient mortality exceeding 10% [34, 35]. Bacterial colonization precedes development of infection and invasive diseases in humans and in experimental animal models [36, 37]. However, few studies have assessed the impact of colonization with antibiotic resistant bacteria on the development of infection in HIV-infected population [37, 38]. Several studies have reported fecal carriage of ESBL producers as a risk factor for BSI caused by ESBL producing bacteria in hospitalized patients [39-41], although these studies have not established the genetic relatedness of the isolates. On the other hand, a meta-analysis reported that MRSA colonized individuals had a relative risk of 24.2 of developing MRSA infection during hospital admission [42]. One study of HIV-infected individuals was able to establish genetic relatedness of colonizing MRSA isolates and isolates causing subsequent skin and soft tissue infections [37]. A study among HIV-infected persons in South Africa showed that *Streptococcus pneumoniae* colonization density correlate with disease severity and poor outcome in patients with pneumonia [43]. Colonization with multi-drug resistant bacteria may pose a greater risk to HIV-infected compared to HIV-negative people. Recent studies have shown HIV positive individuals were at high risk of being colonized with multidrug resistant bacteria

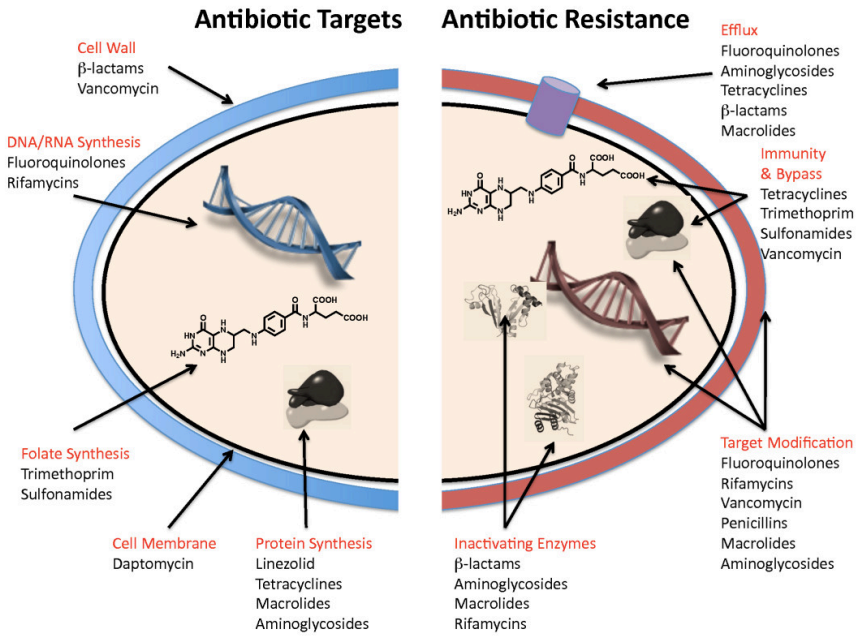
compared to those who are HIV negative [44-47]. Previous studies from HIV-infected children in Africa have reported high rates of antibiotic resistant bacteria including MRSA and ESBL producing Enterobacterales isolated from nasopharyngeal swabs [27, 48]. Another recent study among HIV-infected children from Zimbabwe reported high prevalence (13.7%) of fecal carriage of ESBL producing Enterobacterales, and carriage was associated with a history of recent hospitalization [49]. However, a study from Asia reported a low rate of gut colonization of ESBL producing Enterobacterales among HIV-infected compared to HIV uninfected population [50].

1.2.2. Mechanisms for resistance to antibiotic

Antimicrobial resistance (AMR) occurs when changes in bacteria cause the antibiotics used to treat infections to become ineffective. It remains a major public health concern world-wide. Recent systematic analysis estimated there to be 495 million (3·62–6·57) deaths associated with bacterial AMR in 2019, with the estimated all-age death rate attributable to resistance to be highest in Sub-Saharan Africa, at 23·7 deaths per 100,000 (18·2–30·7) [51]. It is estimated that unless action is taken, the burden of AMR deaths will rise to 10 million per year by 2050, surpassing cancer and any other disease [52].

The main mechanisms bacteria develop resistance to antibiotics include 1) Reduce permeability of drug, 2) Enzymatic inactivation of the drug (β-lactamases), 3) Modification of antibiotic targets and 4) active efflux of drugs using efflux pump, Figure 5.

WHO has published a priority list of AMR pathogens categorized as critical, high, and medium priority, Table 1. These bacteria frequently display a multidrug-resistant (MDR) phenotype and associated with poor treatment outcome [53].



Source: Wright *BMC Biology* 2010.

Figure 5: Mechanism of actions of antibiotics and resistance mechanisms to resistance

Table 1: WHO priority pathogens list

Priority	Name of the pathogens
Priority 1: Critical	carbapenem resistant Enterobacterales
	3rd generation cephalosporin-resistant Enterobacterales (ESBL),
	carbapenem-resistant *Pseudomonas aeruginosa*,
	carbapenem-resistant *Acinetobacter baumannii*
Priority 2: High	vancomycin-resistant *Enterococcus faecium*
	methicillin-resistant *Staphylococcus aureus*, vancomycin intermediate and resistant *Staphylococcus aureus*
	Clarithromycin-resistant *Helicobacter pylori*
	Fluoroquinolone-resistant Campylobacter
	Fluoroquinolone-resistant Salmonella spp.
	3rd generation cephalosporin-resistant *Neisseria gonorrhoeae*, fluoroquinolone-resistant *Neisseria gonorrhoeae*
Priority 3: Medium	Penicillin-non-susceptible *Streptococcus pneumoniae*
	Ampicillin-resistant *Haemophilus influenzae*
	Fluoroquinolone-resistant Shigella spp.

1.3. MRSA in HIV infected population

1.3.1. Historical background

Methicillin resistance in *S. aureus* is mediated by acquisition of genes encoding for penicillin-binding protein 2a (PBP 2a), that has reduced affinity to penicillin and other beta-lactam antibiotics. PBP 2a is encoded by the *mecA* gene, that is carried in a mobile genetic element known as the Staphylococcal cassette chromosomes (SCC) *mec* element.

Methicillin was first introduced into clinical practice in 1959. Less than one year after its first discovery, MRSA was first described in United Kingdom [54] and widespread use of methicillin was thought to have driven emergence of this resistant strain. However, recent whole genome sequencing of early MRSA isolates, suggests MRSA emerged in the mid-1940s, and that methicillin was not a driving factor for its emergence [55]. Since its discovery, MRSA has undoubtedly caused a severe global health threat and contributed to mortality. It has been a leading cause of health-care and community associated infections.

Since their initial description MRSA has been spread world-wide with marked geographical variations in burden [56]. The burden of MRSA infections is reported to be low in Europe and highest in some parts of America and Asia [57]. The few available studies from the African continent indicate a high prevalence of MRSA infections [58]. In Europe, the Scandinavian countries have low prevalence of MRSA, in 2020, only 1.4% (19/1367) of Norwegian blood culture isolates of *S. aureus* were MRSA [59]. Southern Europe has comparatively much higher prevalence of MRSA (25–50% of *S. aureus*) [60]. In the United States of America, a laboratory-based surveillance study reported that 57.8% of *S. aureus* of clinical isolates were MRSA in 2007 [61]. Asia has been reported to have the highest prevalence of MRSA infections, although there are variations among countries. It has been observed in Asia that over 50% of *S. aureus* causing blood stream infections were MRSA, ranging from 28% in Indonesia to >70% in Korea [62].

There is paucity of data on MRSA from Africa, however, it appears that the prevalence of MRSA infections is on the rise on the continent. A recent meta-analysis,

reports an estimated prevalence approaching 50% of MRSA among clinical isolates obtained from most African countries [58].

1.1.1 MRSA colonization in HIV infected population

MRSA is a common cause of both invasive and non-invasive disease in the general population. Previously, MRSA infections were largely associated with health-care settings (healthcare-associated MRSA, HA-MRSA) and limited to hospital settings [63]. However, in the last decade community acquired MRSA (CA-MRSA) has become more prevalent causing infection both in community and hospital settings. In recent years, MRSA bacteremia caused by CA-MRSA has increased steadily tending to replace HA-MRSA [64].

CA-MRSA colonization/infections are disproportionally high in HIV-infected people compared to the general population [30, 65-67]. In people living with HIV infection, CA-MRSA infection is an important contributor to mortality, morbidity, prolonged hospitalization, and increased health care cost [19, 26, 37, 68-70]. Prospective studies in HIV-infected populations show that MRSA colonization is a risk factor for the development of MRSA infections [37, 38, 71-73]. Szumowski *et al*. reported that, in HIV-infected participants colonized with MRSA at study baseline, 36.7% developed skin and soft tissue MRSA infection compared to 8.1% of those who were not colonized [38]. Another prospective molecular study on MRSA colonization and subsequent infection in the HIV positive population, found that genetically similar colonizing strains caused skin and soft tissue infection in the same individual [37]. A multicenter study of HIV negative individuals found that nasal *S. aureus* colonization appeared to be a source for *S. aureus* bloodstream infection [74]. However, not all studies support the hypothesis that prior *S. aureus* colonization precedes infections. A South African study of HIV-infected children found that *S. aureus* bacteremia was not associated with prior *S. aureus* nasopharyngeal colonization [75].

MRSA strains can colonize anyone; however, HIV-infected people are more susceptible compared to their counterparts, mainly resulting from low CD4 count. The prevalence of colonization of MRSA in HIV-positive people varies according to the geographical locations where the studies have been conducted. A review and meta-analysis on the global prevalence of MRSA colonization in the HIV-infected

population has reported an estimated prevalence between 5% and 9%, with the highest prevalence being in Southeast Asia, followed by the American region and Europe [76]. While few studies have assessed the burden of MRSA colonization in people living with HIV in the African region, a meta-analysis reported a pooled prevalence of 7% (95%CI 4 –10%) [76]. The few available studies from Sub-Saharan Africa, the region where two-thirds of the world's HIV-infected people live, have reported varying prevalence of MRSA colonization ranging from 2.4% – 20.8% among different populations [77-79]. Differences in study methodologies could account for some of this varying prevalence. Screening for MRSA colonization from multiple body sites and at different time points increases the rates of colonization [80], all these studies from Sub-Saharan Africa screened only one site (nasal) and sampled at only one time point. A study by Kyaw WM et.al. showed that including throat and/or peri-anal swabs increased the sensitivity of MRSA colonization by 20% compared to only swabbing nose, axilla and groin [80].

The reason for the high burden of MRSA colonization in HIV-infected population compared to general population is not well known. However, frequent visits to health care facilities coupled with frequent exposure to antibiotics is likely to increase the probability of MRSA colonization in the HIV-infected population. Most literature have reported low CD4 counts to be an independent risk factor for MRSA colonization as well as infection [78, 81, 82]. However, MRSA colonization also occurs in HIV-infected individuals without evidence of immunosuppression and in HIV negative populations [37]. This suggests that there are other factors than CD4 T-lymphocyte-mediated immunity that plays a role in MRSA colonization. Similar to findings from the general population, prior antibiotic use and previous history of hospitalization have been reported as risk factors for increased carriage of MRSA in people living with HIV [83]. However, other authors have suggested that current use of trimethoprim-sulfamethoxazole is protective against MRSA colonization [81]. Some authors have argued that certain behavior risk factors increase the risk of MRSA colonization, including incarceration, illicit drug abuse and risky sexual behavior. Despite several risk factors being associated with nasopharyngeal colonization, HIV itself, has remained an independent risk factor for MRSA colonization [66].

In Sub-Saharan Africa, data on circulating CA-MRSA genotypes in the HIV-infected population are scanty. However, predominance of Panton-Valentine ST8 CA-MRSA has been reported to colonize people living with HIV in other parts of world. This genotype has been associated with skin and soft tissue infection, commonly seen in the HIV-infected population.

1.1.2 MRSA infections in HIV infected population

In the HIV-infected population, invasive MRSA infections remains of particular concern. In the era of highly active antiretroviral therapy (HAART), the incidence of CA-MRSA infections in HIV-infected individuals have reported to increase compared to the general population [84-86]. A facility based retrospective study found that HIV-infected persons have 6 –18-fold higher risk of developing CA-MRSA infections compared to the general population, with skin and soft tissue infections accounting for the majority of infections [30, 65, 67]. A 15 years follow up study found that MRSA infections were diagnosed in 11.0% of HIV-infected patients compared to in 1.4% of HIV-uninfected patients. Similarly, a seven-years follow-up study found that 8% (19.8 infections/100-person years) of HIV-infected individuals developed MRSA infection [87]. All these studies reported low CD4 counts, high plasma viral load, injection drug use, absence of cotrimoxazole prophylaxis and high-risk sexual behavior as risk factors for development of MRSA infection in the HIV positive population [30, 65, 87]. In addition, a recent study observed that deficient MRSA-specific IFNγ+ CD4 T-cell responses in HIV-infected people are associated with development of MRSA skin and soft tissue infections (SSTIs) [88].

The HIV epidemic in Sub-Saharan Africa may increase the incidence of MRSA infections. However, data on the incidence of MRSA infections in the HIV-infected population in Sub-Saharan Africa are scanty and could be accounted for by lack of diagnosis and identification of MRSA infections. A prospective study in South Africa studying community onset *S. aureus* bacteremia in HIV-infected children found an incidence of 26/100,000, with 39% of isolates identified as MRSA [19].

Literature from developed countries indicate that, in the HIV-infected population, skin and soft tissue infections accounts for the majority of CA-MRSA infections, mirroring

the trend observed in the general population [30, 89]. Bacteremia is the second most frequent MRSA infection, followed by pneumonia and necrotizing fasciitis [30, 90]. Reviews and studies from developed countries have observed that 80-90% of CA-MRSA infections in HIV-infected population are SSTI [65]. A 12 years follow up study in a HIV-infected population found that 90% of CA-MRSA infections were skin and soft tissue infections with predilection for the buttocks and scrotal areas [65]. Similarly, a review by Shadyab *et al*. echoed the same trend of HIV-infected persons having a propensity for MRSA SSTI and a high rate of recurrent disease [91]. The tendency of MRSA SSTIs to affect the perineum and buttocks seems to be related to sexual risk behavior, as compared to other types of CA-MRSA infections [65, 91]. HIV patients with CA-MRSA SSTI infections have increased genital and perineal MRSA colonization, partly attributable to sexual risk behavior. Studies on the etiology of CA-MRSA SSTI infection in HIV positive populations have observed predominance of the PVL-positive ST8 CA-MRSA (USA300) clone as causative strain [85, 89, 92]. The USA300 clone has been found to be the predominant clone in HIV-infected people in USA and some parts of Asia [83, 89]; and is associated with SSTI and necrotizing pneumonia [85]. In contrast, it is infrequently responsible for other infections, such as bloodstream infection and endocarditis. Studies from Sub-Saharan Africa report varying epidemiology of USA300 like clones in the general population. Studies from Gabon and Cameroon have reported sporadic MRSA strains of USA300 like clones, which do not belong to the classic spa-t008 observed in USA300 clone [93, 94], but recent studies from the Democratic Republic of Congo (DRC) and East Africa have observed predominance of non-USA300 clones and PVL negative MRSA [95-97]. This might suggest that the epidemiology of SSTI in Africa could be different from that of developed countries.

Bloodstream infections are the second most frequent type of MRSA infections in the HIV-infected population. Previous studies have reported that its incidence rate has increase during the HAART era. In a 4-year retrospective follow up study, the incidence of MRSA bacteremia increased from 5.3 per 1000 year at baseline to 11.9 per 1000 year at the end of the study period [70]. In addition, a study among HIV-infected children from South Africa has reported a high rate of community onset

MRSA bacteremia [19]. In contrast, more recent studies have observed decreasing trends of MRSA bacteremia in the HIV-infected population [69, 98]. Moreover, independent predictors for MRSA bacteremia in the HIV-infected population includes injection drug use, end stage renal disease, and low CD4 count [70, 98]. In CA-MRSA bacteremia, SSTI has rarely been documented as the source of infection. Recent studies indicate that PVL positive CA-MRSA strains infecting skin and soft tissue [89] rarely are responsible for other types of infection. Studies from Sab-Saharan Africa have reported that MRSA and multidrug resistant bacteria predict mortality in bloodstream infection [99, 100]. Overall mortality of MRSA bloodstream infection in the HIV-infected population has been reported to range from 10% to 40% [17]. However, studies on the outcome of MRSA bloodstream infections found no significant differences in mortality between HIV-infected compared to HIV negative patients [98].

MRSA pneumonia in the HIV-infected population is responsible for both community and hospital acquired pneumonia. Hospital acquired MRSA is becoming a more frequent cause of hospital acquired pneumonia and is associated with high mortality. A retrospective study in a HIV-infected population found that 65% of *S. aureus* causing nosocomial pneumonia were methicillin resistant and the sole predictor for mortality was isolation of MRSA [101]. In contrast, another hospital-based study examining predictors of mortality found no significant differences in mortality between MRSA and MRSA, despite a higher rate of MRSA (57%) infection than MSSA [102].

1.1.3 Laboratory detection of MRSA

Specimens for detection of MRSA

S. aureus including MRSA colonizes mucosa and skin of all animals. Strategies that limit both MRSA colonization and infection in HIV-infected persons are more likely to be successful in infection prevention and control. Nasal carriage of MRSA in HIV varies between studied populations, but increased colonization in other parts of the body including perineum, groins and axilla has been observed in HIV-infected persons. Screening for MRSA nasal colonization alone may result in low recovery

[103]. Studies on MRSA colonization found that a combination of nares/throat/inguinal/perirectal sampling increases the recovery of MRSA [80, 104].

Methods for screening for MRSA

Previously, screening of MRSA was processed on non-selective medium (sheep blood agar) and selective medium (mannitol salt agar), followed by confirmation of MRSA. In recent decades the use of chromogenic media for isolation of MRSA have gained popularity in clinical microbiology. In principle, these media are simple to use, have low turnaround time and makes it easy to detect MRSA colonies. Chromogenic media employ an enzyme which, when hydrolyzed by bacteria, produce a color change around the bacterial colonies. Studies have reported that MRSA chromogenic media exhibit high specificities and varying sensitivities [105, 106]. In addition, chromogenic media show good diagnostic performance in detection of MRSA from different clinical specimens. Enrichment into broth media and plating into chromogenic media have been found to increase recovery of MRSA from clinical specimens [107].

Recent molecular methods including polymerase chain reaction (PCR) and whole genome sequencing have become more available and gained popularity for screening for MRSA with increased sensitivity and specificity [108]. Apart from identifying MRSA from clinical isolates, these methods have increased the ability to detect MRSA directly from clinical specimens with minimal turnaround time. Studies have shown that real-time polymerase chain reaction reduces the time of detection of MRSA from 48–72 hours to 2–5 hours [109]. However, cost and technical requirements are bottlenecks of the molecular methods, particularly for developing countries.

Phenotypic and molecular confirmation of MRSA

Phenotypic methods

Phenotypic methods including cefoxitin disk diffusion, oxacillin disk diffusion and oxacillin agar dilution remains the main stay for confirmation of MRSA strains in resource limited settings. However, these methods present with low sensitivity and specificity, inconsistent results, and are time-consuming. Bhutia *et al*. found that the sensitivities of oxacillin disk diffusion, cefoxitin disk diffusion and oxacillin agar

dilution were 71%, 86%, 92%, respectively [110]. Likewise, the specificities for oxacillin disk diffusion, cefoxitin disk diffusion and oxacillin agar dilution were 76%; 83%; and 91%, respectively.

Of the disk diffusion methods, most literature have recommended cefoxitin disk diffusion compared to oxacillin disk diffusion, because it is a good discriminator and easier to interpret than oxacillin disk diffusion [111]. In addition, cefoxitin is a better inducer of *mecA* and *mecC* genes than oxacillin. Clinical and laboratory standards institute [111] recommends disk diffusion to be performed with strict incubation at 33–35°C and 24 hours duration of incubation to avoid false negative results [44]. This is because two subpopulations of all isolates with *mecA* genes may co-exist but only a few isolates in these populations may express resistance in vitro, the phenomena referred to as hetero-resistance [112].

Molecular methods

Polymerase chain reaction detecting *mecA,* which encode for low affinity penicillin binding protein 2B (PBP 2B), is considered the gold standard for confirmation of MRSA. PCR based methods offer more benefits compared to culture based methods, including shorter turnaround time and high throughput in screening [113]. Availability of results in hours rather than days, can significantly strengthen infection prevention and control programs at health facilities. Several multiplex PCR based platforms have been validated for the use of clinical specimens or isolates; and simultaneously detect the presence of a S. aureus specific target gene and the *mecA* gene [114]. Current commercially available PCR base methods include BD GeneOhmTM MRSA assay (BD GeneOhm, San Diego, CA, USA), the Hyplex StaphyloResist PCR (BAG, Lich, Germany), the GenoType MRSA direct assay (Hain Lifescience, Nehren, Germany) LightCycler *Staphylococcus* and MRSA detection kit (LC assay; Roche Diagnostics, Mannheim, Germany), and the GeneXpert MRSA assay (Cepheid, Sunnyvale, CA). These platforms commonly use *S. aureus* gene targets genes that include the *nuc* gene encoding heat-stable DNA nuclease, *femA* and *femB* encoding enzymes important for cross-linking peptidoglycan and *spa* encoding *S. aureus*-specific protein A [114]. Several previous and recent studies have analyzed the performance of multiplex real-time PCR using clinical isolates and specimens, all have showed high sensitivity and

specificity for detection of MRSA and *S. aureus* [113, 115, 116]. Furthermore, results were available within 2 hours of setting an assay [113].

Recent development of next generation sequencing (NGS) has revolutionized diagnostic microbiology, outbreak investigation and surveillance of diseases. Sequencing of entire genomes provide information on the identification of bacteria, resistance genes, virulence genes and epidemiological typing. Several NGS systems are currently used in clinical diagnostics and research, however, they differ in several parameters including costs, read length, sequence quality and sequence coverage and depth [117]. Illumina, Pacific Biosciences and Nanopore sequencing are being commonly used in published research [118, 119]. Recent studies using Illumina Miseq on whole genome sequencing for MRSA were able to reveal SCC*mecA*, antibiotic resistance genes, virulence genes, sequence type, and single nucleotide polymorphism differences between isolates [120]. Nanopore technology has also been validated for identification of virulence genes and antibiotic resistance genes [121]. Analyzing the performance of Oxford Nanopore MinION, Judge K et.al found that the technology was able to detect all expected ESBL and carbapenemase genes in Gram-negative bacteria and the *mecA* gene in MRSA [122]. Each of the three commonly used NGS systems has its own advantages and disadvantages. Although there has been a decline in costs in recent years, all NGS systems are still expensive compared to conventional molecular methods.

Sequencing read length is an important parameter in analysis of whole genome sequencing data. Long read sequencing is more desired than short read sequencing, long read sequencing on DNA/RNA eliminate amplification bias while preserving base modifications [123]. Short read generated contigs may be more accurate at the base-pair level, but the short-read sequences have an inability to fully reconstruct the whole gene and detect multiple instances of genes. Illumina sequencing technology is a short-read sequencing system commonly used in diagnostic microbiology, while Pacific Biosciences and Nanopore sequencing technologies' system is the most commonly used long-read sequencing platform [119, 124].

Molecular typing of MRSA

Molecular typing is key for epidemiological investigation and the control of MRSA outbreaks. Understanding of the MRSA evolution, transmission dynamics and population structures are critical for the prevention and control of MRSA infection. Several molecular typing techniques have been developed.

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is a restriction fragment length technology, based on digestion of chromosomal DNA generating large fragments which are separated by pulsed-field gel electrophoresis, followed by clonal assignment. The bands produced allow for the identification of specific clones. PFGE has been widely used for the typing of MRSA and other bacterial isolates as it remains affordable [125]. However, it is laborious and time consuming. A limitation of PFGE is that it has poor discriminatory power, inter-laboratory variability and does not provide information on genealogy of the organisms.

Multilocus Sequence Typing (MLST)

MLST is a sequence based method which allows for the unambiguous assignment of isolates from infectious agents using sequences of internal fragments of seven housekeeping genes (i.e., constitutive genes required for the maintenance of basic cellular functions) [126]. The method is based on nucleotide sequences analysis of internal gene fragments with lengths of 450–500 base pairs. Since mutations accumulate slowly in housekeeping genes, the MLST is used to allocate clusters of closely related strains. The sequence of each gene allows obtaining the allelic profile or sequence type (ST). Organisms which share all seven alleles are defined as a clone, those sharing five or more out of seven alleles are defined as a clonal complex [127] and those sharing less than five of seven alleles are considered unrelated. The method has been used widely in molecular epidemiology, phylogenetics, and studies of population structures and dynamics. However, one of the limitations of MLST, is that it has low discriminatory power to characterize differences in strains in the case of outbreaks.

Whole genome sequencing

Development of next generation sequencing has revolutionized molecular epidemiology, as it is based on determining the whole genome of the microbial pathogens [128]. The technique provides excellent discrimination between closely related strains and has high throughput. PFGE, MVLA and MLST methods are based on amplification of only small parts of the genome and have inherent low discriminatory power; often the methods fail to distinguish closely related strains in case of an outbreak [129, 130]. Madigan and colleagues reported that WGS was able to identify two major clusters of MRSA that PFGE failed to discriminate in their examination of an outbreak in the neonatal intensive care unit [130]. Development of next generation sequencing has revolutionized the detailed understanding of bacterial characteristics based on determination of the entire genome of the microbial pathogens [128]. The technique provides great discrimination between closely related strains and has high throughput. NGS has inherent high resolution in discriminating highly related strains compared to other molecular methods [131, 132]. WGS is very useful in resolving MRSA outbreaks, according to an assessment by Humphreys and colleagues. WGS investigations are now revealing the nature of MRSA transmission from the community to hospitals, inside and between hospitals, and from hospitals to the community [128].

1.1.4 Prevention and control of MRSA infection

MRSA infection is known to cause severe infections associated with increased mortality and health care costs. Efficient strategies to control its spread remains critical to reduce its clinical impact. Strategies for control relies on the identification of MRSA carriers which allows for contact isolation and prevention of transmission. Then, decontamination and treatment of carriers can help reducing MRSA reservoirs. Third, practicing hand hygiene can reduce transmission. Fourth, rational use of antibiotics can help reducing the selection pressure which increases the emergence of MRSA [133].

Screening to detect asymptomatic MRSA carriers has been advocated as a cost-effective prevention strategy. However, several issues remain un-addressed including identifying the appropriate populations for targeted versus universal screening, the

choice of the screening methods; intervention strategies and cost issues. Studies on universal screening of MRSA in intensive care units and in surgical patients followed by intervention either in terms of contact isolation or decolonization have resulted in significant reduction in MRSA infection [134, 135]. The downside in one study was an increase in mupirocin resistant *S. aureus* were mupirocin was used to decolonize MRSA positive patients [136]. In light of the costs related to universal screening for MRSA, targeted screening of specific groups at risks (ICU patients, patient undergoing surgery) remain the cornerstone for control and prevention of MRSA infection [137]. Studies have found that HIV infection is a risk factor for MRSA infection/ colonization [30, 65]; however, the benefit of targeted screening of MRSA in HIV-infected patients at admission has not been studied.

Unprecedented spread of MRSA in the community poses a great challenge for its control, and whether targeted or universal screening would be the best approach remained to be resolved.

1.2 ESBL-PE in HIV infected population

1.2.1 ESBL-PE background

ESBL are a group of enzymes capable of hydrolyzing third-generation cephalosporins penicillins, and aztreonam (but not cephamycin or carbapenems) and are inhibited by β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam and other newer inhibitors [138]. Although the strict definition of an ESBL remains, the inhibition by clavulanate is a requirement for designation in this group. Based on the updated functional classification of beta-lactamases, ESBLs are classified in group 2 serine beta-lactamases, subgroup 2be [139]. Classification based on molecular are classified as amber class A [140].

The first and largest members of subgroup 2be are mutants of TEM-1, TEM-2 and SHV-1 beta-lactamases. To date, there are over 223 TEM type and 193 SHV type beta-lactamases derived from substitution of amino acids [141].

The second largest and rapidly growing member of subgroup 2be is CTX-M, as the name implies it hydrolyzes more efficiently cefotaxime than ceftazidime. Furthermore, CTX-M is inhibited better by tazobactam compared to clavulanic acid [142]. The first CTX-M type enzyme was described from Enterobacterales in Europe

in 1980 [143]. Since its first discovery, CTX-M type ESBLs have rapidly disseminated world-wide; and there are more than 179 CTX-M variants described to date clustered into five groups CTX-M 1, CTX-M-2, CTX-M-8, CTX-M 9, and CTX-M-25 [141].

In Tanzania, the first report of discovery of CTX-M genotype was reported in 2004 from children with septicemia with predominant of *bla*$_{\text{CTX-M-15}}$ [144]. After this first report, several studies have reported *bla*$_{\text{CTX-M-15}}$ producing Enterobacterales associated with community and hospital acquired infections in Tanzania [145-147].

However, there several other genes encoding for ESBL and others are predominant in Pseudomonas and Acinetobacter, including GES, PER, VEB, TLA, BEL, SFO and OXY [148].

1.2.2 ESBL-PE colonization in HIV

Due to suppressed immunity, ESBL-PE gut colonization in the HIV-infected population increases vulnerability to ESBL-PE infection. Recent studies from developing countries have found an increasing prevalence of ESBL fecal carriage in HIV-infected people [49, 50, 149]. A previous Tanzanian study found a significantly higher prevalence of fecal carriage ESBL-PE among HIV positive (89.7%) children compared to HIV negative (16.9%) children [149]. Furthermore, a study of outpatient HIV-infected children in Zimbabwe documented a 13.7 percent prevalence of fecal carriage. Using ART for less than a year and recent hospitalization were identified as risk factors for ESBL-PE fecal carriage in this study [49]. Interestingly, a more recent Nepalese study found a lower rate of ESBL-PE gut colonization in HIV positive individuals compared to HIV negative individuals. Contact with livestock and over-the-counter medication were found to be associated with ESBL-PE fecal colonization in healthy individuals but not in HIV-infected individuals [50].

1.2.3 ESBL-PE infections in HIV

Infections due to ESBL-PE has been increasing globally. In the general population, they are associated with prolonged hospital stay as compared to non-ESBL infections [150, 151]. Furthermore, studies have found that the mortality in ESBL-PE infections is almost 2-3-fold compared to non ESBL-PE infections [100, 151-154]. Likewise, ESBL production has been found as an independent risk factor for mortality in blood-

stream infections [100, 151, 153, 155]. In addition, ESBL-PE infections have been found to increase health care costs both to patients and health facilities [151]. Understanding the risk factors for ESBL-PE infections in a population at risk remains vital for the prevention of its spread. Recent history of antibiotics use and previous hospitalization have been well studied as an important risks factor for ESBL infections [156, 157]. These factors are thought to increase ESBL-PE colonization which is a prerequisite for infection. HIV infection by itself has been documented as an independent risk factor for both ESBL-PE colonization and infection by some authors [149, 158, 159]; while another study found no association [50].

HIV infection was found to be a significant risk factor for the development of ESBL-*K. pneumoniae* blood-stream infections and mortality among patients with blood-stream infections in South Africa [159, 160]. A more recent study in India found ESBL *Klebsiella spp* infection was significantly higher in HIV-infected individuals as compared to HIV negative individuals, concluding immunosuppression renders HIV-infected individuals more susceptible to ESBL infection [158]. Likewise, it has been documented in India, that *E. coli* producing CTX-M is a growing threat to the HIV-infected population. Padmavanthy *et al*. found that a significant number *E. coli* causing urinary tract infection were carrying CTX-M enzyme and the majority displayed multi-drug resistance [161].

Surprisingly, a recent 10-year follow-up study of ESBL and non-ESBL-PE blood-stream infections in children in South Africa documented that HIV infection was not a risk factor for the development of ESBL blood-stream infection [160].

Several studies have found a link between ESBL-PE colonization and subsequent ESBL-PE infections [162, 163]. The risk of ESBL infections in patients colonized by ESBL varies depending on the population studied and the location of admissions [164]. Several studies in intensive care units (ICUs) have identified prior gut ESBL-PE colonization as the only independent risk factor for ESBL-PE ventilator-associated pneumonia, with high sensitivity and negative predictive value in predicting ESBL-PE VAP [165, 166]. Another study found that prior ESBL colonization was an independent risk factor for ESBL blood-stream infection in the general population among admitted patients in French hospitals [163]. It has been reported that ESBL-PE

gut colonization was independently associated with the development of both ESBL-PE surgical site infections and bloodstream infections in patients with gastrointestinal and gynecological malignancies [164].

1.2.4 Laboratory detection of ESBL producing Enterobacterales

Specimens for detection of ESBL -PE

Stool specimens are the gold standard for detecting digestive tract colonization of ESBL producing Enterobacterales and are more sensitive than swabs for detecting ESBL-PE carriage in the gut [167].

Rectal and perineal swabs, however, have been used to detect gut ESBL-PE colonization due to the difficulty of obtaining stool in a timely manner. Rectal and perineal swabs have the advantage of being easier to obtain and can be collected immediately from a large group of patients. Rectal swabs, on the other hand, have been shown to be more effective than perineal swabs in detecting gut ESBL-PE colonization [168].

Screening methods

Chromogenic ESBL screening agar plates

Reliable screening methods are required for infection control measures for ESBL-PE infections. Several selective chromogenic media have been used for targeted or universal ESBL-PE colonization screening. With varying specificities, these media have improved sensitivity for isolating ESBL-PE from clinical specimens. ChromID ESBL (bioMérieux, Marcy l'Etoile, France), Brilliance Oxoid (Oxoid Ltd, Basingstoke, United Kingdom), CHROMagar ESBL (CHROMagar, Paris, France), and BD Drigalski agar with ceftazidime (Becton, Dickinson and Company, New Jersey, US) are among these media. In a study that evaluated the performance of these media, it was shown that all of them had a sensitivity of greater than 95%. For the four types of media, specificity was 72.3 percent, 72.9 percent, 57.9 percent, and 63.9 percent, respectively [169]. Several studies have shown that pre-enriching rectal swabs in broth media improves the detection of ESBL-PE fecal carriage [170-172]. Similarly, Rondinaud *et al*. found that pre-enrichment followed by screening on ChromID ESBL improved detection of ESBL – Enterobacterales from traveler stool samples by 11.7 percent [170].

Media supplemented by antibiotics for ESBL screening

MacConkey agar supplemented with either ceftazidime, cefotaxime, or ceftriaxone, aztreonam, has been used for screening of ESBL production among Enterobacterales with varying sensitivities and specificities [173-175].

The screening concentration of 1µg/mL for cefotaxime or ceftriaxone, ceftazidime, aztreonam or 4µg/mL concentration for cefpodoxime has been recommended by CLSI [111]. Growth at or above the concentrations listed may be presumed as ESBL production. However, one drawback of this medium is that it has low specificity, which allows growth of not only ESBL producers but inducible AmpC beta-lactamase producers and Gram-negative bacteria as well [176]. The addition of cloxacillin into the medium to inhibit AmpC beta lactamase producing bacteria has been recommended by some authors and increases its specificity [177]. On the other hand, CLSI and previous studies recommended, the use of two screening plates, one containing ceftazidime and another containing cefotaxime, to increase the sensitivity of ESBL detection [111, 174].

ESBL confirmation methods

Phenotypic confirmation methods

Combination double disks diffusion method

For phenotypic confirmation of ESBL production in Enterobacterales, the Clinical and Laboratory Standards Institute recommends using double combination disks [111]. The test is dependent on the presence of clavulanic acid and the use of cephalosporins alone (primarily ceftazidime and cefotaxime). Clavulanic acid has the ability to bind irreversibly to ESBL, preventing ESBL from hydrolyzing the beta-lactam ring. As a result, the zone of inhibition in the disk with clavulanate increased. Ceftazidime (30g) plus ceftazidime-clavulanate (30/10µg) and cefotaxime (30µg) plus ceftazidime-clavulanate (30/10 µg) are both recommended.

A ≥ 5mm increase in zone of inhibition for cephalosporins with clavulanate vs cephalosporin alone confirms ESBL production [111]. One disadvantage of the test is that it produces false negative ESBL results when Enterobacterales contain both ESBL and AmpC beta-lactamases. Clavulanic acid does not inhibit AmpC beta-lactamase [178]. The addition of cefepime, which is stable against AmpC beta-

lactamase to cefotaxime and ceftazidime confirmatory tests, could address the AmpC beta-lactamase drawback [178].

Double disk approximation method

The Double disk approximation test has been evaluated for confirmation of ESBL production with varying sensitivities and specificities[179-181]. In this test, ceftazidime/cefotaxime and amoxycillin clavulanic acid are placed 30mm apart, center to center. An enhanced zone of inhibition towards these cephalosporins indicates ESBL production [179]. As for combination disks methods, coexistence of ESBL and AmpC beta-lactamases may give false negative test results.

Broth microdilution method

The CLSI also recommends using the broth microdilution method for confirming ESBL production, in which ceftazidime and cefotaxime are used alone and in combination with clavulanic acid. A ≥3 two-fold decrease in the MIC for either combination with clavulanic acid compared to the MIC for cefotaxime/ceftazidime alone confirms ESBL production [111].

Epsilometer test method

The Epsilometer test (E-test) has been used for confirmation of ESBL production in some studies [182, 183]. The strip carries two gradients: cephalosporines alone (ceftazidime, cefotaxime) and combinations of cephalosporines and clavulanate (ceftazidime-clavulanate and cefotaxime-clavulanate) [183]. A ratio of cephalosporine MIC to cephalosporine-clavulanic acid MIC equal to or greater than 8 confirms ESBL production. The use of both ceftazidime and cefotaxime strips improves detection of different types of ESBL enzymes, as ESBL types differ in hydrolyzing strength to different cephalosporines [138].

Molecular methods for confirmation ESBL production

Using various primers, several polymerase chain reaction methods for detection of cefotaxime-Munich (CTX-M), sulfhydryl variable (SHV), and Temoniera [184] have been developed [185-188]. However, detecting specific variants of bla_{TEM} and bla_{SHV} has proven more difficult because all variants result from single point mutations in the narrow-spectrum beta-lactamases $\text{bla}_{\text{SHV-1}}$, $bla_{\text{TEM-1}}$, or $bla_{\text{TEM-2}}$. Multiplex PCR methods have been validated for detecting different types of SHV and TEM using

universal primers, followed by sequencing to detect specific variant of SHV or TEM [185, 187, 189].

The rising prevalence of *bla*$_{CTX-M}$ among ESBL, which is linked to both hospital and community-acquired infections, necessitates the development of reliable and rapid molecular methods for detection. Multiplex and simplex PCR have been used to detect the *bla*$_{CTX-M}$ genotype using both conventional and real-time PCR approaches [185, 188]. Several previous studies [185, 190], used multiplex PCR with CTX-M group specific primers, followed by sequencing or gel electrophoresis, to detect different *bla*$_{CTX-M}$ genotypes. In a study of 633 Enterobacterales, Woodfold and colleagues were able to detect all common *bla*$_{CTX-M}$ genotypes using multiplex PCR and a set of CTX-M group specific primers, followed by sequencing [190].

A simplex Real-Time PCR approach with one set of primers targeting internal regions present in all five CTX-M phylogenetic groups[149], was recently used, followed by sequencing. The approach was able to detect the most common CTX-M genotypes of ESBL by sequencing the PCR products.

1.2.5 Prevention and control of ESBL-PE infections

Identifying risk factors for ESBL infection and addressing mitigating measures remain critical components of ESBL infection control. Several studies have found that prior antibiotic use, prior hospitalization, and immunosuppression all increase the risk of ESBL-PE infection/colonization. ESBL-PE colonization remains a requirement for ESBL infection [162, 163]. ESBL-PE dissemination has previously been reported in hospital outbreak infections. However, the recent spread of ESBL-PE in the community [191], demonstrates the need for improved infection prevention and control. Identifying patients with ESBL-PE colonization during hospitalization is critical for infection prevention and control. Screening for ESBL-PE colonization, pre-emptive isolation of patients at risk on admission, and contact precautions have all been found to be effective ESBL-PE infection control measures in the developed world [192, 193]. A four-year multicenter study from developed countries documented that ESBL-PE admission screening was associated with lower hospital onset ESBL-PE infection [192]. However, in Sub-Saharan Africa, where HIV is endemic and antibiotic overuse is common, all of these are risk factors for ESBL-PE

infection/colonization. Screening of ESBL-PE at admission as a preventive measure for infection control has rarely been documented.

1.3 Carbapenemase-producing Enterobacterales in HIV

1.3.1 Carbapenemase production among members of Enterobacterales

Carbapenem resistant Enterobacterales have been listed as critical priority pathogens in the WHO list of Priority pathogens for their association with mortality and morbidity [194]. Mechanisms of resistance to carbapenems in Enterobacterales may be conferred by decreased permeability, efflux pump, and production of carbapenemase [195]. The production of carbapenemase is the most worrying mechanism. The carbapenemases have the ability to hydrolyze most of the β-lactam antibiotics and frequently are encoded in mobile genetic elements (plasmids, transposons, integrons).

Among the 4 classes of β-lactamases based on molecular structures (amber classification system), carbapenemases belong to three of the four classes. Amber class A serine-dependent include *Klebsiella pneumoniae* carbapenemase (KPC). Amber class B carbapenemases metal-dependent include metallo-lactamases (MBL), such as the New Delhi metallo-lactamases [196], the IMP family of carbapenemases, the Verona integron–encoded metallo-lactamases (VIM) and the Guiana-extended spectrum (GES). The amber class D serine-dependent include oxacillinase-48 (OXA-48) [140, 197]. Most carbapenemase genes are carried on mobile genetic elements, which are used as vehicles of dissemination intraspecies and interspecies [198-201].

1.3.2 Carbapenemases producing Enterobacterales in the era of HIV

An increased prevalence of ESBL infection has led to an increased consumption of carbapenems. [202, 203]. The increased use of carbapenems has been the driving force behind the rising rate of carbapenem-resistant Enterobacterales [204, 205]. The increasing prevalence of carbapenem-resistant Enterobacterales has necessitated the use of colistin in severe infections. This might drive resistance to colistin in Gram-negative bacteria. Recent studies have already noted the co-existence of the carbapenemase gene and the mcr-1 gene (colistin-resistant gene) in the same plasmid [206, 207].

CPE infections have been linked to a high rate of mortality, which is largely due to the severity of the infections and the limited therapeutic options available [208-210]. CPE infection has been associated to an increased risk of death in patients living with HIV in a recent study [211]. A recent study from India observed that 43% of gram-negative bacteria from HIV patients with infections were resistant to carbapenem [212]. In this study, resistance to carbapenems was conferred by the production of metallo β-lactamases and OXA-48.

Another study in an HIV-infected community in Nigeria found that 25.2% of gram-negative bacteria isolated from bacterial infections produced carbapenemase [213].

It's been proposed that CPE colonization precede CPE infection. According to recent studies, individuals colonized with CPE at the time of admission to an intensive care unit had a higher risk of CPE infection and mortality [214, 215].

People living with HIV tend to have neutrophil and mucus membrane defects, which predispose them to bacterial infections. CPE colonization in this population predisposes to serious CPE infections. However, a recent study in healthy people living with HIV found no carbapenemase producing Gram-negative bacteria, despite high colonization with ESBL-PE [50].

1.3.3 Detection of carbapenemase producing Enterobacterales

Phenotypic screening for CPE

Several methods have been proposed for screening carbapenemase producing Enterobacterales from clinical samples, including those recommended by the CDC and commercially available media ChromID Carba and ChromID OXA-48 (bioMérieux, Marcy l'Etoile, France), CHROMagar KPC (CHROMagar), Remel Spectra CRE (Remel, Lenexa, KS) and Brilliance CRE (Oxoid). Variations in sensitivity and specificity have been reported with these media [216]. However, one drawback of these media has been their failure to detect carbapenemase-producing Enterobacterales with reduced MIC (imipenem and/or meropenem MICs of ≤1 mg/ml) [217]. A recent study assessing seven commercial screening media, including 69 CPE and 40 CPE negatives, found the sensitivity of CRE agars for CPE detection ranged from 34.8 to 98.6%. The OXA-48 producers were the most difficult to detect; only 4/9 agars detected all isolates [216]. This study suggests the addition of

ESBL screening media increases sensitivity for detection of CPE, but would reduce the specificity.

Confirmation of CPE

Phenotypic confirmation methods

Different methods have been used for confirmation of carbapenemase production in Gram-negative bacteria. Carbapenems-carbapenemase inhibitor disk diffusion methods have been recommended by EUCAST for detection of carbapenemase [218]. Different inhibitors are known to inhibit specific carbapenemases. Meropenem or ertapenem with and without boric acid, dipicolinic acid disk, EDTA, phenylboronic acid, or aminophenylboronic acid disk (which inhibits KPC) have been used to detect carbapenemases.

Currently, CLSI recommends Carbapenemase Nordmann–Poirel (CarbaNP), carbapenem inactivation method, and modified carbapenem inactivation method for phenotypic detection of carbapenemase production in Enterobacterales and *Pseudomonas aeruginosa* [111]. The CarbaNP is based on the hydrolysis of imipenem by bacterial lysate, which leads to an acidic pH change determined by the change of the phenol red indicator from red to yellow. The test was reported previously to have high sensitivity and specificity [219, 220].

Carbapenem inactivation methods determines the carbapenemase production based on measuring the diameter of zone of inhibition of *E. coli* ATCC 25922 after the carbapenem disk has been hydrolyzed by the test bacterium [221]. The presence of a carbapenemase is indicated by the absence of a zone of inhibition. A zone of inhibition, on the other hand, means that the carbapenem disk was not inactivated, hence the absence of carbapenemase. Studies indicated CIM have high sensitivity and specificity, ranging 86% to 94% and 95% to 100%, respectively [222, 223].

Molecular confirmation methods

Molecular methods are considered the gold standard for the detection of carbapenemase genes, as genes are not always expressed phenotypically. Previously, simplex PCR was traditionally used to detect carbapenemases genes using both conventional and real-time PCR approaches [224]. But it would be time-consuming, requiring several PCR runs. Recent studies have used multiplex PCR to detect

carbapenemase genes in Enterobacterales [225-227]. The major advantages of real-time multiplex PCR include simple operation, multiplicity, robustness, and speed. Swayne *et al*. validated real-time TaqMan PCR, which was able to detect control strains known to produce five serine carbapenemase genes [228].
Recently, a study indicated that real-time multiplex PCR appropriately differentiated strains harboring each carbapenemase gene (class A, B, and D) without cross-reactivity [225].
Whole genome sequencing has now established itself as the most reliable method for detecting carbapenemase genes, with the ability to detect novel carbapenemase genes and predict phenotypic resistance to carbapenems [229].

1.3.4 Prevention and control of CPE infections

Practicing infection and prevention measures are the robust tools for prevention of the spread CPE infections. Early detection of colonized patients through screening followed by isolation and decolonization has been recommended [230]. Infection surveillance system has been recommended as well for identification and control of CPE infections [231].

1.4 *Streptococcus pneumoniae* in HIV infected population

1.4.1 *Streptococcus pneumoniae* colonization and infection in HIV

Streptococcus pneumoniae is a common bacterium that infects HIV-positive adults and children in Africa. Pneumococcal invasive diseases are much more common in HIV-infected people than in HIV-negative people [232-234]. In a recent South African study, the incidence of invasive pneumococcal diseases was found to be 43 times higher in HIV-infected individuals than in HIV-seronegative individuals (52 per 100 000 vs. 1.2 per 100 000) [235]. In the United States, an adult study found that the rate of invasive pneumococcal disease per 100,000 person-years was 160 for HIV-infected adults and 8 for HIV-uninfected subjects [236]. Most studies on the topic have found that pneumonia, and meningitis and bacteremia without focus are the common invasive pneumococcal diseases in HIV patients, and bacteremia is a more common in HIV-positive than HIV negative patients [235, 237]. A 5-year South African surveillance study found that the majority of HIV-infected people had bacteremia, accounting for 74% of all invasive pneumococcal diseases [235].

According to studies, the overall mortality rate of invasive pneumococcal diseases in HIV patients ranges between 12 and 15% [238], and the mortality rate is higher in HIV positive than HIV negative patients [235]. In a study assessing the clinical outcome of bacteriaemic pneumococcal pneumonia, Feldman *et al*. found that HIV patients had significantly higher mortality than HIV negative patients [24]. Pneumococcal meningitis was found to be a predictor of mortality in HIV-infected patients in a previous study in Malawi [237].

Invasive pneumococcal diseases can occur at any clinical stage and at any CD4 count in people with HIV infection. Some studies have found that low CD4 count is a risk factor for invasive pneumococcal infection [239, 240]. However, it is evident that also HIV patients with high CD4 counts suffer high rates of invasive pneumococcal disease [232]. On the other hand, high viral load has been associated to invasive pneumococcal pneumonia [236, 238]. But, Bordon and colleagues, found that CD+ cell counts and HIV RNA levels do not predict outcomes in invasive pneumococcal diseases [241].

Previous research has found that nasopharyngeal colonization is a major risk factor for invasive pneumococcal disease[43, 242]. Recent studies from Uganda, Kenya, and Zambia have found higher prevalence of *Streptococcus pneumoniae* nasopharyngeal colonization in HIV-infected than HIV-uninfected adults[243-245]. HIV infection has been linked to an increased risk of nasopharyngeal colonization and repeated colonization, as well as a shorter time to new colonization [243, 245]. However, Rodriguez-Barradas and colleagues found that CD4 cell count at baseline had no effect on *Streptococcus pneumoniae* nasopharyngeal colonization [246]. Furthermore, studies have revealed that pneumococcal serotypes 6, 19, and 23 are the strains most commonly colonizing HIV-infected people in Africa. These African studies showed a predominance of non-vaccine serotypes in the majority of study settings [243-245, 247].

Penicillin has long been used to treat pneumococcal infections. Several studies, however, have found an increase in resistance to penicillin [248, 249]. The risk of infection or colonization with multi-drug resistant *Streptococcus pneumoniae* has been reported in the HIV-infected population [14, 244]. This could be due to the increased

use of antibiotics in HIV patients. Previous research from Sub-Saharan Africa has shown that resistance to penicillin is increasing in *Streptococcus pneumoniae* isolated from HIV-infected patients compared to non-HIV-infected [233, 250].
Feldman *et al*. showed that *Streptococcus pneumoniae* isolated from South African HIV-infected patients were more resistant to penicillin (13 percent vs 2.5 percent) than those from HIV-negative patients[250]. *Streptococcus pneumoniae* colonizing the nasopharyngeal tracts of HIV-infected people in Sub-Saharan Africa has also been found to have a high rate of resistance to penicillin [243, 244].
Streptococcus pneumoniae resistance to commonly used antibiotics such as tetracyclines, macrolides, and cephalosporins is now common [251, 252].
A study of HIV-infected adults in Uganda showed that 72% of *Streptococcus pneumoniae* colonizing the nasopharynx were resistant to penicillin and 99% were resistant to trimethoprim-sulfamethoxazole (TMP-SMX) [243]. High rates of resistance to cotrimoxazole and tetracyclines has also been observed in pneumococcus from HIV-infected populations in Tanzania and other African settings [247, 253]. Furthermore, a recent Ghanaian study of an HIV-infected population found that 18.5% of *Streptococcus pneumoniae* nasopharynx were multidrug resistant [247].

1.4.2 Laboratory detection of *Streptococcus pneumoniae*

Specimen collection for detection of *Streptococcus pneumoniae*

The Nasopharyngeal swab is the generally recommended specimen for the detection of *Streptococcus pneumoniae*, though nasal and nasopharyngeal washing are as effective as nasopharyngeal swabs for detection of *Streptococcus pneumoniae*. Nevertheless, nasal and nasopharyngeal washing are difficult to standardize and are not well tolerated. Skim milk, tryptone, glucose, and glycerin (STGG) has been recommended by WHO experts as the medium of choice for transport and storage of nasopharyngeal swabs for isolation of *Streptococcus pneumoniae* [254]. In a study comparing the detection of nasopharyngeal carriage using STGG and silica descant packages as transport media, it was found that silica descant packages underestimated the prevalence of *Streptococcus pneumoniae* carriage compared to STGG (40.9% vs 58.7%) [255]. According to Rubin *et al*., the composition of swabs influences the detection of Streptococcus pneumoniae from the nasopharynx [256]. They found that the number of colonies cultured from rayon swabs was significantly higher than the number of colonies cultured from calcium alginate swabs. Similarly, the number of

colonies on Dacron polyester swabs was significantly lower than on calcium alginate or rayon swabs [256].

Isolation of *Streptococcus pneumoniae*

Streptococcus pneumoniae has been isolated successfully from nasopharynx secretion using sheep or horse blood agar as a primary medium. Previous research has shown that sheep blood agar supplemented with 5 micrograms of gentamicin per ml increases the isolation of *Streptococcus pneumoniae* colonizing the nasopharynx specimen by 25–40% compared to plain sheep blood agar [257].

Identification of *Streptococcus pneumoniae*

Identification of S. pneumoniae is based on colonial morphology, microscopic characteristics, and biochemical tests as described by Murray *et al*. [258]. *Streptococcus pneumoniae* confirmation depends on susceptibility to optochin and bile salt solubility [258].

Serotyping of *Streptococcus pneumoniae*

Understanding the circulating serotypes of *Streptococcus pneumoniae* is critical for the effective formulation of pneumococcal vaccine based on serotype predominance in specific geographic areas. Several studies have shown that the quelling reaction remains the gold standard for detecting pneumococcal capsular serotypes of Streptococcus pneumoniae [259]. However, the test is time-consuming, requires trained experts, and the reagents are prohibitively expensive. Several other methods for serotyping *Streptococcus pneumoniae* based on latex agglutination have been documented. Latex agglutination is a simple, quick, and low-cost method. It is highly recommended in resource-constrained settings compared to quelling swelling reaction [260]. Previous research has shown that the latex agglutination test is highly sensitive (Sensitivity 92.5–95.5 percent) in detecting *Streptococcus pneumoniae* serotypes when using the quelling swelling reaction as a gold standard [260, 261].

1.4.3 Prevention of *Streptococcus pneumoniae* infections

Vaccines continue to be the mainstay of *Streptococcus pneumoniae* infection prevention. Currently, both capsular and polysaccharide pneumococcal vaccines are used on children and vulnerable populations around the world. Since the introduction of pneumococcal capsular vaccines (PCV) 7 and PCV 13 into national childhood

immunization programs in developing countries, the incidence of invasive pneumococcal diseases and mortality has decreased dramatically [262, 263]. Immunization in children has provided herd immunity, preventing infection in at-risk adults. The emergence of non-vaccine serotypes invasive pneumococcal diseases, on the other hand, has been on the rise, reversing the remarkable success of vaccination.[264, 265]. Nevertheless, continued surveillance of circulating *Streptococcus pneumoniae* serotypes colonizing the nasopharynx remains critical for formulation of the pneumococcal vaccine in developing countries.

2. Rationale for the study

The emergence and spread of antibiotic resistant bacteria in the community and hospitals is regarded as a major public health concern. Preventing the emergence and spread of resistant pathogens in vulnerable populations is critical. Because of their immune suppression, HIV-infected people are more vulnerable to MDR bacterial infections. [15, 250]. Infections with resistant pathogens in the HIV-infected population have serious consequences because they are difficult to treat and are associated with a high mortality rate [24, 25]. Understanding the magnitude, dynamics of colonization, and routes of transmission of MDR pathogens is critical for developing strategies for effective MDR bacteria prevention and control in the HIV-infected population. Currently, information on MDR bacteria on HIV-infected individuals is scarce in Tanzania.

This study focuses on the carriage and molecular characterization of multidrug resistant bacteria in HIV-infected people in the community, as well as the risk factors that influence its carriage. The discovery will aid in the development of strategies for preventing and controlling MDR spread in the HIV-infected population at risk of severe bacterial infections, such as isolation of colonized patients and active AMR surveillance culture. Furthermore, the study highlights the resistance pattern of bacteria colonizing HIV-infected individuals, and this finding may help to inform the development of empiric antibiotic treatment guidelines in HIV-infected individuals with suspected bacterial infections.

3. Hypothesis

Does HIV infections associate with increased colonization of antibiotic resistant bacteria in the gut and nasopharynx/nose.

4. Aims and objectives

General aim

To describe the prevalence and characteristics of antibiotic resistant bacteria colonizing the gut and nasopharynx/nose in newly HIV diagnosed adults in community setting, Tanzania

Objectives

1. To determine the prevalence and molecular characteristics of methicillin resistant *Staphylococcus aureus* isolated from newly HIV diagnosed adults in Dar es Salaam, Tanzania (Paper I)
2. To determine the prevalence of fecal carriage for extended spectrum β-lactamases producing Enterobacterales in newly HIV diagnosed adults in Dar es Salaam, Tanzania (Paper II)
3. To determine the molecular characteristics of carbapenemase producing Enterobacterales isolated from the gut of HIV-infected adults in Dar es Salaam, Tanzania (Paper III)
4. To determine phenotypic and genotypic characteristics of *Streptococcus pneumoniae* isolated from HIV diagnosed adults in Dar es Salaam Tanzania (Paper IV)

5. Materials and methods

5.1 Study area/settings

The cross-sectional study was conducted in Dar es Salaam, Tanzania (Figure 1, map of Dar es Salaam), the largest city and economic capital of Tanzania with a population of over 6 million. The city has an overall HIV prevalence of 4.3% among adults aged 15–49 years old [4]. Participants were residents of the five main municipalities of Dar es Salaam city, namely Kinondoni, Temeke, Ilala, Ubungo, and Kigamboni, and were recruited through six HIV care and treatment clinics at Mwananyamala Hospital, Amana Hospital, Temeke Hospital, Pastoral Activities and Services for People with AIDS Dar es Salaam Archdiocese (PASADA), Mbagala Hospital, and Mnazi Mmoja

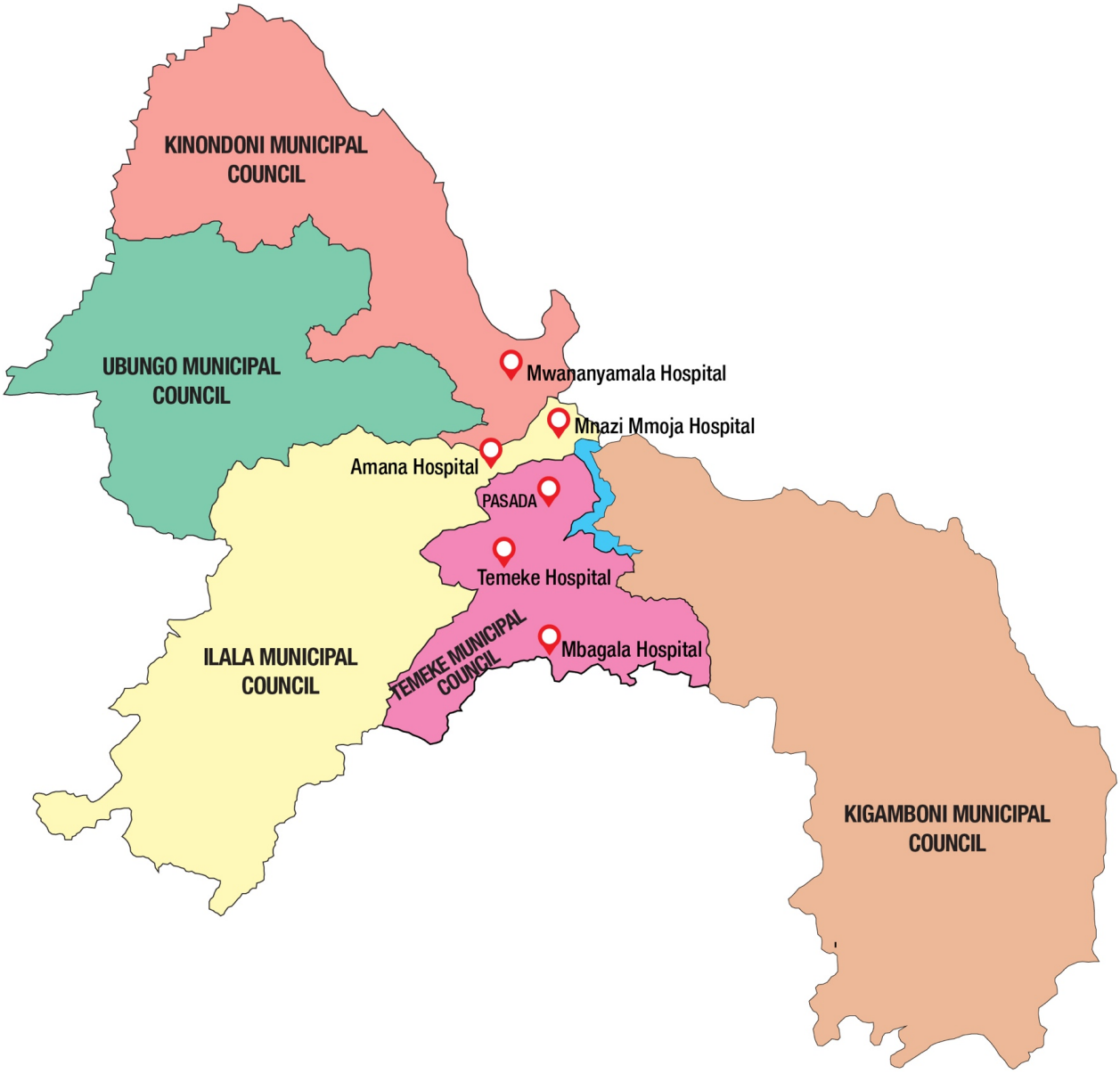


Figure 1 Map of Dar es Salaam showing 5 Municipals and clinics were participants were recruited

5.2 Study population

This study was conducted between April, 2017 and May 2018 as part of the randomized clinical trial CoTrimResist (ClinicalTrials.gov identifier: NCT03087890). It included newly diagnosed HIV-infected people aged 18 and up who had not yet started antiretroviral therapy (ART) and were attending one of the HIV care and treatment outpatient clinics in Dar es Salaam, Tanzania, for the first time. Participants were recruited when they came in for HIV testing, and those who tested positive were invited to participate in the study.

Pregnant women were excluded from the study.

The aim of the CoTrimResist trial is to assess the impact of long-term cotrimoxazole prophylaxis in HIV on colonization with multidrug-resistant bacteria such as ESBL-PE, MRSA, PRSP and VRE (vancomycin-resistant enterococci). The trial part of the study was limited to newly diagnosed HIV-patients with CD4-counts ≥ 350. However, HIV-positive persons with CD4-count < 350, as well as HIV-negative persons were added as control groups. The outcome of the trial has not been published yet.

5.3 Study subjects for paper I, II, III and IV

For paper II and III, the studies included a total of 595 rectal swabs from newly diagnosed HIV-infected aged 18 years or older. The study included subjects with CD4 counts <350 cells/μL in addition to those with CD4 counts of ≥350 cells/μL (participants in CoTrimResist trial).

For paper IV, we included a total of 1877 nasopharyngeal swabs collected at baseline, at Day 14 and at Weeks 24 and 48, from HIV-infected adults with CD4 counts ≥350 cells/μL, during their one year follow up.

Paper I included a total of 537 individuals newly diagnosed with HIV infection with CD4 counts of ≥350 cells/μL.

5.4 Collection and storage of clinical information

Each participant's demographic and clinical information, including age, gender, height, weight, current and previous hospitalizations, clinical staging, CD4 cell counts, and history of antiretroviral therapy and antimicrobial treatment, including anti-TB treatment, was recorded. The data was entered using the electronic data capturing tools REDCap (Vanderbilt University, Tennessee, US) on tablet computers,

which were then synchronized daily to a main server hosted at MUHAS. Only investigators had access to the server's information. A key file containing research numbers as well as corresponding patient names and hospital record numbers was stored separately on a storage medium (USB disk) that was not connected to the internet and was password-protected and locked down in a primary investigator's office. The key file was deleted once data collection was completed.

5.5 Microbiological procedures

5.5.1 Specimen collection, transport and storage

Specimen collection for detection of MRSA and *Streptococcus pneumoniae*

Streptococcus pneumoniae (paper IV) and S. aureus (MRSA) in paper I were detected using nasal/nasopharyngeal swabs. Both the nasopharynx and the anterior nares were swabbed with a single swab. The nasopharynx was swabbed first, followed by the anterior nares while retracting the swab. A trained clinician collected nasopharyngeal/anterior nares swabs with Sigma Transwab® and immediately transported them in liquid Amies transport medium [Sigma Transwab® PF with Liquid Amies; MWE Co (Bath) Ltd., Corsham, UK] to the Muhimbili University of Health and Allied Sciences (MUHAS) bacteriology research laboratory in a cool box at 4 °C.

Specimen collection for detection of ESBL and CPE

Stool or rectal swabs are recommended for detecting ESBL-PE (paper II) and CPE (paper III) fecal carriage. Rectal swabs were used in this study because they were the most convenient way to obtain the specimen. A rectal swab was collected from each participant and transported in liquid Cary-Blair medium (Fecal Transwab, MWE Co Bath Ltd., Corsham, United Kingdom) in a cool box at 4°C with an icepack to the MUHAS bacteriology research laboratory. Rectal swabs in liquid Cary-Blair medium were immediately frozen at -70°C upon arrival in the laboratory. At the conclusion of the study, all rectal swabs were shipped on dry ice to Bergen, Norway, for further laboratory analysis.

5.5.2 Bacterial isolation

***S. aureus*/MRSA isolation**

Swabs from the nasopharynx/anterior nares were cultured in sheep blood agar as soon as they arrived at MUHAS bacteriology research laboratory. The plates were incubated at 33°C for 24 hours.

Isolation of *Streptococcus pneumoniae*

Immediately upon arrival at MUHAS bacteriology research laboratory, nasopharyngeal/anterior nares swabs were cultured in 7 percent sheep blood agar with optochin disk. Plates were incubated for 24 hours at 37°C in a 5 percent CO2 atmosphere before being read.

Screening for ESBL-PE and CPE colonization

CHROMID ESBL (bioMérieux, Marcy l'Etoile, France) was used for ESBL-PE screening and CHROMID CARBA SMART (bioMérieux, Marcy l'Etoile, France) for CPE screening. Rectal swabs were first placed in enrichment medium (brain heart infusion broth) and incubated at 37°C for one night. Two drops (0.1mL) of brain heart infusion broth were sub-cultured on CHROMID ESBL, CHROMID CARBA SMART, and in-house made lactose agar for 24 hours at 37°C. Lactose agar, a Gram-negative bacterium selective medium, was used as a quality control to ensure that the specimen contained viable bacteria. Bacterial growth was read and interpreted according to manufacturer's instructions.

Screening for vancomycin resistant Enterococcus

Rectal swabs were screened for fecal carriage of vancomycin resistant Enterococcus (VRE) using in-house made VRE agar. Two drops from overnight rectal swabs cultured into brain heart infusion broth were sub-cultured into VRE agar and sheep blood agar, and incubated at 37°C for 24 hours. The growth of mauve colonies was considered suspect of VRE. Sheep blood agar was used as a quality control for rectal swabs if they contained viable bacteria. Identification of VRE was done by MALDI-TOF MS using the Microflex LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany), MIC for vancomycin was performed following CLSI guidelines [111] and PCR as previously described [266].

5.5.3 Identification of bacteria

In this study, initial identification of bacteria was based on colonial morphology, microscopic characteristics, and biochemical tests as described by Murray *et al*. [258].

In paper IV, *Streptococcus pneumoniae* was further confirmed by susceptibility to optochin and bile salt solubility [258]. Further identification of bacterial isolates was done by MALDI-TOF MS using the Microflex LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany. *Staphylococcus aureus* was identified in paper I using PCR targeting the thermonuclease (*nuc*) gene, as previously described [267].

5.5.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out in accordance with the guidelines of Clinical & Laboratory Standards Institute [111]. Papers I, III, and IV used disk diffusion (Oxoid,UK) and an E-test (bioMérieux, Marcy l'Etoile, France) to determine antimicrobial susceptibility. Details on the choice of media and disks for susceptibility testing have been described in papers I, III and IV. MDR bacteria were defined as those that were resistant to three or more antibiotic classes/categories [268].

5.5.5 Detection and characterization of resistance genes

DNA extraction

For papers II (Enterobacterales) and I (*S. aureus*), genomic DNA was extracted by the boiling method. The DNA templates were prepared by re-suspending 10-20 bacteria colonies in 0.5ml of TE-buffer in an Eppendorf tube. The mixture was mixed by vortexing for about 10 seconds and boiled at 100 ^{0}C for 10 minutes. The tubes were then centrifuged for 5 minutes at 15700 rpm and 200 µL of supernatant was stored at –20 ^{0}C and used as a DNA template.

The DNA template from *Streptococcus pneumoniae* in the paper IV, was extracted using a MagNA Pure LC instrument (Roche Diagnostics, Mannheim, Germany) using a Total Nucleic Acid Isolation Kit (Roche Diagnostics). Details of extraction are described in paper IV.

PCR for detection of resistant genes

PCR for detection of DHFR gene and sequencing paper IV

Conventional PCR for detection of the DHFR gene was performed using 2 × QuantiTect® Multiplex PCR NoROX Master Mix (QIAGEN) and amplification was carried out on a GeneAmp^{TM} 9700 Thermocycler (Applied Biosystems, Foster City, CA, USA). Details on primers and PCR conditions are described in detail in

paper IV. The amplicons were analyzed by gel electrophoresis. The amplified PCR products were sequenced using the same pairs of primers used in PCR. SnapGene® v.5.0.7 software (GSL Biotech LLC, Chicago, IL, USA) was used to assemble, edit, and analyze the sequences.

PCR for detection of *nuc* and *mecA* genes in paper I

Multiplex real-time PCR was used to detect both the nuc and mecA genes for the identification of S. aureus and the presence of methicillin resistance in S. aureus, respectively. PCR was carried out in a 25 µL reaction volume using a LightCycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). 12.5 µL 2x QuantiTect Multiplex PCR NoROX Master Mix (Qiagen), 1µL mecA-F (0.4 M), primers 1µL mecA-R (0.4 M), 0.5µL mecA-FAM (0.2 M), 1L Nuc-F (0.4 M), 1µL Nuc-R (0.4 M), 0.5µL Nuc-YAK (0.2 M) (Table 1), and 2µL of DNA template were used in the reaction. The PCR was performed with an initial activation temperature of 95°C for 15 minutes, followed by 40 cycles of 94°C for 15 seconds and 60°C for 60 seconds [267]. Melting curve analysis was done to determine the specific gene (s) detected from the samples. A molecular characterized *S. aureus* carrying the mecA gene was used as a positive control and RNAse free water was used as a negative control.

Table 1: Primers for detection of *mecA* and *nuc* genes

Gene	Primers
mecA-F	5′-TGC-TAA-AGT-TCA-AAA-GAG-TAT-TTA-TAA-CAA-CA-3′
mecA-R	5′-TGT-GCT-TAC-AAG-TGC-TAA-TAA-TTC-ACC-3′
mecA-FAM	5′-CAA-AGC-ATC-CTA-AAA-AAG-GTG-TAG-AGA-3′
nuc-F	5′-TTC-AAT-TTT-CTT-TGC-ATT-TTC-TAC-CA-3′
nuc-R	5′-ATT-ATG-GCT-CAG-GTA-CTG-CTA-TCC-ACC-CTC-AAA-3′
nuc-YAK	5′-TTT-TCG-TAA-ATG-CAC-TTG-CTT-CAG-GAC-CA-3′

PCR and sequencing for detection and identification of ESBL genes

Real-time PCR was used for detection of cefotaxime-Munich (CTX-M) encoding genes using a LightCycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). Forward primer CTXM-F 5′-ATGTGCAGYACCAGTAAR GT-3′, and reverse primers CTXM-R1 5′-TGGGTGAAGTAA GTGACCAGA-3 and CTXM-R2

5′-TGGGTAAARTAGG TCACCAGA-3′ (TIB Molbiol, Berlin, Germany) were used as previously described [149]. Details on PCR conditions and sequencing of PCR products have been explained in paper II.

5.5.6 Whole genome sequencing and analysis

DNA extraction and whole genome sequencing (WGS) for MRSA (paper I) and CPE (paper III) was performed by MicrobesNG (MicrobesNG, Birmingham, UK) using 2x250 bp paired-end reads on Illumina Hiseq (Illumina, San Diego, CA, USA). Trimming and quality filtering of the sequencing reads were assembled using SPAdes and annotated in GenBank. In addition, long read sequencing was performed for CPE isolates using MinION (Oxford Nanopore Technologies).

Assignment of multilocus sequence typing (MLST), was based on sequencing of seven housekeeping genes. Sequence type (ST) and clonal complex were determined by submission of sequence files to an online MLST database website (**https://pubmlst.org/**).

For identification of antimicrobial resistance genes, virulence genes, SCCmec and *spa*-type, ResFinder v3.2, virulenceFinder 2.0, SCCmecFinder 1.2 and spaTyper 1.0 of the Center for Genomic Epidemiology GEE server (http://www.genomicepidemiology.org/). For analysis of inter-strain whole genome single nucleotide polymorphism (SNPs) we used CSI phylogeny 1.4 (**https://cge.cbs.dtu.dk/services/CSIPhylogeny/**).

5.6 Statistical analysis

Statistical analysis was performed using STATA v.16.0 (Stata Corp LLC, College Station, TX, USA). A significance level of 0.05 was used and all p-values refer to two-sided tests.

In paper IV, categorical variables were presented as proportions, and continuous variables were presented using the median and range. The difference of proportions of resistant bacteria between vaccine-and non-vaccine serotype isolates were compared by $\chi 2$ test, and the medians of MICs were compared by the Wilcoxon rank-sum test.

In paper II, we used χ^2 test to assess the proportions of fecal carriage of ESBL producing Enterobacterales by patient characteristics. We analyzed the association

between risk factors of interest and fecal carriage of ESBL producers, adjusting for age, gender, educational status, district of residence, and study sites. For each main exposure variable, we controlled for confounders using both logistic regression and the Mantel–Haenszel method, defining a confounder as a factor that changes the effect size by ≥10%. The effect of modification of factors between different groups was examined by the Mantel–Haenszel method.

In Paper I and III, mainly descriptive analysis was performed.

5.7 Ethical approval and informed consent

Ethical approval to conduct the study in Tanzania was obtained from Muhimbili University of Health and Allied Sciences senate research and publication committee (Ref. No. 2015-10-27/Vol.X/54)—Muhimbili University of Health and Allied Sciences, National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2144)—Tanzania, Ministry of Health, Community development, Gender, Elderly, and Children. The clinical trial was also registered by Tanzania Food and Drugs Authority (TZ16CT007) and Clinical trial.org (NCT03087890). In Norway, the study was approved by the Regional Committee for Medical and Health Research Ethics of Western Norway (REK2015/540). Only study participants providing informed written consent were included in the study.

6. Summary of results of papers

6.1 Paper I

Predominance of PVL-negative community associated methicillin resistant *Staphylococcus aureus* sequence type 8 in newly diagnosed HIV-infected adults, Tanzania.

Joel Manyahi, Sabrina J Moyo, Said Aboud, Nina Langeland, Bjørn Blomberg

European Journal of Clinical Microbiology & Infectious Diseases. 2021 Jul; 40:1477–1485

Difficult-to-treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are of concern in people living with HIV infection as they are more vulnerable to infection. We aimed to identify molecular characteristics of MRSA colonizing newly diagnosed HIV-infected adults in Tanzania. Individuals newly diagnosed with HIV infection were recruited in Dar es Salaam, Tanzania, from April 2017 to May 2018, as part of the randomized clinical trial CoTrimResist (ClinicalTrials.gov identifier: NCT03087890). Nasal/nasopharyngeal isolates of *S. aureus* were susceptibility tested by disk diffusion method, and cefoxitin-resistant isolates were characterized by short-read whole genome sequencing. Four percent (22/537) of patients carried MRSA in the nose/nasopharynx. MRSA isolates were frequently resistant towards gentamicin (95%), ciprofloxacin (91%), and erythromycin (82%) but less often towards trimethoprim-sulfamethoxazole (9%). Seventy-three percent had inducible clindamycin resistance. Erythromycin-resistant isolates harbored *ermC* (15/18) and *LmrS* (3/18) resistance genes. Ciprofloxacin resistance was mediated by mutations of the quinolone resistance-determining region (QRDR) sequence in the *gyrA* (S84L) and *parC* (S80Y) genes. All isolates belonged to the CC8 and ST8-SCCmecIV MRSA clone. Ninety-five percent of the MRSA isolates were spa-type t1476, and one exhibited spa-type t064. All isolates were negative for Panton-Valentine leucocidin (PVL) and arginine catabolic mobile element (ACME) type 1. All ST8-SCCmecIV-spa-t1476 MRSA clones from Tanzania were unrelated to the globally successful USA300 clone. Carriage of ST8 MRSA (non-USA300) was common among newly diagnosed HIV-infected adults in Tanzania. Frequent co-resistance to non-beta lactam antibiotics limits therapeutic options when infection occurs.

6.2 Paper II

High Prevalence of Fecal Carriage of Extended Spectrum β-Lactamase-Producing Enterobacterales Among Newly HIV-Diagnosed Adults in a Community Setting in Tanzania

Joel Manyahi, Sabrina J Moyo, Said Aboud, Nina Langeland, Bjorn Blomberg

Microb Drug Resist. 2020 Dec; 26(12)

Colonization in HIV-infected populations with extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) is particularly worrisome in low-income settings. This study describes the prevalence of ESBL-PE carriage and associated risk factors among newly HIV-diagnosed adults in a community setting in Tanzania. A total of 595 newly diagnosed HIV-infected adults with a median age of 35 years with interquartile range (IQR) 29-42 years and a median CD4 count of 492 cells/μL (IQR 390-666 cells/μL) were recruited. Among these, 194/595 (32.6%, 95% confidence interval [CI] 28.9-36.6) were ESBL-PE carriers. Participants with low CD4 count (<350 cells/μL) had significantly higher prevalence of ESBL-PE carriage compared to those with CD4 count ≥350 cells/μL (26/58, 44.8%, vs. 168/537, 31.3%, $p = 0.04$). Antibiotic use in last 4 weeks (odds ratio [OR] 1.55, 95% CI 1.08-2.22, $p = 0.02$) and CD4 count <350 cells/μL (OR 1.78, 95% CI 1.03-3.09, $p = 0.04$) were independent risk factors for fecal carriage of ESBL-PE. In total, 244 isolates of ESBL-PE were isolated from 194 participants. Of these, 238/244 (97.5%) harbored $bla_{\text{CTX-M}}$ genes, with $bla_{\text{CTX-M-15}}$ being predominant (219/238 (92%), followed by $bla_{\text{CTX-M-27}}$ (9/238 (3.8%), $bla_{\text{CTX-M-14}}$ (8/238 (3.4%), $bla_{\text{CTX-M-55}}$ (1/238), and $bla_{\text{CTX-M-211/3}}$ (1/238). $bla_{\text{SHV-2a}}$ genes were detected in four isolates, whereas the $bla_{\text{SHV-12}}$ gene was detected in one isolate. Phenotypic carbapenemase-producing Enterobacterales was detected in one HIV-positive person with CD4 count 132 cells/μL. In conclusion, the prevalence of ESBL-PE carriage is high among newly diagnosed HIV adults in Dar es Salaam, and is significantly associated with antibiotic use and low CD4 count.

6.3 Paper III

First identification of *bla*$_{\text{NDM-5}}$ producing *Escherichia coli* from neonates and a HIV infected adult in Tanzania.

Authors: Joel Manyahi, Sabrina J. Moyo, Upendo Kibwana, Richard N. Goodman, Ellie Allman, Alasdair T.M. Hubbard, Bjørn Blomberg, Nina Langeland and Adam P. Roberts

Journal of Medical Microbiology. 2022 Feb; 71(2).

Introduction. Carbapenem-resistant members of the family Enterobacterales are emerging as a global public-health threat and cause substantial challenges in clinical practice.

Gap Statement. There is a need for increased and continued genomic surveillance of antimicrobial resistance genes globally in order to detect outbreaks and dissemination of clinically important resistance genes and their associated mobile genetic elements in human pathogens.

Aim. To describe the resistance mechanisms of carbapenem-resistant *Escherichia coli*.

Methods. Rectal swabs from neonates and newly diagnosed human immunodeficiency virus [4] infected adults were collected between April 2017 and May 2018 and screened for faecal carriage of carbapenamases and OXA-48 producing members of the family Enterobacterales. Bacterial isolates were identified using matrix assisted laser desorption ionization time of flight mass spectrometry. Antimicrobial susceptibility testing was performed by E-test. Whole genomes of carbapenem-resistant *E. coli* were investigated using a hybrid assembly of Illumina and Oxford Nanopore Technologies sequencing reads.

Results. Three carbapenem-resistant *E. coli* were detected, two from neonates and one from an HIV infected adult. All three isolates carried *bla*$_{\text{NDM-5}}$. Two *E. coli* from neonates belonged to ST167 and *bla*$_{\text{NDM-5}}$ co-existed with *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{OXA-}}$

01, and all were carried on IncFIA type plasmids. The *E. coli* from the HIV infected adult belonged to ST2083, and carried *bla*$_{\text{NDM-5}}$ on an IncX3 type plasmid and *bla*$_{\text{CMY-42}}$ on an IncI type plasmid. All *bla*$_{\text{NDM-5}}$ carrying plasmids contained conjugation related genes. In addition, *E. coli* from the HIV infected adult carried three more plasmid types; IncFIA, IncFIB and Col(BS512). One *E. coli* from a neonate also carried one extra plasmid Col(BS512). All three *E. coli* harbored resistance genes to fluoroquinolone, aminoglycosides, sulfamethoxazole, trimethoprim, macrolides and tetracycline, carried on the IncFIA type plasmid. Furthermore, *E. coli* from the neonates carried a chloramphenicol resistance gene (*catB3*), also on the IncFIA plasmid. All three isolates were susceptible to colistin.

Conclusion. This is the first report, to our knowledge, from Tanzania detecting *bla*$_{\text{NDM-5}}$ producing *E. coli.* The carbapenemase gene was carried on an IncFIA and IncX3 type plasmids. Our findings highlight the urgent need for a robust antimicrobial resistance (AMR) surveillance system to monitor and rapidly report on the incidence and spread of emerging resistant bacteria in Tanzania.

6.4 Paper IV

High rate of antimicrobial resistance and multiple mutations in the dihydrofolate reductase gene among *Streptococcus pneumoniae* isolated from HIV-infected adults in a community setting in Tanzania

Joel Manyahi, Sabrina J Moyo, Said Aboud, Nina Langeland, Bjorn Blomberg

J Global Antimicrob Resist. 2020, Sept; 22:749-753

Objectives: The aim of this study was to characterize molecular mechanisms of resistance to trimethoprim and other antibiotics in *Streptococcus pneumoniae* isolate from HIV-infected adults in Dar es Salaam, Tanzania.

Results: A total of 76 pneumococcal isolates were obtained. Of the 70 isolates that could be serotyped, 42 (60.0%) were vaccine serotypes included in pneumococcal conjugate vaccine 23 (PCV23). The majority of isolates (73.7%; 56/76) were non-susceptible to penicillin (MICs of 0.06-2µg/mL). Isolates were frequently resistant to co-trimoxazole (trimethoprim/sulfamethoxazole) (71.1%) but less so to azithromycin

(22.4%), erythromycin (21.1%), chloramphenicol (18.4%), tetracycline (14.5%), clindamycin (10.5%) and levofloxacin (0%). Moreover, 26.3% were multidrug-resistant (resistant to ≥3 antibiotic classes). Vaccine-type pneumococci were resistant to more classes of antibiotics, were more frequently resistant to erythromycin, azithromycin, clindamycin and tetracycline, and had higher MICs to penicillin (median, 0.19µg/mL; range, 0.002-1.5µg/mL) compared to non-vaccine serotypes (median, 0.125µg/mL; range, 0.012-0.25µg/mL, P=0.003). Co-trimoxazole-resistant isolates carried from 1 to 11 different mutations in the dihydrofolate reductase (DHFR) gene, most commonly Ile100Leu (100%), Glu20Asp (91.8%), Glu94Asp (61.2%), Leu135Phe (57.1%), His26Tyr (53.1%), Asp92Ala (53.1%) and His120Gln (53.1%).

Conclusion: *Streptococcus pneumoniae* isolated from HIV-diagnosed patients were frequently non-susceptible to penicillin and co-trimoxazole. Most isolates carried multiple mutations in DHFR.

7. Discussion

7.1 Prevalence of MRSA ESBL, and CPE and MRSA in HIV-infected adults

7.1.1 Prevalence of carriage of MRSA in newly diagnosed HIV-infected adults

The prevalence of nasal/nasopharyngeal carriage of MRSA colonization in newly diagnosed HIV-infected adults from the community in Tanzania was 4% (paper I). This prevalence is in line with a pooled prevalence of 7% (95%CI 4 –10%) from a metanalysis of studies done among people living with HIV in Africa [76]. However, although there are relatively few studies from Sub-Saharan Africa, they have reported varying prevalence of MRSA colonization ranging from 2 to 21% [77-79]. Studies show that swabbing multiple sites increases the likelihood of recovering MRSA [80, 269]. The low prevalence of MRSA in our study could have been attributed to including only nasal and nasopharynx swabs in screening for MRSA. The 4% of newly diagnosed HIV-infected adults colonized with MRSA in our setting are at risk for developing subsequent MRSA infections. Furthermore, they could act as a reservoir for spread of MRSA in the community. On the other hand, the prevalence of MRSA observed in paper I warrant the need for screening of HIV-infected adults on diagnosis and enrollment in treatment programs in order to reduce the risk of cross-transmission between patients. The findings also raise the question of whether MRSA-colonized patients should be given appropriate antibiotics for decolonization/eradication therapy.

7.1.2 Prevalence of ESBL-PE in HIV-infected adults

The study documented high prevalence of fecal carriage of ESBL-PE among newly diagnosed HIV-infected adults from community settings in Dar es Salaam, Tanzania (paper II). High prevalence of ESBL-PE fecal carriage has also been reported in a recent study from Nepal amongst healthy people living with HIV [50]. However, a previous study from Zimbabwe among HIV-infected children has reported low prevalence of fecal carriage of ESBL-PE [49]. It is alarming to note the high prevalence of fecal carriage of ESBL-PE in this population, as carriage/colonization increases vulnerability to subsequent ESBL infection. Our findings show that a high percentage of newly diagnosed HIV-infected individuals are at high risk of developing ESBL-PE infections. When HIV-infected individuals are co-infected with ESBL-PE,

the risk of morbidity and mortality increases. Previous studies have documented that ESBL colonization is a great risk for subsequent ESBL-PE infections [162, 163]. Furthermore, the high prevalence of fecal carriage of ESBL-PE among newly diagnosed HIV-infected individuals from community settings observed in our study indicates that this population could be a reservoir for ESBL-PE spread in the community.

7.1.3 Risk factors for fecal carriage of ESBL-PE in HIV-infected adults

Previous studies have documented hospitalization and recent use of antibiotics as important independent risk factors for increased fecal carriage of ESBL-PE [156, 157]. Our findings concur with those reported from previous studies [149, 157]. In this study we found the odds of fecal carriage of ESBL was 1.55 times in HIV-infected adults who reported to have used antibiotics in last 4 weeks compared to those who did not use antibiotics (paper II). Prior antibiotic consumption was an independent risk factor for the observed high fecal carriage of ESBL-PE in this study (Paper II). Antibiotics, including third generation cephalosporins are easily accessible over-the-counter without prescription in Tanzania, therefore some of the participants might have used antibiotics for self-treatment because of their HIV related illness but failed to recall. Our study could not confirm whether hospitalization is an independent factor for fecal carriage of ESBL (paper II).

Low CD4 count (<350 cells/μL) was found to be an independent risk factor for fecal carriage of ESBL -PE (paper II). Participants with low CD4 counts were more likely to have been HIV-infected for a prolonged duration than those with high CD4 counts, which could have increased the likelihood of antibiotics use and health facility visits for HIV related illness. In addition, the immune suppressed status in itself could have made these individuals more susceptible to exogenous ESBL-PE colonization. Contrary to our observation, a recent study in Zimbabwe found no association between low CD4 counts and fecal carriage of ESBL-PE among children on ART [49]. Early initiation of antiretroviral treatment in HIV is well documented to improve clinical outcome [270]. Our findings add to the arguments for early testing for HIV infection, so that more infected people can get diagnosed earlier when CD4 counts are

high, with the prospects of decreasing the risk for fecal carriage of ESBL-PE and subsequent infections.

7.1.4 Prevalence of fecal carriage of carbapenemase producing Enterobacterales

One of 595 (0.2%) newly diagnosed HIV-infected adults was found to carry carbapenemase producing *E. coli* (paper III). This finding implies very low prevalence of fecal carriage of CPE among HIV-infected population in Tanzania. Increased use of carbapenems especially at referral hospitals and the national hospital in recent years could further drive the emergence of CPE in Tanzania. This is the first report on fecal carriage of CPE in Tanzania. Despite the low prevalence observed in this population, continued surveillance of CPE needs to be implemented because carbapenemase resistance genes are transmitted by plasmids and could easily spread to other Enterobacterales.

7.2 Antimicrobial resistance

A large proportion of Gram-positive bacteria colonizing the nasopharynx and anterior nares in newly diagnosed HIV-infected adults were resistant to commonly prescribed antibiotics in Tanzania (Paper I and IV). Furthermore, 26.3% of *Streptococcus pneumoniae* colonizing the nasopharynx in this population were multidrug-resistant (resistant to ≥3 antibiotic classes) (paper IV). Observation of Gram-positive bacteria resistant to antibiotics commonly used in this setting is alarming and limits treatment options when HIV-infected individuals develop infections with the same bacteria. Worryingly, these bacteria display multi-drug resistance, and our study showed that 26% and 29% of nasopharyngeal isolates of *Streptococcus pneumoniae* and *S. aureus*, respectively, were multi-drug resistant. Previous studies in Tanzania among children in 2013 and 2015 found that 15.4% and 16.5% of *Streptococcus pneumoniae* isolates were multi-drug resistant, respectively [248, 249]. It appears that there has been an increase in the prevalence of multi-drug resistance in nasopharyngeal isolates of *Streptococcus pneumoniae* in Tanzania compared to what was reported in previous studies. Overuse and irrational use of antibiotics is rampant in Tanzania and is a likely driving force behind the increasing resistance to antimicrobial agents.

The rate of Gram-positive bacteria resistant to penicillin was high in our study (paper I and IV). Non-susceptibility of *Streptococcus pneumoniae* to penicillin was 73.7%

(paper IV) and all MRSA isolated from nasal/nasopharynx were resistant to penicillin (paper I). Penicillins (including ampicillin and amoxicillin) have been the mainstay for treatment of non-severe pneumococcal and other Gram-positive infections in Tanzania. This practice was confirmed during the data collection period as most clinicians were commonly prescribing penicillin for empirical treatment of respiratory infections. Other studies in Tanzania, have reported high rates of Gram-positive bacterial resistance to penicillin [97, 248]. Bles and colleagues reported 69.2% of *Streptococcus pneumoniae* from HIV exposed children were non-susceptible to penicillin [249]. Furthermore, Moyo et. al found that 67.8% of *Streptococcus pneumoniae* isolated from healthy children were non-susceptible to penicillin [248]. Therefore, with increased resistance to penicillin, the practice of empiric prescription of penicillin for treatment of Gram-positive infection in HIV-infected individuals may not guarantee cure anymore in Tanzania.

High rate of *Streptococcus pneumoniae* resistance to cotrimoxazole is worrying in Tanzania, with 71.1% of isolate being resistant to cotrimoxazole (Paper IV). Cotrimoxazole is one of the commonly used antibiotics in Tanzania for the treatment of respiratory tract infections. It is also widely used as prophylaxis for prevention of *Pneumocystis jiroveci* pneumonia, bacterial infections and protozoal infections, and all these could contribute to the high rate of resistance observed. High rates of resistance of *Streptococcus pneumoniae* to cotrimoxazole in the HIV-infected population has been reported previously in Tanzania and other HIV endemic settings [247, 249, 271]. Interestingly, MRSA were less resistant to cotrimoxazole (9%, paper I). Our recent and previous studies of *S. aureus* isolate in Tanzania found low rates of resistance to cotrimoxazole [272-274]. Our findings imply that cotrimoxazole could still be useful in suspected staphylococcal infections, however, should be used more cautiously in suspected pneumococcal infections. Moreover, increased rates of resistance of Gram-negative bacteria toward cotrimoxazole has been reported in our previous studies in Tanzania. Despite cotrimoxazole prophylaxis playing a significant role in the prevention of *Pneumocystis jiroveci* pneumonia and protozoal infection in HIV-infected people, its effectiveness in prevention of bacterial infections is jeopardized by the high resistance rates.

Whereas nasal/nasopharyngeal MRSA isolates from HIV-infected adults were frequently resistant to erythromycin (82%) (paper I), *Streptococcus pneumoniae* displayed a low rate of resistance to erythromycin (21.1%) (paper IV). High rates of resistance to erythromycin have previously been reported among clinical and colonizing *S. aureus* isolates in Tanzania [272]. This observed differences in susceptibility to erythromycin call for bacteriological confirmation and performance of susceptibility testing in suspected bacterial infections in the HIV-infected population.

Interestingly, all *Streptococcus pneumoniae* from nasopharynx were susceptible to levofloxacin and showed low rates of resistance towards chloramphenicol (18.4%), tetracycline (14.5%) and clindamycin (10.5%) (Paper IV). With increased resistance to penicillin and macrolides, fluoroquinolones and other non-beta-lactam antibiotics may get increasing relevance for empirical treatment of respiratory tract infections. Unfortunately, the increased use of such broader spectrum antibiotics may contribute to escalating the AMR problem. Previous studies have documented that resistance to fluoroquinolones has remained relatively low compared to other antibiotics for treatment of pneumococcal infections [275, 276]. However, treatment failure has been reported where fluoroquinolones has been used [277]. Other previous studies from Tanzania have observed low levels of resistance of *Streptococcus pneumoniae* towards fluoroquinolones, chloramphenicol, tetracycline and clindamycin [278]. Our findings in paper IV and results from recent studies in Tanzania, confirm that these antibiotics could still be effective for empirical treatment of suspected pneumococcal infections. Confirmation of susceptibility by performing antimicrobial susceptibility testing is recommended. Unfortunately, respiratory infections frequently do not get confirmed bacteriologically, hindering further susceptibility testing.

It was also observed that vaccine serotypes of *Streptococcus pneumoniae* were significantly more resistant towards tetracycline, clindamycin, erythromycin and azithromycin compared to non-vaccine serotype. The background for their inclusion into vaccine could have been their virulence factors and hence causing more serious diseases, which necessitated increased use of antibiotics and consequently driving emergence of resistance in these vaccine serotypes.

MRSA isolates from nasal/nasopharynx were highly resistant to ciprofloxacin (95%) and gentamicin (91%) (paper I). MRSA co-resistance to non-beta-lactam antibiotics limits empirical treatment options in suspected MRSA infections in the HIV-infected population in Tanzania. An increased trend of resistance to these antibiotics in Tanzania has been reported previously [272, 274], and has been attributed to frequent use of the respective antibiotics for treatment of urinary tract and gastrointestinal infections. All our MRSA isolates were susceptible to vancomycin, linezolid and clindamycin. However, these antibiotics are very expensive and their introduction in Tanzania could further strain the underfunded health care resources.

The carbapenemase producing *E. coli* isolate was resistant to cefotaxime, tetracycline, ciprofloxacin and gentamicin, all with minimum inhibitory concentrations of >256 µg/mL (paper III). This resistance pattern would limit treatment options in the event of CPE infections in newly diagnosed HIV-infected adults. The isolate was only susceptible to colistin, a drug that is not commonly available in Tanzania and is quite expensive. Plasmid-mediated colistin resistance has already spread out of Asia [206, 207]. The introduction of colistin in Tanzania could have negative impact, as increased use could drive the spread of resistance to colistin among Gram-negative bacteria.

7.3 Genomic resistance traits of bacteria colonizing HIV-infected adults in Tanzania

Cefotaximase-Munich (CTX-M) ESBL has been extraordinarily disseminated among members of the family Enterobacterales worldwide. Results presented in paper II show predominance of the *bla*$_{\text{CTX-M}}$ genotype conferring resistance in Enterobacterales towards the third generation cephalosporins, with *bla*$_{\text{CTX-M-15}}$ being the commonest. Since its first documentation in Tanzania in early 2000 [100, 144], *bla*$_{\text{CTX-M-15}}$ has been widely reported from clinical and colonizing Enterobacterales isolates [145, 149, 156]. The *bla*$_{\text{CTX-M-15}}$ is carried in plasmids, which confer easy intra-species and inter-species transferability [279]. Plasmids carrying *bla*$_{\text{CTX-M}}$ are likely to carry co-resistance genes as well [280, 281], hence, bacteria become multi-drug resistant. Spread of ESBL-PE harboring *bla*$_{\text{CTX-M-15}}$ in newly diagnosed HIV-infected adults in the community is of great concern. Since *bla*$_{\text{CTX-M-15}}$ is carried on plasmids, ESBL-PE

with *bla*$_{\text{CTX-M-15}}$ can easily disseminate in the community. Therefore, it is imperative to understand community drivers for spread of the ESBL-PE harboring *bla*$_{\text{CTX-M-15}}$ in the communities in order to generate appropriate mitigation measures to limit the spread of this resistant strain.

In *Streptococcus pneumoniae*, mutation to the dihydrofolate reductase gene (DHFR) rather than the dihydropteroate synthetase gene is more correlated with resistance to trimethoprim/sulfamethoxazole (cotrimoxazole) [282]. A total of 11 amino acid substitutions in the DHFR gene were observed in cotrimoxazole resistant *Streptococcus pneumoniae* that had available nucleotide sequences (paper IV). Previous studies have demonstrated that a single substitution of amino acid isoleucine at position 100 with leucine in DHFR is sufficient to confer resistance to trimethoprim [282, 283]. Results presented in paper IV concur with previous studies [282, 284], in that all sequenced *Streptococcus pneumoniae* isolates that were phenotypically resistant cotrimoxazole had substitutions at Ile100Leu. A recent study shows that an increased number of DHFR gene mutations correlates with an increased MIC of trimethoprim resistant *Streptococcus pneumoniae* [283]. In paper IV we could not confirm this hypothesis as isolates with this mutation did not show increased MIC of trimethoprim compared to those with less mutations. From our finding we could draw the conclusion that there is a high prevalence of known mutations in the DHFR gene associated with trimethoprim resistance in *Streptococcus pneumoniae* isolated from HIV-infected adults. Therefore, cotrimoxazole might not be effective for treatment of *Streptococcus pneumoniae* infections in HIV-infected people in Tanzania.

Phenotypic resistance towards cotrimoxazole in MRSA isolates was very low as presented in paper I. However, all MRSA isolates carried acquired *dfrG*, which mediates high level resistance to trimethoprim. This confirms the concept that acquired *dfrG* is the main mechanism of resistance to trimethoprim in *S. aureus*. A multicenter cross-sectional study in Africa (Tanzania included) and Europe including travelers from Africa demonstrated that *dfrG* was the most predominant determinant gene conferring resistance to trimethoprim in *S. aureus* [285]. A recent study suggests that *dfrG* is located on a mobile genetic element, and successful transfer of plasmid-

encoded *dfrG* has been documented [286]. Spread of the *dfrG* genetic determinant for resistance to trimethoprim in MRSA limits the empiric use of trimethoprim and cotrimoxazole, as well, for the treatment of SSTI in the HIV-infected population in Tanzania. Cotrimoxazole is a fixed combination of trimethoprim and sulfamethoxazole, which act synergistically in inhibiting bacterial folic acid synthesis. It may be hypothesized that bacteria resistant to one ingredient might acquire resistance to cotrimoxazole. It was surprising to observe a low rate of phenotypical resistance to cotrimoxazole in MRSA; despite all carrying *dfrG* mutations, which determine resistance to trimethoprim. Due to limited use of beta-lactam antibiotics, non-beta-lactam drugs like cotrimoxazole could be a viable option for MRSA infections in Tanzania, since a low rate of resistance was observed. However, it should be emphasized that in case of its use, careful clinical treatment monitoring is needed to prevent treatment failure.

Macrolides and lincosamides are non-beta-lactam alternative antibiotics for empirical treatment of MRSA infections. Resistance to macrolides, lincosamides and streptogramins type B (MLS_B) antibiotics in *S. aureus* are mainly mediated by target modification conferred by *erm* genes encoding for 23S rRNA methylase and MS_B by presence of macrolides efflux pump encoded by *msrA/B*. Results in paper I show that *ermC* was the most predominant gene in MRSA conferring resistance to MLS_B, none of isolates carried *ermA* or *ermB*. Previous studies from Tanzania and the Democratic Republic of Congo showed similar results of predominant *ermC* in MRSA resistant to MLS_B [97, 120]. Studies analyzing the predominance of *erm* genes, have found that *ermA* is mainly observed in MRSA expressing constitutive MLS_B ($cMLS_B$); whereas *ermC* is common in MRSA expressing inducible MLS_B ($iMLS_B$) [287, 288]. Seventy-three percent of MRSA expressed iMLSB, explaining the predominance of *ermC* in paper I. Despite all MRSA isolates in paper I being susceptible to clindamycin, the use of MLS_B antibiotics, particularly macrolides could select for constitutive MLS_B mutants in apparently susceptible MRSA carrying the *ermC* gene, leading to treatment failure due to global MLS_B resistance [289]. This underscores the need for performing susceptibility testing, including detection of both $cMLS_B$ and $iMLS_B$, in conjunction with use of MLS_B antibiotics in MRSA infections in Tanzania.

Resistance of *S. aureus* to aminoglycosides is mainly mediated by enzymatic modification. Aminoglycosides modifying enzymes 6´-N-acetyltransferase-2@-O-phosphotransferase" (*aac6'-aph2"*), which is encoded by the *aac (6')-Ie-aph(2*") gene inactivating gentamicin and other aminoglycosides was detected in all gentamicin resistant MRSA (paper I). The *aac6'-aph2"* gene has been detected in plasmids and is encoded by transposons [290, 291]. Detection of the *aac6'-aph2"* gene in plasmids, means plasmids could be a major vehicle for spread and transmission of this gene in *S. aureus* and other species. Fluoroquinolones, essentially ciprofloxacin, are common non-beta-lactam antibiotics used for empirical treatment of MRSA infections. Resistance to ciprofloxacin limits further options for managing MRSA infections. Results from paper I show that all ciprofloxacin resistant MRSA isolates harbored serine mutation of the quinolone resistance determining regions (QRDRs) sequences in the *gyrA* (S84L) and *parC* (S80Y) genes. Double mutations in QRDRs sequences have demonstrated high levels of resistance to ciprofloxacin with elevated MIC value [292, 293]. A previous study from East Africa observed similar type of mutation in ciprofloxacin resistant *S. aureus* [97].

7.4 Genetic population structure of MRSA strains

The PVL positive ST8-SCC*mec*IV (USA300) CA-MRSA clone has been successfully spread and is predominant in HIV-infected populations in the developed world, where it has been associated with SSTI and necrotizing pneumonia [85, 89]. In contrast, it is infrequently responsible for other infections, such as bloodstream infection and endocarditis in HIV-infected persons [89]. A PVL negative ST8-SCC*mec*IV CA-MRSA strain was the predominant clone colonizing nose/nasopharynx of newly diagnosed HIV-infected adults in Tanzania (paper I). The association of nasal PVL-negative ST8-SCC*mec*IV MRSA colonization with any specific MRSA infections in HIV-infected people has not been investigated. However, studies show that HIV patients with PVL positive ST8-SCC*mec*IV -CA MRSA SSTI infections have increased genital and perineal MRSA colonization compared to nasal MRSA colonization [82, 85]. Therefore, our stud could have underestimated the rate of colonization by this clone. It is possible that the epidemiology of diseases caused by PVL negative ST8 MRSA CA-MRSA differ from that of PVL positive ST8 CA-

MRSA. Further studies need to be conducted to explore the epidemiology of PVL negative ST8 CA-MRSA infections.

The ST8-spa-t1476 has been reported circulating in clinical and colonizing MRSA isolates in the Democratic Republic of Congo [95, 96]. Similarly, the spa-t1476 type was the predominant spa type among ST8-MRSA colonizing the nose/nasopharynx of newly diagnosed HIV-infected adults in Tanzania (paper I). The spa-1476 among ST8 CA-MRSA has been reported once outside Africa, in UK, in an outbreak in patients with no evidence of travel to the African region [294]. It is not well known if specific spa-types are associated with specific types of disease or colonization. This needs to be investigated, because it will help in further understanding of the MRSA/ *S. aureus* epidemiology.

Results from paper I show that all ST8-SCC*mec*IV-spa-t1476 MRSA from HIV-infected adults from Tanzania were not related to already known ST8-CA MRSA (USA300) from Africa and USA. Furthermore, on phylogenetic analysis, all ST8-SCC*mec*IV-spa-t1476 MRSA were clustered into one clade and may have shared a common ancestor. Evolutionary study suggest that ST8-MRSA evolved from an ST8 Methicillin susceptible *Staphylococcus aureus* (MSSA) clone by acquisition of *SCCmec* type [295]. On the other hand, studies support MRSA to have no single common ancestor, rather SCC*mec* were introduced several times to different *S. aureus* lineages [196, 296]. Findings from the present study support those from a previous evolutionary study comprising ST8 MRSA isolates from several regions of the world, which found that no African ST8 had direct ancestry to USA300 clones, and that the African MRSA ST8 did not belong to spa-t008 which is seen in USA300 [295].

7.5 Molecular characteristics of *bla*$_{\text{NDM-5}}$ Producing *E. coli* isolate

Carbapenems have been widely considered as a last resort for treatment of multi drug resistant Enterobacterales infections. Lately, the use of carbapenems in Tanzania has increased and this is potentiated by weak regulation on prescriptions of antimicrobial agents. There is increased risk for emergence of resistance to carbapenems in the country. Resistance to carbapenems in Enterobacterales is mediated by carbapenemases, which hydrolyze carbapenems and other beta-lactam antibiotics. The *bla*$_{NDM-5}$ carried on the IncX3 type plasmid was detected in one *E. coli* isolate

colonizing the gut of a newly diagnosed HIV-infected adult (paper III). The *bla*$_{NDM-5}$ has preferably been located on an IncX3 type plasmid [297-299]; the IncX3 type plasmid has been reported as an important vehicle for spread and dissemination of *bla*$_{NDM-5}$ among Enterobacterales [298]. Furthermore, the isolate contained an IncFIA plasmid type which carried genes conferring resistance towards multiple drugs, including fluoroquinolones (*aac(6')-Ib-cr*), aminoglycosides (*aac(6')-Ib-cr)*; sulfamethoxazole (*sul1* and *sul*2); trimethoprim (*dfr*17); aminoglycoside *aac*[257]-IId, *aph*(3")-lb, *aadA*5); tetracycline (*tetB*); macrolides *mph*(A) and *mdfA*. The finding in paper III of an IncX3 type plasmid carrying *bla*$_{NDM-5}$ and IncFIA carrying multidrug resistance genes in a newly diagnosed HIV patient from the community setting in Tanzania is of great concern, since these plasmids have great potential to efficiently disseminate in the community. Spread of *bla*$_{NDM-5}$ in the community has serious implication since *bla*$_{NDM-5}$ carrying isolates frequently are resistant to almost all clinically relevant drugs for the treatment of Gram-negative infections.

7.6 Methodological discussion

7.6.1 Study design and sample size

The study design was cross-sectional involving only newly diagnosed HIV-infected adults. An ideal study design could have been a comparative cross-sectional study comparing cohorts of HIV-infected and HIV non-infected adults on outcome of fecal ESBL-producers carriage. This could have highlighted the difference in prevalence of carriage for multidrug resistant bacteria between HIV-infected and non-infected individuals. The comparative cross-sectional study design could also have provided an insight into whether HIV infection in itself could be a risk factor for carriage of multidrug resistant bacteria like ESBL, MRSA etc. Despite lacking a suitable, comparative group in this study, findings from our study represent a true picture of multidrug resistant bacterial colonization in newly diagnosed HIV-infected adults in Tanzania. This could be accounted for by a large sample size of newly diagnosed HIV-infected adults enrolled in this study. Furthermore, in paper II we compared the proportion of fecal carriage of ESBL in our study to other studies from adults with unknown HIV status; and found a high proportion of fecal carriage of ESBL in our study compared to the others.

7.6.2 Microbiological methods

Specimen collection and transport

Collection, transport and storage of specimens for culture are critical for isolation of the bacteria colonizing any anatomical site. The anterior nares are the primary reservoir for *S. aureus* and swabbing of the anterior nares is appropriate for detection of *S. aureus* colonization. In collection of nasal swabs, we swabbed the nasopharynx first, and the anterior nares next while retracting the swab. This procedure may have led to falsely low prevalence of MRSA observed in our study (paper I). However, the prevalence of nasal carriage of *S. aureus* in our study is similar to the range of 12% to 30%, which is commonly documented in healthy individuals [300, 301]. This suggests that our prevalence of MRSA could represent the true rate in this population. On the other hand, a previous study on MRSA colonization among HIV-infected people found that swabbing a combination of nares/throat/inguinal/perirectal sites increases recovery of MRSA and subsequently the estimated prevalence [80].

Recent studies have documented that MRSA chromogenic media exhibit high specificities with varying degree of sensitivities [105-107].

Skimmed milk, tryptone, glucose, and glycerin (STGG) medium has been recommended by WHO experts as the medium of choice for transport and storage of nasopharyngeal swabs for isolation of *Streptococcus pneumoniae* [254]. In paper IV, liquid Amies transport medium [Sigma Transwab® PF with Liquid Amies; MWE Co (Bath) Ltd., Corsham, UK] was used for transport and storage of nasopharyngeal swabs for detection of *Streptococcus pneumoniae*. This medium has been shown to maintain the viability of *Streptococcus pneumoniae* when held at a different temperature for 0, 24 or 48 hours. In paper IV, the nasopharyngeal swabs were cultured immediately on arrival at our laboratory; and besides not using STGG we trust the swabs and medium used had good recovery and that our culture results presented a true picture for nasopharyngeal colonization of *Streptococcus pneumoniae*.

Processing of Specimen in the Laboratory

Enrichment of specimen into broth media and plating into chromogenic media have been found to increase the recovery of MRSA from clinical specimens [107]. In paper

I, screening for MRSA colonization was processed on sheep blood agar and mannitol salt agar incubated at 33–35°C ambient air. On the other hand, enrichment on broth media was not performed. This could have led to lower recovery of MRSA in circumstances of mixed growth of MRSA and MSSA. Selective medium could have been an ideal technique for detection of MRSA.

Generally, stool culture rather than rectal swabs culture is considered superior for detection of fecal enteric pathogens [302, 303]. On the hand prior broth enrichment increases the sensitivity for recovery of ESBL isolates from stool/rectal swabs [304]. In paper II, rectal swabs were collected and were used for culture. However, prior to culture onto selective media, overnight enrichment in brain heart infusion broth were performed for all swabs. Overnight broth enrichment of the rectal swabs might have increased the recovery rate of fecal carriage of ESBL-PE observed in paper II.

Several culture-based screening media for carbapenemase producing Enterobacterales have been evaluated with varying sensitivities and specificities [305, 306]. Commercially available screening media including Brilliance CRE (Oxoid), Chromogenic chromID® CARBA (bioMérieux) and CHROMagar KPC (CHROMagar) have been used for screening for CPE in clinical samples. However, culture-based screening for CPE may have difficulties in detecting Enterobacterales with low carbapenems MIC. Previous studies have document that CPE with meropenem and/or imipenem MIC of ≤ 1µg/ml are common and may fail to be detected in CPE screening media [305, 307]. In paper III Chromogenic chromID® CARBA (bioMérieux) was used for screening fecal carriage for CPE, and overnight broth enrichment was performed before subculture into the selective media. However, only one carbapenemase producing *E. coli* was isolated out of 595 rectal swabs screened. It is difficult to know the reason for the low yield observed, whether it was due to poor recovery of the media or low fecal carriage of CPE in our study setting.

Whole genome sequencing

In papers I and III, whole genome sequencing was used to determine antimicrobial resistant genes. Concordance of phenotypic and whole genome sequencing in predicting susceptibility was high in most antibiotics tested. However, in paper I, it was observed that all isolates carried the *dfr*G gene, which confers resistance to

trimethoprim (constituents of cotrimoxazole), but only 9% of MRSA were resistant to trimethoprim-sulfamethoxazole. This means, the whole genome sequencing did not predict susceptibility to trimethoprim-sulfamethoxazole.

In paper III, one of the *E. coli* strains was categorized as susceptible according to EUCAST clinical breakpoint [218]. Whole genome sequencing, on the other hand, detected blaNDM-5, which encodes for New Delhi metallo--lactamase; thus, whole genome sequencing predicted susceptibility differed from phenotypic susceptibility results. From our findings, whole genome sequencing should be used to complement phenotypic testing but not for clinical guidance. However, whole genome sequencing facilitated understanding the genetic basis of AMR mechanisms in papers I and III and the location of AMR determinants on the chromosomes or plasmids, which is valuable information on the pathways of AMR spread.

8. Limitations

This study dealt only with newly diagnosed HIV-infected adults from the community in a cross-sectional study design, with no comparative group like HIV non-infected individuals. This could have overestimated or underestimated the burden of antibiotic resistant bacteria. Therefore, it is difficult to generalize if an HIV-infected population has a high burden of carriage of antibiotic resistant bacteria. However, in this study, we recruited 537 newly diagnosed HIV-infected patients from several geographically separated treatment centers, and we believe that with such a large sample size, our findings are truly representative of the HIV-infected population.

On the other hand, one caveat of this study is that it did not investigate if HIV infection was a risk factor for carriage/colonization of antibiotic resistant bacteria. This would have been important in addressing mitigation measures of antibiotic resistance in this population.

The antimicrobial susceptibility pattern for ESBL-PE in paper II was not available for analysis. Therefore, we could not establish alternative antibiotic treatment options for ESBL suspected infections in HIV-infected populations. This would have been important as alternative antibiotics such as meropenem used in the developed world are very expensive. Therefore, finding a cheap alternative could have been helpful in this setting.

For determination of fecal carriage of antibiotic resistant bacteria, rectal swabs were used instead of stool because the rectal swab has the advantage of immediate and uncomplicated specimen collection, while stool samples may not be readily available and can hamper the sample collection process. However, stool is recommended as the gold standard for detection of microbial gut colonization and failure to collect stool samples could have contributed to the low detection of ESBL and CRE and no detection of VRE. Despite this limitation, in papers II and III, all rectal swabs were incubated overnight and plated on lactose agar and sheep blood agar for quality checking if the swabs carried viable bacteria. Therefore, our findings represent a true picture of fetal carriage of ESBL and CRE.

Another limitation of this study is that confirmation of ESBL was done by PCR to detect the CTX-M gene as a surrogate marker for ESBL carriage. Only those negative

for CTX-M were tested for SHV-12 and 12. This did not mean these isolates were not producing other SHV and TEM genes; and CTX-M was not the only predominant ESBL gene in Tanzania. However, most of the phenotypically characterized ESBL isolates were carrying the *bla*$_{\text{CTX-M-15}}$ genotype, which correlates with previous findings from Tanzania [144, 145, 156].

In this study only, anterior nares swabs were used to screen for MRSA carriage. Other anatomical sites, including the axilla, groins, and perineum, have been found to increase detection rates for colonization of MRSA in HIV-infected populations. Screening anterior nares only could have led to a low estimate for the prevalence of MRSA observed in this study. Furthermore, the use of sheep blood agar and mannitol salt agar for isolation of MRSA followed by confirmation using cefoxitin disk, could have missed MRSA colonies (not able to differentiate between MSSA and MRSA). Selective media like chromogenic agar, which have high sensitivity and specificity, could have been used instead of general-purpose medium.

Another drawback of this study was the failure to isolate vancomycin-resistant Enterococcus from rectal swabs. We could not ascertain the reasons for this, whether the in-house prepared medium used contained low/high MIC value of vancomycin or other inhibitors for this isolate. However, the medium had high sensitivity as it allowed growth of other Enterococcus species, but confirmatory tests suggested there was no VRE.

9. Conclusion

Findings from this thesis show that:

Streptococcus pneumoniae isolated from HIV-infected adult patients were highly resistant to penicillin and cotrimoxazole; and were frequently multi-drug resistant.

Resistance to cotrimoxazole in *Streptococcus pneumoniae* was mediated by multiple mutations in the DHFR gene and all had the amino acid substitution Ile100Leu.

We found a high prevalence of fecal ESBL-PE carriage among people newly diagnosed with HIV, with a predominance of the $bla_{CTX\text{-}M\text{-}15}$ genotype.

Antibiotic use during the last 4 weeks and low CD4 counts were found to be associated with fecal carriage of ESBL producers.

This is the first report of $bla_{NDM\text{-}5}$ producing *E. coli* carried on IncFA type and IncX3 type plasmids in Tanzania. The $bla_{NDM\text{-}5}$ producing *E. coli* also carried multiple other resistance determinants on the IncFA type plasmids.

ST8 CA-MRSA (non-USA300) was found to be the commonest circulating population structure in newly diagnosed HIV-infected adults in Tanzania. The circulating ST8 CA-MRSA isolates were not related to other common, successful circulating ST8 lineages. The spa-type t1476 is predominant in this case of CA-MRSA. The majority of CA-MRSA showed high rates of resistance to non-beta lactam antibiotics.

10. Recommendations

Based on our finding of a high prevalence of fecal carriage of ESBL in newly diagnosed HIV-infected populations in the community, we recommend the establishment and strengthening of an AMR surveillance system to monitor the spread of emerging antibiotic-resistant bacteria in community settings.

The discovery of *bla*$_{\text{NDM-5}}$ carried in plasmids both from community and hospital settings in paper III calls for intensification of infection prevention and control to curb the further spread of this resistant bacterium. Furthermore, the country needs to restrict and enforce regulation on the use of carbapenems, a driver of carbapenem resistant Enterobacterales in order to reduce the spread of CRE in the country. Unpublished data shows an increased use of carbapenems in Tanzania, posing a great risk for the further spread of CRE.

In Tanzania, universal or targeted surveillance screening of antibiotic resistant bacteria like ESBL, MRSA, and CRE among risk groups during hospitalization is not routinely practiced either due to resource constraints or lack of evidence to support intervention. Based on our findings and previous studies from Tanzania showing a high burden of antibiotic resistant bacteria, we recommend the need for the country to integrate surveillance screening for colonization of antibiotic resistant bacteria like MRSA and ESBL among high-risk groups, including HIV-infected people when they are hospitalized, especially in areas like the intensive care unit.

Further studies should investigate transmission dynamics of ESBL-PE in the community, complex factors driving the emergence of multidrug-resistant bacteria, and the relationship between ESBL-PE carriage and invasive disease.

11. References

1. UNAIDS Data: **Report**. *Retrieved February* 2021.
2. Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S: **HIV virology and pathogenetic mechanisms of infection: a brief overview**. *Ann Ist Super Sanita* 2010, **46**(1):5-14.
3. Country progress report - United Republic of Tanzania, Global AIDS Monitoring 2020. https://www.unaids.org/sites/default/files/country/documents/TZA_2020_countryreport.pdf
4. Tanzania Commission for AIDS (TACAIDS), Zanzibar A IDS Commission (ZAC). Tanzania HIV Impact Survey (THIS) 2016-2017: Final Report. Dar es Salaam, Tanzania. December 2018. https://phia.icap.columbia.edu/wp-content/uploads/2019/06/FINAL_THIS-2016-2017_Final-Report__06.21.19_for-web_TS.pdf
5. Levy JA: **HIV pathogenesis: 25 years of progress and persistent challenges**. *Aids* 2009, **23**(2):147-160.
6. Huson MA, Grobusch MP, van der Poll T: **The effect of HIV infection on the host response to bacterial sepsis**. *Lancet Infect Dis* 2015, **15**(1):95-108.
7. Michailidis C, Giannopoulos G, Vigklis V, Armenis K, Tsakris A, Gargalianos P: **Impaired phagocytosis among patients infected by the human immunodeficiency virus: implication for a role of highly active anti-retroviral therapy**. *Clin Exp Immunol* 2012, **167**(3):499-504.
8. Klatt NR, Brenchley JM: **Th17 cell dynamics in HIV infection**. *Curr Opin HIV AIDS* 2010, **5**(2):135-140.
9. Dandekar S, George MD, Bäumler AJ: **Th17 cells, HIV and the gut mucosal barrier**. *Curr Opin HIV AIDS* 2010, **5**(2):173-178.
10. Moreno García M: **[Neutropenia in HIV infection]**. *An Med Interna* 1997, **14**(4):199-208.
11. Kuritzkes DR: **Neutropenia, neutrophil dysfunction, and bacterial infection in patients with human immunodeficiency virus disease: the role of granulocyte colony-stimulating factor**. *Clin Infect Dis* 2000, **30**(2):256-260.

12. Elbim C, Prevot MH, Bouscarat F, Franzini E, Chollet-Martin S, Hakim J, Gougerot-Pocidalo MA: **Polymorphonuclear neutrophils from human immunodeficiency virus-infected patients show enhanced activation, diminished fMLP-induced L-selectin shedding, and an impaired oxidative burst after cytokine priming**. *Blood* 1994, **84**(8):2759-2766.
13. Rubinstein DB, Farrington GK, O'Donnell C, Hartman KR, Wright DG: **Autoantibodies to leukocyte alphaMbeta2 integrin glycoproteins in HIV infection**. *Clin Immunol* 1999, **90**(3):352-359.
14. Bédos JP, Chevret S, Chastang C, Geslin P, Régnier B: **Epidemiological features of and risk factors for infection by *Streptococcus pneumoniae* strains with diminished susceptibility to penicillin: findings of a French survey**. *Clin Infect Dis* 1996, **22**(1):63-72.
15. Hidron AI, Kourbatova EV, Halvosa JS, Terrell BJ, McDougal LK, Tenover FC, Blumberg HM, King MD: **Risk factors for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage**. *Clin Infect Dis* 2005, **41**(2):159-166.
16. Gilks CF, Ojoo SA, Ojoo JC, Brindle RJ, Paul J, Batchelor BI, Kimari JN, Newnham R, Bwayo J, Plummer FA *et al*: **Invasive pneumococcal disease in a cohort of predominantly HIV-1 infected female sex-workers in Nairobi, Kenya**. *Lancet* 1996, **347**(9003):718-723.
17. Huson MA, Stolp SM, van der Poll T, Grobusch MP: **Community-acquired bacterial bloodstream infections in HIV-infected patients: a systematic review**. *Clin Infect Dis* 2014, **58**(1):79-92.
18. Castro-Lima VAC, Borges IC, Joelsons D, Sales VVT, Guimaraes T, Ho YL, Costa SF, Moura MLN: **Impact of human immunodeficiency virus infection on mortality of patients who acquired healthcare associated-infection in critical care unit**. *Medicine (Baltimore)* 2019, **98**(23):e15801.
19. Groome MJ, Albrich WC, Wadula J, Khoosal M, Madhi SA: **Community-onset *Staphylococcus aureus* bacteraemia in hospitalised African children:**

high incidence in HIV-infected children and high prevalence of multidrug resistance. *Paediatr Int Child Health* 2012, **32**(3):140-146.

20. Todd J, Slaymaker E, Zaba B, Mahy M, Byass P: **Measuring HIV-related mortality in the first decade of anti-retroviral therapy in sub-Saharan Africa**. *Glob Health Action* 2014, **7**:24787.
21. Crump JA, Ramadhani HO, Morrissey AB, Msuya LJ, Yang LY, Chow SC, Morpeth SC, Reyburn H, Njau BN, Shaw AV *et al*: **Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected children and infants in northern Tanzania**. *Trop Med Int Health* 2011, **16**(7):830-837.
22. Meremo A, Mshana SE, Kidenya BR, Kabangila R, Peck R, Kataraihya JB: **High prevalence of Non-typhoid salmonella bacteraemia among febrile HIV adult patients admitted at a tertiary Hospital, North-Western Tanzania**. *Int Arch Med* 2012, **5**(1):28.
23. Nathoo KJ, Chigonde S, Nhembe M, Ali MH, Mason PR: **Community-acquired bacteremia in human immunodeficiency virus-infected children in Harare, Zimbabwe**. *Pediatr Infect Dis J* 1996, **15**(12):1092-1097.
24. Feldman C, Klugman KP, Yu VL, Ortqvist A, Choiu CC, Chedid MB, Rello J, Wagener M: **Bacteraemic pneumococcal pneumonia: impact of HIV on clinical presentation and outcome**. *J Infect* 2007, **55**(2):125-135.
25. Japiassú AM, Amâncio RT, Mesquita EC, Medeiros DM, Bernal HB, Nunes EP, Luz PM, Grinsztejn B, Bozza FA: **Sepsis is a major determinant of outcome in critically ill HIV/AIDS patients**. *Crit Care* 2010, **14**(4):R152.
26. Furuno JP, Johnson JK, Schweizer ML, Uche A, Stine OC, Shurland SM, Forrest GN: **Community-associated methicillin-resistant *Staphylococcus aureus* bacteremia and endocarditis among HIV patients: a cohort study**. *BMC Infect Dis* 2011, **11**:298.
27. Cotton MF, Wasserman E, Smit J, Whitelaw A, Zar HJ: **High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* in nasopharyngeal and blood isolates of HIV-**

infected children from Cape Town, South Africa. *BMC Infect Dis* 2008, **8**:40.

28. Taramasso L, Tatarelli P, Di Biagio A: **Bloodstream infections in HIV-infected patients**. *Virulence* 2016, **7**(3):320-328.
29. Bliss SJ, O'Brien KL, Janoff EN, Cotton MF, Musoke P, Coovadia H, Levine OS: **The evidence for using conjugate vaccines to protect HIV-infected children against pneumococcal disease**. *Lancet Infect Dis* 2008, **8**(1):67-80.
30. Mathews WC, Caperna JC, Barber RE, Torriani FJ, Miller LG, May S, McCutchan JA: **Incidence of and risk factors for clinically significant methicillin-resistant *Staphylococcus aureus* infection in a cohort of HIV-infected adults**. *J Acquir Immune Defic Syndr* 2005, **40**(2):155-160.
31. Palepu A, Sun H, Kuyper L, Schechter MT, O'Shaughnessy MV, Anis AH: **Predictors of early hospital readmission in HIV-infected patients with pneumonia**. *J Gen Intern Med* 2003, **18**(4):242-247.
32. Madeddu G, Porqueddu EM, Cambosu F, Saba F, Fois AG, Pirina P, Mura MS: **Bacterial community acquired pneumonia in HIV-infected inpatients in the highly active antiretroviral therapy era**. *Infection* 2008, **36**(3):231-236.
33. Aston SJ, Ho A, Jary H, Huwa J, Mitchell T, Ibitoye S, Greenwood S, Joekes E, Daire A, Mallewa J *et al*: **Etiology and Risk Factors for Mortality in an Adult Community-acquired Pneumonia Cohort in Malawi**. *Am J Respir Crit Care Med* 2019, **200**(3):359-369.
34. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP: **Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children**. *Clin Infect Dis* 2000, **31**(1):170-176.
35. Benito N, Rañó A, Moreno A, González J, Luna M, Agustí C, Danés C, Pumarola T, Miró JM, Torres A *et al*: **Pulmonary infiltrates in HIV-infected patients in the highly active antiretroviral therapy era in Spain**. *J Acquir Immune Defic Syndr* 2001, **27**(1):35-43.

36. Gilsdorf JR, Ferrieri P: **Role of nasopharyngeal colonization with and without bacteremia in the protection of infant rats against *Haemophilus influenzae* type b challenge**. *Infect Immun* 1985, **47**(3):648-653.
37. Shet A, Mathema B, Mediavilla JR, Kishii K, Mehandru S, Jeane-Pierre P, Laroche M, Willey BM, Kreiswirth N, Markowitz M *et al*: **Colonization and subsequent skin and soft tissue infection due to methicillin-resistant *Staphylococcus aureus* in a cohort of otherwise healthy adults infected with HIV type 1**. *J Infect Dis* 2009, **200**(1):88-93.
38. Szumowski JD, Wener KM, Gold HS, Wong M, Venkataraman L, Runde CA, Cohen DE, Mayer KH, Wright SB: **Methicillin-resistant *Staphylococcus aureus* colonization, behavioral risk factors, and skin and soft-tissue infection at an ambulatory clinic serving a large population of HIV-infected men who have sex with men**. *Clin Infect Dis* 2009, **49**(1):118-121.
39. Reddy P, Malczynski M, Obias A, Reiner S, Jin N, Huang J, Noskin GA, Zembower T: **Screening for extended-spectrum beta-lactamase-producing Enterobacteriaceae among high-risk patients and rates of subsequent bacteremia**. *Clin Infect Dis* 2007, **45**(7):846-852.
40. Kiddee A, Assawatheptawee K, Na-Udom A, Boonsawang P, Treebupachatsakul P, Walsh TR, Niumsup PR: **Risk Factors for Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae Carriage in Patients Admitted to Intensive Care Unit in a Tertiary Care Hospital in Thailand**. *Microb Drug Resist* 2019, **25**(8):1182-1190.
41. Denkel LA, Maechler F, Schwab F, Kola A, Weber A, Gastmeier P, Pfäfflin F, Weber S, Werner G, Pfeifer Y *et al*: **Infections caused by extended-spectrum β-lactamase-producing Enterobacterales after rectal colonization with ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae***. *Clin Microbiol Infect* 2020, **26**(8):1046-1051.
42. Zervou FN, Zacharioudakis IM, Ziakas PD, Mylonakis E: **MRSA colonization and risk of infection in the neonatal and pediatric ICU: a meta-analysis**. *Pediatrics* 2014, **133**(4):e1015-1023.

43. Albrich WC, Madhi SA, Adrian PV, van Niekerk N, Telles J-N, Ebrahim N, Messaoudi M, Paranhos-Baccalà G, Giersdorf S, Vernet G *et al*: **Pneumococcal colonisation density: a new marker for disease severity in HIV-infected adults with pneumonia**. *BMJ Open* 2014, **4**(8):e005953.
44. Chabala F, Madubasi M, Mutengo MM, Banda N, Yamba K, Kaonga P: ***Escherichia coli* Antimicrobial Susceptibility Reduction amongst HIV-Infected Individuals at the University Teaching Hospital, Lusaka, Zambia**. *Int J Environ Res Public Health* 2020, **17**(10).
45. Reinheimer C, Keppler OT, Stephan C, Wichelhaus TA, Friedrichs I, Kempf VA: **Elevated prevalence of multidrug-resistant gram-negative organisms in HIV positive men**. *BMC Infect Dis* 2017, **17**(1):206.
46. Mulu W, Yizengaw E, Alemu M, Mekonnen D, Hailu D, Ketemaw K, Abera B, Kibret M: **Pharyngeal colonization and drug resistance profiles of *Morraxella catarrrhalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae* among HIV infected children attending ART Clinic of Felegehiwot Referral Hospital, Ethiopia**. *PLoS One* 2018, **13**(5):e0196722.
47. Marbou WJT, Kuete V: **Bacterial resistance and immunological profiles in HIV-infected and non-infected patients at Mbouda AD LUCEM Hospital in Cameroon**. *J Infect Public Health* 2017, **10**(3):269-276.
48. Sampane-Donkor E, Badoe EV, Annan JA, Nii-Trebi N: **Colonisation of antibiotic resistant bacteria in a cohort of HIV infected children in Ghana**. *Pan Afr Med J* 2017, **26**:60.
49. Wilmore SMS, Kranzer K, Williams A, Makamure B, Nhidza AF, Mayini J, Bandason T, Metcalfe J, Nicol MP, Balakrishnan I *et al*: **Carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in HIV-infected children in Zimbabwe**. *J Med Microbiol* 2017, **66**(5):609-615.
50. Hosuru Subramanya S, Bairy I, Nayak N, Padukone S, Sathian B, Gokhale S: **Low rate of gut colonization by extended-spectrum β-lactamase producing Enterobacteriaceae in HIV infected persons as compared to healthy individuals in Nepal**. *PLoS One* 2019, **14**(2):e0212042.

51. Collaborators AR: **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis**. *Lancet* 2022, **399**(10325):629-655.
52. O'Neill J: **Tackling drug-resistant infections globally: final report and recommendations**. 2016.
53. Zhanel GG, Decorby M, Nichol KA, Baudry PJ, Karlowsky JA, Lagace-Wiens PR, McCracken M, Mulvey MR, Hoban DJ: **Characterization of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and extended-spectrum beta-lactamase-producing *Escherichia coli* in intensive care units in Canada: Results of the Canadian National Intensive Care Unit (CAN-ICU) study (2005-2006)**. *Can J Infect Dis Med Microbiol* 2008, **19**(3):243-249.
54. Jevons MP: **"Celbenin" - resistant Staphylococci**. *British Medical Journal* 1961, **1**(5219):124-125.
55. Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, de Lencastre H, Bentley SD, Kearns AM, Holden MTG: **Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice**. *Genome Biol* 2017, **18**(1):130.
56. Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, Harbarth S: **Methicillin-resistant *Staphylococcus aureus***. *Nat Rev Dis Primers* 2018, **4**:18033.
57. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M: **Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999**. *Clin Infect Dis* 2001, **32 Suppl 2**:S114-132.
58. Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP: **MRSA in Africa: filling the global map of antimicrobial resistance**. *PLoS One* 2013, **8**(7):e68024.

59. NORM/NORM-VET 2020. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2021. ISSN:1502-2307 (print) / 1890-9965 (electronic)
60. European Antimicrobial Resistance Surveillance Network (EARS-Net). European Centre for Disease Prevention and Control http://ecdc.europa.eu/en/activities/surveillance/.
61. Tillotson GS, Draghi DC, Sahm DF, Tomfohrde KM, Del Fabro T, Critchley IA: **Susceptibility of *Staphylococcus aureus* isolated from skin and wound infections in the United States 2005-07: laboratory-based surveillance study**. *J Antimicrob Chemother* 2008, **62**(1):109-115.
62. Chen CJ, Huang YC: **New epidemiology of *Staphylococcus aureus* infection in Asia**. *Clin Microbiol Infect* 2014, **20**(7):605-623.
63. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK: **NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007**. *Infect Control Hosp Epidemiol* 2008, **29**(11):996-1011.
64. Popovich KJ, Weinstein RA, Hota B: **Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains?** *Clin Infect Dis* 2008, **46**(6):787-794.
65. Crum-Cianflone NF, Burgi AA, Hale BR: **Increasing rates of community-acquired methicillin-resistant *Staphylococcus aureus* infections among HIV-infected persons**. *Int J STD AIDS* 2007, **18**(8):521-526.
66. Popovich KJ, Hota B, Aroutcheva A, Kurien L, Patel J, Lyles-Banks R, Grasso AE, Spec A, Beavis KG, Hayden MK *et al*: **Community-associated methicillin-resistant *Staphylococcus aureus* colonization burden in HIV-infected patients**. *Clin Infect Dis* 2013, **56**(8):1067-1074.
67. Popovich KJ, Weinstein RA, Aroutcheva A, Rice T, Hota B: **Community-associated methicillin-resistant *Staphylococcus aureus* and HIV: intersecting epidemics**. *Clin Infect Dis* 2010, **50**(7):979-987.

68. Ouko TT, Ngeranwa JN, Orinda GO, Bii CC, Amukoye E, Lucy M, Wamae CN: **Oxacillin resistant *Staphylococcus aureus* among HIV infected and non-infected Kenyan patients**. *East Afr Med J* 2010, **87**(5):179-186.

69. Kempker RR, Farley MM, Ladson JL, Satola S, Ray SM: **Association of methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 genotype with mortality in MRSA bacteremia**. *J Infect* 2010, **61**(5):372-381.

70. Burkey MD, Wilson LE, Moore RD, Lucas GM, Francis J, Gebo KA: **The incidence of and risk factors for MRSA bacteraemia in an HIV-infected cohort in the HAART era**. *HIV Med* 2008, **9**(10):858-862.

71. Peters PJ, Brooks JT, McAllister SK, Limbago B, Lowery HK, Fosheim G, Guest JL, Gorwitz RJ, Bethea M, Hageman J *et al*: **Methicillin-resistant *Staphylococcus aureus* colonization of the groin and risk for clinical infection among HIV-infected adults**. *Emerg Infect Dis* 2013, **19**(4):623-629.

72. Yang ES, Tan J, Eells S, Rieg G, Tagudar G, Miller LG: **Body site colonization in patients with community-associated methicillin-resistant *Staphylococcus aureus* and other types of S. aureus skin infections**. *Clin Microbiol Infect* 2010, **16**(5):425-431.

73. Weinke T, Schiller R, Fehrenbach FJ, Pohle HD: **Association between *Staphylococcus aureus* nasopharyngeal colonization and septicemia in patients infected with the human immunodeficiency virus**. *Eur J Clin Microbiol Infect Dis* 1992, **11**(11):985-989.

74. von Eiff C, Becker K, Machka K, Stammer H, Peters G: **Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group**. *N Engl J Med* 2001, **344**(1):11-16.

75. McNally LM, Jeena PM, Gajee K, Sturm AW, Tomkins AM, Coovadia HM, Goldblatt D: **Lack of association between the nasopharyngeal carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in HIV-1-infected South African children**. *J Infect Dis* 2006, **194**(3):385-390.

76. Sabbagh P, Riahi SM, Gamble HR, Rostami A: **The global and regional prevalence, burden, and risk factors for methicillin-resistant**

Staphylococcus aureus **colonization in HIV-infected people: A systematic review and meta-analysis**. *Am J Infect Control* 2019, **47**(3):323-333.

77. Manilal A, Shewangizaw M, Mama M, Gezmu T, Merdekios B: **Methicillin-resistant *Staphylococcus aureus* colonization in HIV patients of Arba Minch province, Ethiopia: Carriage rates, antibiotic resistance, and biofilm formation**. *Acta Microbiol Immunol Hung* 2019, **66**(4):469-483.
78. Reid MJA, Steenhoff AP, Mannathoko N, Muthoga C, McHugh E, Brown EL, Fischer RSB: ***Staphylococcus aureus*** **nasal colonization among HIV-infected adults in Botswana: prevalence and risk factors**. *AIDS Care* 2017, **29**(8):961-965.
79. Lemma MT, Zenebe Y, Tulu B, Mekonnen D, Mekonnen Z: **Methicillin Resistant *Staphylococcus aureus* among HIV Infected Pediatric Patients in Northwest Ethiopia: Carriage Rates and Antibiotic Co-Resistance Profiles**. *PLoS One* 2015, **10**(9):e0137254.
80. Kyaw WM, Lee LK, Siong WC, Ping AC, Ang B, Leo YS: **Prevalence of and risk factors for MRSA colonization in HIV-positive outpatients in Singapore**. *AIDS Res Ther* 2012, **9**(1):33.
81. Cenizal MJ, Hardy RD, Anderson M, Katz K, Skiest DJ: **Prevalence of and risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization in HIV-infected ambulatory patients**. *J Acquir Immune Defic Syndr* 2008, **48**(5):567-571.
82. Ramsetty SK, Stuart LL, Blake RT, Parsons CH, Salgado CD: **Risks for methicillin-resistant *Staphylococcus aureus* colonization or infection among patients with HIV infection**. *HIV Med* 2010, **11**(6):389-394.
83. Ferreira Dde C, Silva GR, Cavalcante FS, Carmo FL, Fernandes LA, Moreira S, Passos MR, Colombo AP, Santos KR: **Methicillin-resistant *Staphylococcus aureus* in HIV patients: risk factors associated with colonization and/or infection and methods for characterization of isolates - a systematic review**. *Clinics (Sao Paulo)* 2014, **69**(11):770-776.
84. Delorenze GN, Horberg MA, Silverberg MJ, Tsai A, Quesenberry CP, Baxter R: **Trends in annual incidence of methicillin-resistant *Staphylococcus***

***aureus* (MRSA) infection in HIV-infected and HIV-uninfected patients**. *Epidemiol Infect* 2013, **141**(11):2392-2402.

85. Srinivasan A, Seifried S, Zhu L, Bitar W, Srivastava DK, Shenep JL, Bankowski MJ, Flynn PM, Hayden RT: **Short communication: methicillin-resistant *Staphylococcus aureus* infections in children and young adults infected with HIV**. *AIDS Res Hum Retroviruses* 2009, **25**(12):1219-1224.
86. Cole J, Popovich K: **Impact of community-associated methicillin resistant *Staphylococcus aureus* on HIV-infected patients**. *Curr HIV/AIDS Rep* 2013, **10**(3):244-253.
87. Vyas KJ, Shadyab AH, Lin CD, Crum-Cianflone NF: **Trends and factors associated with initial and recurrent methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft-tissue infections among HIV-infected persons: an 18-year study**. *J Int Assoc Provid AIDS Care* 2014, **13**(3):206-213.
88. Utay NS, Roque A, Timmer JK, Morcock DR, DeLeage C, Somasunderam A, Weintrob AC, Agan BK, Estes JD, Crum-Cianflone NF *et al*: **MRSA Infections in HIV-Infected People Are Associated with Decreased MRSA-Specific Th1 Immunity**. *PLoS Pathog* 2016, **12**(4):e1005580.
89. Hirota K, Watanabe D, Koizumi Y, Sakanashi D, Ueji T, Nishida Y, Takeda M, Taguri T, Ozawa K, Mikamo H *et al*: **Observational study of skin and soft-tissue *Staphylococcus aureus* infection in patients infected with HIV-1 and epidemics of Panton-Valentine leucocidin-positive community-acquired MRSA infection in Osaka, Japan**. *J Infect Chemother* 2020.
90. Hidron AI, Kempker R, Moanna A, Rimland D: **Methicillin-resistant *Staphylococcus aureus* in HIV-infected patients**. *Infect Drug Resist* 2010, **3**:73-86.
91. Shadyab AH, Crum-Cianflone NF: **Methicillin-resistant *Staphylococcus aureus* (MRSA) infections among HIV-infected persons in the era of highly active antiretroviral therapy: a review of the literature**. *HIV Med* 2012, **13**(6):319-332.

92. Skiest D, Brown K, Hester J, Moore T, Crosby C, Mussa HR, Hoffman-Roberts H, Cooper T: **Community-onset methicillin-resistant *Staphylococcus aureus* in an urban HIV clinic**. *HIV Med* 2006, **7**(6):361-368.
93. Ateba Ngoa U, Schaumburg F, Adegnika AA, Kösters K, Möller T, Gaus E, Fernandes JF, Alabi A, Issifou S, Becker K *et al*: **Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa**. *Acta Trop* 2012, **124**(1):42-47.
94. Breurec S, Zriouil SB, Fall C, Boisier P, Brisse S, Djibo S, Etienne J, Fonkoua MC, Perrier-Gros-Claude JD, Pouillot R *et al*: **Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones**. *Clin Microbiol Infect* 2011, **17**(2):160-165.
95. De Boeck H, Vandendriessche S, Hallin M, Batoko B, Alworonga JP, Mapendo B, Van Geet C, Dauly N, Denis O, Jacobs J: ***Staphylococcus aureus* nasal carriage among healthcare workers in Kisangani, the Democratic Republic of the Congo**. *Eur J Clin Microbiol Infect Dis* 2015, **34**(8):1567-1572.
96. Vandendriessche S, De Boeck H, Deplano A, Phoba MF, Lunguya O, Falay D, Dauly N, Verhaegen J, Denis O, Jacobs J: **Characterisation of *Staphylococcus aureus* isolates from bloodstream infections, Democratic Republic of the Congo**. *Eur J Clin Microbiol Infect Dis* 2017, **36**(7):1163-1171.
97. Kumburu HH, Sonda T, Leekitcharoenphon P, van Zwetselaar M, Lukjancenko O, Alifrangis M, Lund O, Mmbaga BT, Kibiki G, Aarestrup FM: **Hospital Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Tertiary Care Hospital in Moshi, Tanzania, as Determined by Whole Genome Sequencing**. *Biomed Res Int* 2018, **2018**:2087693.
98. Tumbarello M, de Gaetano Donati K, Tacconelli E, Citton R, Spanu T, Leone F, Fadda G, Cauda R: **Risk factors and predictors of mortality of**

methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia in HIV-infected patients. *J Antimicrob Chemother* 2002, **50**(3):375-382.

99. Steinhaus N, Al-Talib M, Ive P, Boyles T, Bamford C, Davies MA, Mendelson M, Wasserman S: **The management and outcomes of *Staphylococcus aureus* bacteraemia at a South African referral hospital: A prospective observational study**. *Int J Infect Dis* 2018, **73**:78-84.
100. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, Msangi V, Tellevik MG, Holberg-Petersen M, Harthug S *et al*: **Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study**. *BMC Infect Dis* 2007, **7**:43.
101. Franzetti F, Grassini A, Piazza M, Degl'innocenti M, Bandera A, Gazzola L, Marchetti G, Gori A: **Nosocomial bacterial pneumonia in HIV-infected patients: risk factors for adverse outcome and implications for rational empiric antibiotic therapy**. *Infection* 2006, **34**(1):9-16.
102. Everett CK, Subramanian A, Jarisberg LG, Fei M, Huang L: **Characteristics of Drug-Susceptible and Drug-Resistant *Staphylococcus aureus* Pneumonia in Patients with HIV**. *Epidemiology (Sunnyvale)* 2013, **3**(1).
103. Ide L, Lootens J, Thibo P: **The nose is not the only relevant MRSA screening site**. *Clin Microbiol Infect* 2009, **15**(12):1192-1193.
104. Senn L, Basset P, Nahimana I, Zanetti G, Blanc DS: **Which anatomical sites should be sampled for screening of methicillin-resistant *Staphylococcus aureus* carriage by culture or by rapid PCR test?** *Clin Microbiol Infect* 2012, **18**(2):E31-33.
105. Morris K, Wilson C, Wilcox MH: **Evaluation of chromogenic meticillin-resistant *Staphylococcus aureus* media: sensitivity versus turnaround time**. *J Hosp Infect* 2012, **81**(1):20-24.
106. Lee S, Park YJ, Park KG, Jekarl DW, Chae H, Yoo JK, Seo SW, Choi JE, Lim JH, Heo SM *et al*: **Comparative evaluation of three chromogenic media combined with broth enrichment and the real-time PCR-based Xpert MRSA assay for screening of methicillin-resistant *Staphylococcus aureus* in nasal swabs**. *Ann Lab Med* 2013, **33**(4):255-260.

107. Nonhoff C, Denis O, Brenner A, Buidin P, Legros N, Thiroux C, Dramaix M, Struelens MJ: **Comparison of three chromogenic media and enrichment broth media for the detection of methicillin-resistant *Staphylococcus aureus* from mucocutaneous screening specimens : Comparison of MRSA chromogenic media**. *Eur J Clin Microbiol Infect Dis* 2009, **28**(4):363-369.

108. Tübbicke A, Hübner C, Kramer A, Hübner NO, Fleßa S: **Transmission rates, screening methods and costs of MRSA--a systematic literature review related to the prevalence in Germany**. *Eur J Clin Microbiol Infect Dis* 2012, **31**(10):2497-2511.

109. Stürenburg E: **Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from clinical samples: methods, effectiveness and cost considerations**. *Ger Med Sci* 2009, **7**:Doc06.

110. Bhutia KO, Singh TS, Biswas S, Adhikari L: **Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in *Staphylococcus aureus***. *Int J Appl Basic Med Res* 2012, **2**(2):84-91.

111. Clinical and Laboratory Standards Institute: **Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement CLSI Document M100–S20**. Wayne, PA: Clinical and Laboratory Standards Institute; 2019

112. Ryffel C, Strässle A, Kayser FH, Berger-Bächi B: **Mechanisms of heteroresistance in methicillin-resistant *Staphylococcus aureus***. *Antimicrob Agents Chemother* 1994, **38**(4):724-728.

113. Costa AM, Kay I, Palladino S: **Rapid detection of mecA and nuc genes in staphylococci by real-time multiplex polymerase chain reaction**. *Diagn Microbiol Infect Dis* 2005, **51**(1):13-17.

114. Carroll KC: **Rapid diagnostics for methicillin-resistant *Staphylococcus aureus*: current status**. *Mol Diagn Ther* 2008, **12**(1):15-24.

115. Wang HY, Kim S, Kim J, Park SD, Uh Y, Lee H: **Multiplex real-time PCR assay for rapid detection of methicillin-resistant staphylococci directly from positive blood cultures**. *J Clin Microbiol* 2014, **52**(6):1911-1920.

116. Rocchetti TT, Martins KB, Martins PYF, Oliveira RA, Mondelli AL, Fortaleza C, Cunha M: **Detection of the mecA gene and identification of Staphylococcus directly from blood culture bottles by multiplex polymerase chain reaction**. *Braz J Infect Dis* 2018, **22**(2):99-105.
117. Quainoo S, Coolen JPM, van Hijum S, Huynen MA, Melchers WJG, van Schaik W, Wertheim HFL: **Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial Outbreak Analysis**. *Clin Microbiol Rev* 2017, **30**(4):1015-1063.
118. Besser J, Carleton HA, Gerner-Smidt P, Lindsey RL, Trees E: **Next-generation sequencing technologies and their application to the study and control of bacterial infections**. *Clin Microbiol Infect* 2018, **24**(4):335-341.
119. De Maio N, Shaw LP, Hubbard A, George S, Sanderson ND, Swann J, Wick R, AbuOun M, Stubberfield E, Hoosdally SJ *et al*: **Comparison of long-read sequencing technologies in the hybrid assembly of complex bacterial genomes**. *Microb Genom* 2019, **5**(9).
120. Phaku P, Lebughe M, Strauß L, Peters G, Herrmann M, Mumba D, Mellmann A, Muyembe-Tamfum JJ, Schaumburg F: **Unveiling the molecular basis of antimicrobial resistance in *Staphylococcus aureus* from the Democratic Republic of the Congo using whole genome sequencing**. *Clin Microbiol Infect* 2016, **22**(7):644.e641-645.
121. Ferreira FA, Helmersen K, Visnovska T, Jørgensen SB, Aamot HV: **Rapid nanopore-based DNA sequencing protocol of antibiotic-resistant bacteria for use in surveillance and outbreak investigation**. *Microb Genom* 2021, **7**(4).
122. Judge K, Harris SR, Reuter S, Parkhill J, Peacock SJ: **Early insights into the potential of the Oxford Nanopore MinION for the detection of antimicrobial resistance genes**. *J Antimicrob Chemother* 2015, **70**(10):2775-2778.
123. Depledge DP, Srinivas KP, Sadaoka T, Bready D, Mori Y, Placantonakis DG, Mohr I, Wilson AC: **Direct RNA sequencing on nanopore arrays redefines**

the transcriptional complexity of a viral pathogen. *Nat Commun* 2019, **10**(1):754.

124. George S, Pankhurst L, Hubbard A, Votintseva A, Stoesser N, Sheppard AE, Mathers A, Norris R, Navickaite I, Eaton C *et al*: **Resolving plasmid structures in Enterobacteriaceae using the MinION nanopore sequencer: assessment of MinION and MinION/Illumina hybrid data assembly approaches**. *Microb Genom* 2017, **3**(8):e000118.
125. Neoh HM, Tan XE, Sapri HF, Tan TL: **Pulsed-field gel electrophoresis (PFGE): A review of the "gold standard" for bacteria typing and current alternatives**. *Infect Genet Evol* 2019, **74**:103935.
126. Pérez-Losada M, Cabezas P, Castro-Nallar E, Crandall KA: **Pathogen typing in the genomics era: MLST and the future of molecular epidemiology**. *Infect Genet Evol* 2013, **16**:38-53.
127. Giufrè M, Errico G, Accogli M, Monaco M, Villa L, Distasi MA, Del Gaudio T, Pantosti A, Carattoli A, Cerquetti M: **Emergence of NDM-5-producing *Escherichia coli* sequence type 167 clone in Italy**. *Int J Antimicrob Agents* 2018, **52**(1):76-81.
128. Humphreys H, Coleman DC: **Contribution of whole-genome sequencing to understanding of the epidemiology and control of meticillin-resistant *Staphylococcus aureus***. *J Hosp Infect* 2019, **102**(2):189-199.
129. Kanamori H, Parobek CM, Weber DJ, van Duin D, Rutala WA, Cairns BA, Juliano JJ: **Next-Generation Sequencing and Comparative Analysis of Sequential Outbreaks Caused by Multidrug-Resistant *Acinetobacter baumannii* at a Large Academic Burn Center**. *Antimicrob Agents Chemother* 2015, **60**(3):1249-1257.
130. Madigan T, Cunningham SA, Patel R, Greenwood-Quaintance KE, Barth JE, Sampathkumar P, Cole NC, Kohner PC, Colby CE, Asay GF *et al*: **Whole-genome sequencing for methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak investigation in a neonatal intensive care unit**. *Infect Control Hosp Epidemiol* 2018, **39**(12):1412-1418.

131. Salipante SJ, SenGupta DJ, Cummings LA, Land TA, Hoogestraat DR, Cookson BT: **Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology**. *J Clin Microbiol* 2015, **53**(4):1072-1079.
132. Fitzpatrick MA, Ozer EA, Hauser AR: **Utility of Whole-Genome Sequencing in Characterizing Acinetobacter Epidemiology and Analyzing Hospital Outbreaks**. *J Clin Microbiol* 2016, **54**(3):593-612.
133. Harbarth S: **Control of endemic methicillin-resistant *Staphylococcus aureus*--recent advances and future challenges**. *Clin Microbiol Infect* 2006, **12**(12):1154-1162.
134. Robicsek A, Beaumont JL, Paule SM, Hacek DM, Thomson RB, Jr., Kaul KL, King P, Peterson LR: **Universal surveillance for methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals**. *Ann Intern Med* 2008, **148**(6):409-418.
135. Pofahl WE, Goettler CE, Ramsey KM, Cochran MK, Nobles DL, Rotondo MF: **Active surveillance screening of MRSA and eradication of the carrier state decreases surgical-site infections caused by MRSA**. *J Am Coll Surg* 2009, **208**(5):981-986; discussion 986-988.
136. Kluytmans J, Harbarth S: **Methicillin-resistant *Staphylococcus aureus* decolonization: "yes, we can," but will it help?** *Infect Control Hosp Epidemiol* 2009, **30**(7):633-635.
137. Harbarth S, Masuet-Aumatell C, Schrenzel J, Francois P, Akakpo C, Renzi G, Pugin J, Ricou B, Pittet D: **Evaluation of rapid screening and pre-emptive contact isolation for detecting and controlling methicillin-resistant *Staphylococcus aureus* in critical care: an interventional cohort study**. *Crit Care* 2006, **10**(1):R25.
138. Paterson DL, Bonomo RA: **Extended-spectrum beta-lactamases: a clinical update**. *Clin Microbiol Rev* 2005, **18**(4):657-686.
139. Bush K, Jacoby GA: **Updated functional classification of beta-lactamases**. *Antimicrob Agents Chemother* 2010, **54**(3):969-976.
140. Ambler RP: **The structure of beta-lactamases**. *Philos Trans R Soc Lond B Biol Sci* 1980, **289**(1036):321-331.

141. Ramadan AA, Abdelaziz NA, Amin MA, Aziz RK: **Novel blaCTX-M variants and genotype-phenotype correlations among clinical isolates of extended spectrum beta lactamase-producing *Escherichia coli***. *Sci Rep* 2019, **9**(1):4224.
142. Walther-Rasmussen J, Høiby N: **Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum beta-lactamases**. *Can J Microbiol* 2004, **50**(3):137-165.
143. Bonnet R: **Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes**. *Antimicrob Agents Chemother* 2004, **48**(1):1-14.
144. Blomberg B, Jureen R, Manji KP, Tamim BS, Mwakagile DS, Urassa WK, Fataki M, Msangi V, Tellevik MG, Maselle SY *et al*: **High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania**. *J Clin Microbiol* 2005, **43**(2):745-749.
145. Manyahi J, Moyo SJ, Tellevik MG, Ndugulile F, Urassa W, Blomberg B, Langeland N: **Detection of CTX-M-15 beta-lactamases in Enterobacteriaceae causing hospital- and community-acquired urinary tract infections as early as 2004, in Dar es Salaam, Tanzania**. *BMC Infect Dis* 2017, **17**(1):282.
146. Mshana SE, Hain T, Domann E, Lyamuya EF, Chakraborty T, Imirzalioglu C: **Predominance of *Klebsiella pneumoniae* ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania**. *BMC Infect Dis* 2013, **13**:466.
147. Mshana SE, Imirzalioglu C, Hain T, Domann E, Lyamuya EF, Chakraborty T: **Multiple ST clonal complexes, with a predominance of ST131, of *Escherichia coli* harbouring blaCTX-M-15 in a tertiary hospital in Tanzania**. *Clin Microbiol Infect* 2011, **17**(8):1279-1282.
148. Castanheira M, Simner PJ, Bradford PA: **Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection**. *JAC Antimicrob Resist* 2021, **3**(3):dlab092.
149. Tellevik MG, Blomberg B, Kommedal Ø, Maselle SY, Langeland N, Moyo SJ: **High Prevalence of Faecal Carriage of ESBL-Producing**

Enterobacteriaceae among Children in Dar es Salaam, Tanzania. *PLoS One* 2016, **11**(12):e0168024.

150. Richelsen R, Smit J, Schønheyder HC, Laxsen Anru P, Gutiérrez-Gutiérrez B, Rodríguez-Bãno J, Nielsen H: **Outcome of community-onset ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* bacteraemia and urinary tract infection: a population-based cohort study in Denmark**. *J Antimicrob Chemother* 2020, **75**(12):3656-3664.
151. Ndir A, Diop A, Ka R, Faye PM, Dia-Badiane NM, Ndoye B, Astagneau P: **Infections caused by extended-spectrum beta-lactamases producing Enterobacteriaceae: clinical and economic impact in patients hospitalized in 2 teaching hospitals in Dakar, Senegal**. *Antimicrob Resist Infect Control* 2016, **5**:13.
152. Melzer M, Petersen I: **Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing *E. coli* compared to non-ESBL producing *E. coli***. *J Infect* 2007, **55**(3):254-259.
153. Kang CI, Song JH, Chung DR, Peck KR, Ko KS, Yeom JS, Ki HK, Son JS, Lee SS, Kim YS *et al*: **Risk factors and treatment outcomes of community-onset bacteraemia caused by extended-spectrum beta-lactamase-producing *Escherichia coli***. *Int J Antimicrob Agents* 2010, **36**(3):284-287.
154. Superti SV, Augusti G, Zavascki AP: **Risk factors for and mortality of extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* nosocomial bloodstream infections**. *Rev Inst Med Trop Sao Paulo* 2009, **51**(4):211-216.
155. Ndir A, Diop A, Faye PM, Cissé MF, Ndoye B, Astagneau P: **Epidemiology and Burden of Bloodstream Infections Caused by Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in a Pediatric Hospital in Senegal**. *PLoS One* 2016, **11**(2):e0143729.
156. Moremi N, Claus H, Rutta L, Frosch M, Vogel U, Mshana SE: **High carriage rate of extended-spectrum beta-lactamase-producing Enterobacteriaceae among patients admitted for surgery in Tanzanian hospitals with a low rate of endogenous surgical site infections**. *J Hosp Infect* 2018, **100**(1):47-53.

157. Ouédraogo AS, Sanou S, Kissou A, Poda A, Aberkane S, Bouzinbi N, Nacro B, Ouédraogo R, Van De Perre P, Carriere C *et al*: **Fecal Carriage of Enterobacteriaceae Producing Extended-Spectrum Beta-Lactamases in Hospitalized Patients and Healthy Community Volunteers in Burkina Faso**. *Microb Drug Resist* 2017, **23**(1):63-70.

158. Bhattacharya G: **Extended spectrum beta lactamase (ESBL) Producing Klebsiella spp in HIV: is it an added threat?**; 2016.

159. Buys H, Muloiwa R, Bamford C, Eley B: ***Klebsiella pneumoniae* bloodstream infections at a South African children's hospital 2006-2011, a cross-sectional study**. *BMC Infect Dis* 2016, **16**(1):570.

160. Malande OO, Nuttall J, Pillay V, Bamford C, Eley B: **A ten-year review of ESBL and non-ESBL *Escherichia coli* bloodstream infections among children at a tertiary referral hospital in South Africa**. *PLoS One* 2019, **14**(9):e0222675.

161. Padmavathy K, Padma K, Rajasekaran S: **Multidrug Resistant CTX-M-Producing *Escherichia coli*: A Growing Threat among HIV Patients in India**. *J Pathog* 2016, **2016**:4152704.

162. Massart N, Camus C, Benezit F, Moriconi M, Fillatre P, Le Tulzo Y: **Incidence and risk factors for acquired colonization and infection due to extended-spectrum beta-lactamase-producing Gram-negative bacilli: a retrospective analysis in three ICUs with low multidrug resistance rate**. *Eur J Clin Microbiol Infect Dis* 2020, **39**(5):889-895.

163. Denis B, Lafaurie M, Donay JL, Fontaine JP, Oksenhendler E, Raffoux E, Hennequin C, Allez M, Socie G, Maziers N *et al*: **Prevalence, risk factors, and impact on clinical outcome of extended-spectrum beta-lactamase-producing *Escherichia coli* bacteraemia: a five-year study**. *Int J Infect Dis* 2015, **39**:1-6.

164. Golzarri MF, Silva-Sánchez J, Cornejo-Juárez P, Barrios-Camacho H, Chora-Hernández LD, Velázquez-Acosta C, Vilar-Compte D: **Colonization by fecal extended-spectrum β-lactamase-producing Enterobacteriaceae and**

surgical site infections in patients with cancer undergoing gastrointestinal and gynecologic surgery. *Am J Infect Control* 2019, **47**(8):916-921.

165. Houard M, Rouzé A, Ledoux G, Six S, Jaillette E, Poissy J, Préau S, Wallet F, Labreuche J, Nseir S *et al*: **Relationship between digestive tract colonization and subsequent ventilator-associated pneumonia related to ESBL-producing Enterobacteriaceae**. *PLoS One* 2018, **13**(8):e0201688.
166. Bruyère R, Vigneron C, Bador J, Aho S, Toitot A, Quenot JP, Prin S, Charles PE: **Significance of Prior Digestive Colonization With Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Patients With Ventilator-Associated Pneumonia**. *Crit Care Med* 2016, **44**(4):699-706.
167. Pilmis B, Cattoir V, Lecointe D, Limelette A, Grall I, Mizrahi A, Marcade G, Poilane I, Guillard T, Bourgeois Nicolaos N *et al*: **Carriage of ESBL-producing Enterobacteriaceae in French hospitals: the PORTABLSE study**. *J Hosp Infect* 2018, **98**(3):247-252.
168. Dyakova E, Bisnauthsing KN, Querol-Rubiera A, Patel A, Ahanonu C, Tosas Auguet O, Edgeworth JD, Goldenberg SD, Otter JA: **Efficacy and acceptability of rectal and perineal sampling for identifying gastrointestinal colonization with extended spectrum β-lactamase Enterobacteriaceae**. *Clin Microbiol Infect* 2017, **23**(8):577.e571-577.e573.
169. Grohs P, Tillecovidin B, Caumont-Prim A, Carbonnelle E, Day N, Podglajen I, Gutmann L: **Comparison of five media for detection of extended-spectrum Beta-lactamase by use of the wasp instrument for automated specimen processing**. *J Clin Microbiol* 2013, **51**(8):2713-2716.
170. Rondinaud E, Ruppé E, Matheron S, Lucet JC, Armand-Lefevre L: **Screening methods for intestinal carriage of multidrug-resistant Enterobacterales: interest of enrichment broth**. *Diagn Microbiol Infect Dis* 2020, **97**(4):115079.
171. Jazmati N, Jazmati T, Hamprecht A: **Importance of pre-enrichment for detection of third-generation cephalosporin-resistant Enterobacteriaceae (3GCREB) from rectal swabs**. *Eur J Clin Microbiol Infect Dis* 2017, **36**(10):1847-1851.

172. Kluytmans-van den Bergh MF, Verhulst C, Willemsen LE, Verkade E, Bonten MJ, Kluytmans JA: **Rectal Carriage of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae in Hospitalized Patients: Selective Preenrichment Increases Yield of Screening**. *J Clin Microbiol* 2015, **53**(8):2709-2712.

173. Swarna SR, Srimathi NN, Madhavan R, Gomathi S: **Performance of extended spectrum beta lactamases (ESBL) screening agar in various clinical specimens**. *Indian J Med Res* 2015, **141**(4):481-482.

174. Wilson G, McCabe D: **The use of antibiotic-containing agars for the isolation of extended-spectrum beta-lactamase-producing organisms in intensive care units**. *Clin Microbiol Infect* 2007, **13**(4):451-453.

175. Miró E, Mirelis B, Navarro F, Rivera A, Mesa RJ, Roig MC, Gómez L, Coll P: **Surveillance of extended-spectrum beta-lactamases from clinical samples and faecal carriers in Barcelona, Spain**. *J Antimicrob Chemother* 2005, **56**(6):1152-1155.

176. Stürenburg E, Sobottka I, Laufs R, Mack D: **Evaluation of a new screen agar plate for detection and presumptive identification of Enterobacteriaceae producing extended-spectrum beta-lactamases**. *Diagn Microbiol Infect Dis* 2005, **51**(1):51-55.

177. Naiemi NA, Murk JL, Savelkoul PH, Vandenbroucke-Grauls CM, Debets-Ossenkopp YJ: **Extended-spectrum beta-lactamases screening agar with AmpC inhibition**. *Eur J Clin Microbiol Infect Dis* 2009, **28**(8):989-990.

178. Tzouvelekis LS, Vatopoulos AC, Katsanis G, Tzelepi E: **Rare case of failure by an automated system to detect extended-spectrum beta-lactamase in a cephalosporin-resistant *Klebsiella pneumoniae* isolate**. *J Clin Microbiol* 1999, **37**(7):2388.

179. MacKenzie FM, Miller CA, Gould IM: **Comparison of screening methods for TEM- and SHV-derived extended-spectrum beta-lactamase detection**. *Clin Microbiol Infect* 2002, **8**(11):715-724.

180. Bedenic B, Randegger C, Boras A, Haechler H: **Comparison of five different methods for detection of SHV extended-spectrum beta-lactamases**. *J Chemother* 2001, **13**(1):24-33.

181. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Hombach M: **Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum β-lactamase production in clinical Enterobacteriaceae isolates**. *J Antimicrob Chemother* 2012, **67**(1):159-166.

182. Mohanty S, Gaind R, Ranjan R, Deb M: **Use of the cefepime-clavulanate ESBL Etest for detection of extended-spectrum beta-lactamases in AmpC co-producing bacteria**. *J Infect Dev Ctries* 2009, **4**(1):24-29.

183. Stürenburg E, Sobottka I, Noor D, Laufs R, Mack D: **Evaluation of a new cefepime-clavulanate ESBL Etest to detect extended-spectrum beta-lactamases in an Enterobacteriaceae strain collection**. *J Antimicrob Chemother* 2004, **54**(1):134-138.

184. Ruppé E, Lixandru B, Cojocaru R, Büke C, Paramythiotou E, Angebault C, Visseaux C, Djuikoue I, Erdem E, Burduniuc O *et al*: **Relative fecal abundance of extended-spectrum-β-lactamase-producing *Escherichia coli* strains and their occurrence in urinary tract infections in women**. *Antimicrob Agents Chemother* 2013, **57**(9):4512-4517.

185. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G: **Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae**. *J Antimicrob Chemother* 2010, **65**(3):490-495.

186. Colom K, Pérez J, Alonso R, Fernández-Aranguiz A, Lariño E, Cisterna R: **Simple and reliable multiplex PCR assay for detection of blaTEM, bla(SHV) and blaOXA-1 genes in Enterobacteriaceae**. *FEMS Microbiol Lett* 2003, **223**(2):147-151.

187. Roschanski N, Fischer J, Guerra B, Roesler U: **Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase**

genes CTX-M, SHV, TEM and CIT-type AmpCs in Enterobacteriaceae. *PLoS One* 2014, **9**(7):e100956.

188. Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE: **Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae**. *Apmis* 2007, **115**(12):1400-1408.
189. Chia JH, Chu C, Su LH, Chiu CH, Kuo AJ, Sun CF, Wu TL: **Development of a multiplex PCR and SHV melting-curve mutation detection system for detection of some SHV and CTX-M beta-lactamases of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* in Taiwan**. *J Clin Microbiol* 2005, **43**(9):4486-4491.
190. Woodford N, Fagan EJ, Ellington MJ: **Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases**. *J Antimicrob Chemother* 2006, **57**(1):154-155.
191. Park SY, Kang CI, Wi YM, Chung DR, Peck KR, Lee NY, Song JH: **Risk factors and molecular epidemiology of community-onset, multidrug resistance extended-spectrum β-lactamase-producing *Escherichia coli* infections**. *Korean J Intern Med* 2017, **32**(1):146-157.
192. Lowe CF, Katz K, McGeer AJ, Muller MP: **Efficacy of admission screening for extended-spectrum beta-lactamase producing Enterobacteriaceae**. *PLoS One* 2013, **8**(4):e62678.
193. Laurent C, Rodriguez-Villalobos H, Rost F, Strale H, Vincent JL, Deplano A, Struelens MJ, Byl B: **Intensive care unit outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* controlled by cohorting patients and reinforcing infection control measures**. *Infect Control Hosp Epidemiol* 2008, **29**(6):517-524.
194. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y *et al*: **Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis**. *Lancet Infect Dis* 2018, **18**(3):318-327.

195. Meletis G, Vavatsi N, Exindari M, Protonotariou E, Sianou E, Haitoglou C, Sofianou D, Pournaras S, Diza E: **Accumulation of carbapenem resistance mechanisms in VIM-2-producing *Pseudomonas aeruginosa* under selective pressure**. *Eur J Clin Microbiol Infect Dis* 2014, **33**(2):253-258.
196. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG: **The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA)**. *Proc Natl Acad Sci U S A* 2002, **99**(11):7687-7692.
197. van Duin D, Doi Y: **The global epidemiology of carbapenemase-producing Enterobacteriaceae**. *Virulence* 2017, **8**(4):460-469.
198. Ho PL, Cheung YY, Lo WU, Li Z, Chow KH, Lin CH, Chan JF, Cheng VC: **Molecular Characterization of an Atypical IncX3 Plasmid pKPC-NY79 Carrying bla KPC-2 in a *Klebsiella pneumoniae***. *Curr Microbiol* 2013, **67**(4):493-498.
199. Sumbana JJ, Santona A, Fiamma M, Taviani E, Deligios M, Zimba T, Lucas G, Sacarlal J, Rubino S, Paglietti B: **Extraintestinal Pathogenic *Escherichia coli* ST405 Isolate Coharboring blaNDM-5 and blaCTXM-15: A New Threat in Mozambique**. *Microb Drug Resist* 2021, **27**(12):1633-1640.
200. Huang Y, Yu X, Xie M, Wang X, Liao K, Xue W, Chan EW, Zhang R, Chen S: **Widespread Dissemination of Carbapenem-Resistant *Escherichia coli* Sequence Type 167 Strains Harboring blaNDM-5 in Clinical Settings in China**. *Antimicrob Agents Chemother* 2016, **60**(7):4364-4368.
201. Mataseje LF, Boyd DA, Fuller J, Haldane D, Hoang L, Lefebvre B, Melano RG, Poutanen S, Van Caeseele P, Mulvey MR: **Characterization of OXA-48-like carbapenemase producers in Canada, 2011-14**. *J Antimicrob Chemother* 2018, **73**(3):626-633.
202. Klein EY, Van Boeckel TP, Martinez EM, Pant S, Gandra S, Levin SA, Goossens H, Laxminarayan R: **Global increase and geographic convergence in antibiotic consumption between 2000 and 2015**. *Proceedings of the National Academy of Sciences* 2018, **115**(15):E3463-E3470.

203. Remschmidt C, Schneider S, Meyer E, Schroeren-Boersch B, Gastmeier P, Schwab F: **Surveillance of Antibiotic Use and Resistance in Intensive Care Units (SARI)**. *Dtsch Arztebl Int* 2017, **114**(50):858-865.

204. McLaughlin M, Advincula MR, Malczynski M, Qi C, Bolon M, Scheetz MH: **Correlations of antibiotic use and carbapenem resistance in enterobacteriaceae**. *Antimicrob Agents Chemother* 2013, **57**(10):5131-5133.

205. Zhang D, Hu S, Sun J, Zhang L, Dong H, Feng W, Lei J, Dong Y: **Antibiotic consumption versus the prevalence of carbapenem-resistant Gram-negative bacteria at a tertiary hospital in China from 2011 to 2017**. *J Infect Public Health* 2019, **12**(2):195-199.

206. Zhou Y, Ai W, Cao Y, Guo Y, Wu X, Wang B, Rao L, Xu Y, Zhao H, Wang X *et al*: **The Co-occurrence of NDM-5, MCR-1, and FosA3-Encoding Plasmids Contributed to the Generation of Extensively Drug-Resistant *Klebsiella pneumoniae***. *Front Microbiol* 2021, **12**:811263.

207. Shen Z, Hu Y, Sun Q, Hu F, Zhou H, Shu L, Ma T, Shen Y, Wang Y, Li J *et al*: **Emerging Carriage of NDM-5 and MCR-1 in *Escherichia coli* From Healthy People in Multiple Regions in China: A Cross Sectional Observational Study**. *EClinicalMedicine* 2018, **6**:11-20.

208. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y: **Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality**. *Antimicrob Agents Chemother* 2008, **52**(3):1028-1033.

209. Wang Z, Qin RR, Huang L, Sun LY: **Risk Factors for Carbapenem-resistant *Klebsiella pneumoniae* Infection and Mortality of *Klebsiella pneumoniae* Infection**. *Chin Med J (Engl)* 2018, **131**(1):56-62.

210. Ramos-Castañeda JA, Ruano-Ravina A, Barbosa-Lorenzo R, Paillier-Gonzalez JE, Saldaña-Campos JC, Salinas DF, Lemos-Luengas EV: **Mortality due to KPC carbapenemase-producing *Klebsiella pneumoniae* infections: Systematic review and meta-analysis: Mortality due to KPC *Klebsiella pneumoniae* infections**. *J Infect* 2018, **76**(5):438-448.

211. Franceschini E, Santoro A, Menozzi M, Bacca E, Venturelli C, Zona S, Bedini A, Digaetano M, Puzzolante C, Meschiari M *et al*: **Epidemiology and Outcomes of Bloodstream Infections in HIV-Patients during a 13-Year Period**. *Microorganisms* 2020, **8**(8).

212. Rameshkumar MR, Arunagirinathan N, Senthamilselvan B, Swathirajan CR, Solomon SS, Vignesh R, Balakrishnan P, Aljowaie RM, Almaary KS, Chen TW: **Occurrence of extended-spectrum β-lactamase, AmpC, and carbapenemase-producing genes in gram-negative bacterial isolates from human immunodeficiency virus infected patients**. *J Infect Public Health* 2021, **14**(12):1881-1886.

213. Adeyemi F, Akinde S: **ESβL, AmpC and carbapenemase co-production in multi-drug resistant Gram-negative bacteria from HIV-infected patients in southwestern Nigeria**. *African Journal of Clinical and Experimental Microbiology* 2021, **22**(1):38-51.

214. McConville TH, Sullivan SB, Gomez-Simmonds A, Whittier S, Uhlemann AC: **Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent risk of infection and 90-day mortality in critically ill patients, an observational study**. *PLoS One* 2017, **12**(10):e0186195.

215. Dickstein Y, Edelman R, Dror T, Hussein K, Bar-Lavie Y, Paul M: **Carbapenem-resistant Enterobacteriaceae colonization and infection in critically ill patients: a retrospective matched cohort comparison with non-carriers**. *J Hosp Infect* 2016, **94**(1):54-59.

216. Göttig S, Walker SV, Saleh A, Koroska F, Sommer J, Stelzer Y, Steinmann J, Hamprecht A: **Comparison of nine different selective agars for the detection of carbapenemase-producing Enterobacterales (CPE)**. *Eur J Clin Microbiol Infect Dis* 2020, **39**(5):923-927.

217. Pauly N, Hammerl JA, Grobbel M, Tenhagen BA, Käsbohrer A, Bisenius S, Fuchs J, Horlacher S, Lingstädt H, Mauermann U *et al*: **ChromID(®) CARBA Agar Fails to Detect Carbapenem-Resistant Enterobacteriaceae With Slightly Reduced Susceptibility to Carbapenems**. *Front Microbiol* 2020, **11**:1678.

218. EUCAST T: **European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameters**: European Society of Clinical Microbiology and Infectious Diseases Basel; 2020.
219. Bayramoğlu G, Uluçam G, Gençoğlu Özgür Ç, Kılıç AO, Aydın F: **[Comparison of the modified Hodge test and the Carba NP test for detection of carbapenemases in Enterobacteriaceae isolates]**. *Mikrobiyol Bul* 2016, **50**(1):1-10.
220. Srisrattakarn A, Lulitanond A, Wilailuckana C, Charoensri N, Wonglakorn L, Piyapatthanakul S, Supajeen A, Chanawong A: **Modification and evaluation of the Carba NP test by use of paper strip for simple and rapid detection of carbapenemase-producing Enterobacteriaceae**. *World J Microbiol Biotechnol* 2016, **32**(7):117.
221. Tamma PD, Simner PJ: **Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates**. *J Clin Microbiol* 2018, **56**(11).
222. Muntean MM, Muntean AA, Gauthier L, Creton E, Cotellon G, Popa MI, Bonnin RA, Naas T: **Evaluation of the rapid carbapenem inactivation method (rCIM): a phenotypic screening test for carbapenemase-producing Enterobacteriaceae**. *J Antimicrob Chemother* 2018, **73**(4):900-908.
223. Aguirre-Quiñonero A, Cano ME, Gamal D, Calvo J, Martínez-Martínez L: **Evaluation of the carbapenem inactivation method (CIM) for detecting carbapenemase activity in enterobacteria**. *Diagn Microbiol Infect Dis* 2017, **88**(3):214-218.
224. Mlynarcik P, Roderova M, Kolar M: **Primer Evaluation for PCR and its Application for Detection of Carbapenemases in Enterobacteriaceae**. *Jundishapur J Microbiol* 2016, **9**(1):e29314.
225. Yoshioka N, Hagiya H, Deguchi M, Hamaguchi S, Kagita M, Nishi I, Akeda Y, Tomono K: **Multiplex Real-Time PCR Assay for Six Major Carbapenemase Genes**. *Pathogens* 2021, **10**(3).
226. van der Zee A, Roorda L, Bosman G, Fluit AC, Hermans M, Smits PH, van der Zanden AG, Te Witt R, Bruijnesteijn van Coppenraet LE, Cohen Stuart J *et al*:

Multi-centre evaluation of real-time multiplex PCR for detection of carbapenemase genes OXA-48, VIM, IMP, NDM and KPC. *BMC Infect Dis* 2014, **14**:27.

227. Poirel L, Walsh TR, Cuvillier V, Nordmann P: **Multiplex PCR for detection of acquired carbapenemase genes**. *Diagn Microbiol Infect Dis* 2011, **70**(1):119-123.
228. Swayne RL, Ludlam HA, Shet VG, Woodford N, Curran MD: **Real-time TaqMan PCR for rapid detection of genes encoding five types of non-metallo- (class A and D) carbapenemases in Enterobacteriaceae**. *Int J Antimicrob Agents* 2011, **38**(1):35-38.
229. Sherif M, Palmieri M, Mirande C, El-Mahallawy H, Rashed HG, Abd-El-Reheem F, El-Manakhly AR, Abdel-Latif RAR, Aboulela AG, Saeed LY *et al*: **Whole-genome sequencing of Egyptian multidrug-resistant *Klebsiella pneumoniae* isolates: a multi-center pilot study**. *Eur J Clin Microbiol Infect Dis* 2021, **40**(7):1451-1460.
230. Puleston R, Brown CS, Patel B, Fry C, Singleton S, Robotham JV, Freeman R: **Recommendations for detection and rapid management of carbapenemase-producing Enterobacterales outbreaks**. *Infect Prev Pract* 2020, **2**(3):100086.
231. Organization WH: **Guidelines on core components of infection prevention and control programmes at the national and acute health care facility level**: World Health Organization; 2016.
232. Jordano Q, Falcó V, Almirante B, Planes AM, del Valle O, Ribera E, Len O, Pigrau C, Pahissa A: **Invasive pneumococcal disease in patients infected with HIV: still a threat in the era of highly active antiretroviral therapy**. *Clin Infect Dis* 2004, **38**(11):1623-1628.
233. Jones N, Huebner R, Khoosal M, Crewe-Brown H, Klugman K: **The impact of HIV on *Streptococcus pneumoniae* bacteraemia in a South African population**. *Aids* 1998, **12**(16):2177-2184.
234. Frankel RE, Virata M, Hardalo C, Altice FL, Friedland G: **Invasive pneumococcal disease: clinical features, serotypes, and antimicrobial**

resistance patterns in cases involving patients with and without human immunodeficiency virus infection. *Clin Infect Dis* 1996, **23**(3):577-584.

235. Meiring S, Cohen C, Quan V, de Gouveia L, Feldman C, Karstaedt A, Klugman KP, Madhi SA, Rabie H, Sriruttan C *et al*: **HIV Infection and the Epidemiology of Invasive Pneumococcal Disease (IPD) in South African Adults and Older Children Prior to the Introduction of a Pneumococcal Conjugate Vaccine (PCV)**. *PLoS One* 2016, **11**(2):e0149104.
236. Marcus JL, Baxter R, Leyden WA, Muthulingam D, Yee A, Horberg MA, Klein DB, Towner WJ, Chao CR, Quesenberry CP, Jr. *et al*: **Invasive Pneumococcal Disease Among HIV-Infected and HIV-Uninfected Adults in a Large Integrated Healthcare System**. *AIDS Patient Care STDS* 2016, **30**(10):463-470.
237. Gordon SB, Chaponda M, Walsh AL, Whitty CJ, Gordon MA, Machili CE, Gilks CF, Boeree MJ, Kampondeni S, Read RC *et al*: **Pneumococcal disease in HIV-infected Malawian adults: acute mortality and long-term survival**. *Aids* 2002, **16**(10):1409-1417.
238. Sadlier C, O'Connell S, Kelleher M, Bergin C: **Incidence and risk factors for invasive pneumococcal disease in HIV-positive individuals in the era of highly active antiretroviral therapy**. *Int J STD AIDS* 2019, **30**(5):472-478.
239. Gebo KA, Moore RD, Keruly JC, Chaisson RE: **Risk factors for pneumococcal disease in human immunodeficiency virus-infected patients**. *J Infect Dis* 1996, **173**(4):857-862.
240. Feikin DR, Feldman C, Schuchat A, Janoff EN: **Global strategies to prevent bacterial pneumonia in adults with HIV disease**. *Lancet Infect Dis* 2004, **4**(7):445-455.
241. Bordon J, Kapoor R, Martinez C, Portela D, Duvvuri P, Klochko A, Ayesu K, Peyrani P, Cillóniz C, Wiemken T *et al*: **CD4+ cell counts and HIV-RNA levels do not predict outcomes of community-acquired pneumonia in hospitalized HIV-infected patients**. *Int J Infect Dis* 2011, **15**(12):e822-827.

242. Bogaert D, de Groot R, Hermans PWM: ***Streptococcus pneumoniae* colonisation: the key to pneumococcal disease**. *The Lancet Infectious Diseases* 2004, **4**(3):144-154.

243. Blossom DB, Namayanja-Kaye G, Nankya-Mutyoba J, Mukasa JB, Bakka H, Rwambuya S, Windau A, Bajaksouzian S, Walker CJ, Joloba ML *et al*: **Oropharyngeal colonization by *Streptococcus pneumoniae* among HIV-infected adults in Uganda: assessing prevalence and antimicrobial susceptibility**. *Int J Infect Dis* 2006, **10**(6):458-464.

244. Conklin LM, Bigogo G, Jagero G, Hampton L, Junghae M, da Gloria Carvalho M, Pimenta F, Beall B, Taylor T, Plikaytis B *et al*: **High *Streptococcus pneumoniae* colonization prevalence among HIV-infected Kenyan parents in the year before pneumococcal conjugate vaccine introduction**. *BMC Infect Dis* 2016, **16**:18.

245. Gill CJ, Mwanakasale V, Fox MP, Chilengi R, Tembo M, Nsofwa M, Chalwe V, Mwananyanda L, Mukwamataba D, Malilwe B *et al*: **Impact of Human Immunodeficiency Virus Infection on *Streptococcus pneumoniae* Colonization and Seroepidemiology among Zambian Women**. *The Journal of Infectious Diseases* 2008, **197**(7):1000-1005.

246. Rodriguez-Barradas MC, Tharapel RA, Groover JE, Giron KP, Lacke CE, Houston ED, Hamill RJ, Steinhoff MC, Musher DM: **Colonization by *Streptococcus pneumoniae* among Human Immunodeficiency Virus-Infected Adults: Prevalence of Antibiotic Resistance, Impact of Immunization, and Characterization by Polymerase Chain Reaction with BOX Primers of Isolates from Persistent S. pneumoniae Carriers**. *The Journal of Infectious Diseases* 1997, **175**(3):590-597.

247. Dayie NT, Baffuor-Asare M, Labi AK, Obeng-Nkrumah N, Olayemi E, Lartey M, Slotved HC, Donkor ES: **Epidemiology of Pneumococcal Carriage among HIV-Infected Individuals in the Conjugate Vaccine Era: A Study in Southern Ghana**. *Biomed Res Int* 2019, **2019**:3427174.

248. Moyo SJ, Steinbakk M, Aboud S, Mkopi N, Kasubi M, Blomberg B, Manji K, Lyamuya EF, Maselle SY, Langeland N: **Penicillin resistance and serotype**

distribution of *Streptococcus pneumoniae* in nasopharyngeal carrier children under 5 years of age in Dar es Salaam, Tanzania. *J Med Microbiol* 2012, **61**(Pt 7):952-959.

249. Bles P, de Mast Q, van der Gaast-de Jongh CE, Kinabo GD, Kibiki G, van de Ven A, de Jonge MI: **Antibiotic resistance of *Streptococcus pneumoniae* colonising the nasopharynx of HIV-exposed Tanzanian infants**. *Trop Med Int Health* 2015, **20**(11):1559-1563.
250. Feldman C, Glatthaar M, Morar R, Mahomed AG, Kaka S, Cassel M, Klugman KP: **Bacteremic pneumococcal pneumonia in HIV-seropositive and HIV-seronegative adults**. *Chest* 1999, **116**(1):107-114.
251. Birindwa AM, Emgård M, Nordén R, Samuelsson E, Geravandi S, Gonzales-Siles L, Muhigirwa B, Kashosi T, Munguakonkwa E, Manegabe JT *et al*: **High rate of antibiotic resistance among pneumococci carried by healthy children in the eastern part of the Democratic Republic of the Congo**. *BMC Pediatr* 2018, **18**(1):361.
252. Kengne M, Lebogo MBB, Nwobegahay JM, Ondigui BE: **Antibiotics susceptibility pattern of *Streptococcus pneumoniae* isolated from sputum cultures of human immunodeficiency virus infected patients in Yaoundé, Cameroon**. *Pan Afr Med J* 2018, **31**:16.
253. Bles P, de Mast Q, van der Gaast-de Jongh CE, Kinabo GD, Kibiki G, van de Ven A, de Jonge MI: **Antibiotic resistance of *Streptococcus pneumoniae* colonising the nasopharynx of HIV-exposed Tanzanian infants**. *Tropical Medicine & International Health* 2015, **20**(11):1559-1563.
254. Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, Henao-Restrepo AM, Leach AJ, Klugman KP, Porter BD *et al*: **Standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group**. *Vaccine* 2013, **32**(1):165-179.
255. Hanieh S, Hamaluba M, Kelly DF, Metz JA, Wyres KL, Fisher R, Pradhan R, Shakya D, Shrestha L, Shrestha A *et al*: ***Streptococcus pneumoniae* carriage**

prevalence in Nepal: evaluation of a method for delayed transport of samples from remote regions and implications for vaccine implementation. *PLoS One* 2014, **9**(6):e98739.

256. Rubin LG, Rizvi A, Baer A: **Effect of swab composition and use of swabs versus swab-containing skim milk-tryptone-glucose-glycerol (STGG) on culture- or PCR-based detection of *Streptococcus pneumoniae* in simulated and clinical respiratory specimens in STGG transport medium**. *J Clin Microbiol* 2008, **46**(8):2635-2640.
257. Converse GM, 3rd, Dillon HC, Jr.: **Epidemiological studies of *Streptococcus pneumoniae* in infants: methods of isolating pneumococci**. *J Clin Microbiol* 1977, **5**(3):293-296.
258. Murray PR BE, Pfaller MA, Tenovar FC, Yolken RH: Manual of Clinical Microbiology. 7th edition. Washington, DC: American Society for Microbiology; 1999.: **Manual of Clinical Microbiology. 7th edition. Washington, DC: American Society for Microbiology; 1999.** 1999.
259. Habib M, Porter BD, Satzke C: **Capsular serotyping of *Streptococcus pneumoniae* using the Quellung reaction**. *J Vis Exp* 2014(84):e51208.
260. Slotved HC, Kaltoft M, Skovsted IC, Kerrn MB, Espersen F: **Simple, rapid latex agglutination test for serotyping of pneumococci (Pneumotest-Latex)**. *J Clin Microbiol* 2004, **42**(6):2518-2522.
261. Sanz JC, Culebras E, Ríos E, Rodríguez-Avial I, Wilhelmi I, Ramos B, Ordobás M, Picazo JJ: **Direct serogrouping of *Streptococcus pneumoniae* strains in clinical samples by use of a latex agglutination test**. *J Clin Microbiol* 2010, **48**(2):593-595.
262. Madhi SA, Nunes MC: **The potential impact of pneumococcal conjugate vaccine in Africa: Considerations and early lessons learned from the South African experience**. *Hum Vaccin Immunother* 2016, **12**(2):314-325.
263. Sanneh B, Okoi C, Grey-Johnson M, Bah-Camara H, Kunta Fofana B, Baldeh I, Papa Sey A, Labbo Bah M, Cham M, Samateh A *et al*: **Declining Trends of Pneumococcal Meningitis in Gambian Children After the Introduction of**

Pneumococcal Conjugate Vaccines. *Clin Infect Dis* 2019, **69**(Suppl 2):S126-s132.

264. Yoo JR, Oh S, Lee JG, Kim YR, Lee KH, Heo ST: **Invasive Pneumococcal Disease Caused by Non-Vaccine Type Multidrug-Resistant *Streptococcus pneumoniae* Transmitted by Close Contact in a Healthy Adult**. *Yonsei Med J* 2019, **60**(11):1103-1107.
265. Setchanova L, Alexandrova A, Pencheva D, Sirakov I, Mihova K, Kaneva R, Mitov I: **Rise of multidrug-resistant *Streptococcus pneumoniae* clones expressing non-vaccine serotypes among children following introduction of the 10-valent pneumococcal conjugate vaccine in Bulgaria**. *J Glob Antimicrob Resist* 2018, **15**:6-11.
266. Asgin N, Otlu B: **Antibiotic Resistance and Molecular Epidemiology of Vancomycin-Resistant Enterococci in a Tertiary Care Hospital in Turkey**. *Infect Drug Resist* 2020, **13**:191-198.
267. Moyo SJ, Aboud S, Blomberg B, Mkopi N, Kasubi M, Manji K, Lyamuya EF, Maselle SY, Langeland N: **High nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthy Tanzanian under-5 children**. *Microb Drug Resist* 2014, **20**(1):82-88.
268. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B *et al*: **Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance**. *Clin Microbiol Infect* 2012, **18**(3):268-281.
269. Chipolombwe J, Török ME, Mbelle N, Nyasulu P: **Methicillin-resistant *Staphylococcus aureus* multiple sites surveillance: a systemic review of the literature**. *Infect Drug Resist* 2016, **9**:35-42.
270. Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, Sharma S, Avihingsanon A, Cooper DA, Fätkenheuer G, Llibre JM *et al*: **Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection**. *N Engl J Med* 2015, **373**(9):795-807.

271. Verani JR, Massora S, Acácio S, Dos Santos RT, Vubil D, Pimenta F, Moura I, Whitney CG, Costa MH, Macete E *et al*: **Nasopharyngeal carriage of *Streptococcus pneumoniae* among HIV-infected and -uninfected children <5 years of age before introduction of pneumococcal conjugate vaccine in Mozambique**. *PLoS One* 2018, **13**(2):e0191113.
272. Manyahi J, Kibwana U, Mgimba E, Majigo M: **Multi-drug resistant bacteria predict mortality in bloodstream infection in a tertiary setting in Tanzania**. *PLoS One* 2020, **15**(3):e0220424.
273. Moyo SJ, Manyahi J, Blomberg B, Tellevik MG, Masoud NS, Aboud S, Manji K, Roberts AP, Hanevik K, Mørch K *et al*: **Bacteraemia, Malaria, and Case Fatality Among Children Hospitalized With Fever in Dar es Salaam, Tanzania**. *Front Microbiol* 2020, **11**:2118.
274. Manyahi J, Matee MI, Majigo M, Moyo S, Mshana SE, Lyamuya EF: **Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili National Hospital, Tanzania**. *BMC Res Notes* 2014, **7**:500.
275. Jones RN, Sader HS, Mendes RE, Flamm RK: **Update on antimicrobial susceptibility trends among *Streptococcus pneumoniae* in the United States: report of ceftaroline activity from the SENTRY Antimicrobial Surveillance Program (1998-2011)**. *Diagn Microbiol Infect Dis* 2013, **75**(1):107-109.
276. Domenech A, Tirado-Vélez JM, Fenoll A, Ardanuy C, Yuste J, Liñares J, de la Campa AG: **Fluoroquinolone-resistant pneumococci: dynamics of serotypes and clones in Spain in 2012 compared with those from 2002 and 2006**. *Antimicrob Agents Chemother* 2014, **58**(4):2393-2399.
277. Anderson KB, Tan JS, File TM, Jr., DiPersio JR, Willey BM, Low DE: **Emergence of levofloxacin-resistant pneumococci in immunocompromised adults after therapy for community-acquired pneumonia**. *Clin Infect Dis* 2003, **37**(3):376-381.
278. Emgård M, Msuya SE, Nyombi BM, Mosha D, Gonzales-Siles L, Nordén R, Geravandi S, Mosha V, Blomqvist J, Franzén S *et al*: **Carriage of penicillin-**

non-susceptible pneumococci among children in northern Tanzania in the 13-valent pneumococcal vaccine era. *Int J Infect Dis* 2019, **81**:156-166.

279. Agyekum A, Fajardo-Lubián A, Ansong D, Partridge SR, Agbenyega T, Iredell JR: **blaCTX-M-15 carried by IncF-type plasmids is the dominant ESBL gene in *Escherichia coli* and *Klebsiella pneumoniae* at a hospital in Ghana**. *Diagn Microbiol Infect Dis* 2016, **84**(4):328-333.
280. Li JJ, Spychala CN, Hu F, Sheng JF, Doi Y: **Complete nucleotide sequences of bla(CTX-M)-harboring IncF plasmids from community-associated *Escherichia coli* strains in the United States**. *Antimicrob Agents Chemother* 2015, **59**(6):3002-3007.
281. Xue M, Wang K, Lu L, Li Z, Li P, Li J, Lin Y, Yang L, Qi K, Song H *et al*: **Characterization of an New Delhi-Metallo-1-Producing *Enterobacter cloacae* ST418 Strain from a Patient in Guangzhou, China**. *Microb Drug Resist* 2021, **27**(5):706-709.
282. Adrian PV, Klugman KP: **Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of *Streptococcus pneumoniae***. *Antimicrob Agents Chemother* 1997, **41**(11):2406-2413.
283. Cornick JE, Harris SR, Parry CM, Moore MJ, Jassi C, Kamng'ona A, Kulohoma B, Heyderman RS, Bentley SD, Everett DB: **Genomic identification of a novel co-trimoxazole resistance genotype and its prevalence amongst *Streptococcus pneumoniae* in Malawi**. *J Antimicrob Chemother* 2014, **69**(2):368-374.
284. He M, Yao K, Shi W, Gao W, Yuan L, Yu S, Yang Y: **Dynamics of serotype 14 *Streptococcus pneumoniae* population causing acute respiratory infections among children in China (1997-2012)**. *BMC Infect Dis* 2015, **15**:266.
285. Nurjadi D, Olalekan AO, Layer F, Shittu AO, Alabi A, Ghebremedhin B, Schaumburg F, Hofmann-Eifler J, Van Genderen PJ, Caumes E *et al*: **Emergence of trimethoprim resistance gene dfrG in *Staphylococcus aureus* causing human infection and colonization in sub-Saharan Africa and its import to Europe**. *J Antimicrob Chemother* 2014, **69**(9):2361-2368.

286. Steinig EJ, Andersson P, Harris SR, Sarovich DS, Manoharan A, Coupland P, Holden MT, Parkhill J, Bentley SD, Robinson DA *et al*: **Single-molecule sequencing reveals the molecular basis of multidrug-resistance in ST772 methicillin-resistant *Staphylococcus aureus***. *BMC Genomics* 2015, **16**(1):388.

287. Khodabandeh M, Mohammadi M, Abdolsalehi MR, Alvandimanesh A, Gholami M, Bibalan MH, Pournajaf A, Kafshgari R, Rajabnia R: **Analysis of Resistance to Macrolide-Lincosamide-Streptogramin B Among mecA-Positive *Staphylococcus Aureus* Isolates**. *Osong Public Health Res Perspect* 2019, **10**(1):25-31.

288. Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Köhrer K, Verhoef J, Fluit AC: **Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals**. *J Antimicrob Chemother* 2000, **45**(6):891-894.

289. Weisblum B: **Insights into erythromycin action from studies of its activity as inducer of resistance**. *Antimicrob Agents Chemother* 1995, **39**(4):797-805.

290. Rouch DA, Byrne ME, Kong YC, Skurray RA: **The aacA-aphD gentamicin and kanamycin resistance determinant of Tn4001 from *Staphylococcus aureus*: expression and nucleotide sequence analysis**. *J Gen Microbiol* 1987, **133**(11):3039-3052.

291. Lyon BR, Gillespie MT, Byrne ME, May JW, Skurray RA: **Plasmid-mediated resistance to gentamicin in *Staphylococcus aureus*: the involvement of a transposon**. *J Med Microbiol* 1987, **23**(2):101-110.

292. Schmitz FJ, Fluit AC, Lückefahr M, Engler B, Hofmann B, Verhoef J, Heinz HP, Hadding U, Jones ME: **The effect of reserpine, an inhibitor of multidrug efflux pumps, on the in-vitro activities of ciprofloxacin, sparfloxacin and moxifloxacin against clinical isolates of *Staphylococcus aureus***. *J Antimicrob Chemother* 1998, **42**(6):807-810.

293. Hashem RA, Yassin AS, Zedan HH, Amin MA: **Fluoroquinolone resistant mechanisms in methicillin-resistant *Staphylococcus aureus* clinical isolates in Cairo, Egypt**. *J Infect Dev Ctries* 2013, **7**(11):796-803.

294. Utsi L, Pichon B, Arunachalam N, Kerrane A, Batten E, Denton M, Townsend R, Agwuh KN, Hughes GJ, Kearns A: **Circulation of a community healthcare-associated multiply-resistant meticillin-resistant *Staphylococcus aureus* lineage in South Yorkshire identified by whole genome sequencing**. *J Hosp Infect* 2019, **103**(4):454-460.

295. Strauß L, Stegger M, Akpaka PE, Alabi A, Breurec S, Coombs G, Egyir B, Larsen AR, Laurent F, Monecke S *et al*: **Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8**. *Proc Natl Acad Sci U S A* 2017, **114**(49):E10596-e10604.

296. Qi W, Ender M, O'Brien F, Imhof A, Ruef C, McCallum N, Berger-Bächi B: **Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Zurich, Switzerland (2003): prevalence of type IV SCCmec and a new SCCmec element associated with isolates from intravenous drug users**. *J Clin Microbiol* 2005, **43**(10):5164-5170.

297. Sun P, Xia W, Liu G, Huang X, Tang C, Liu C, Xu Y, Ni F, Mei Y, Pan S: **Characterization Of bla (NDM-5)-Positive *Escherichia coli* Prevalent In A University Hospital In Eastern China**. *Infect Drug Resist* 2019, **12**:3029-3038.

298. Li X, Fu Y, Shen M, Huang D, Du X, Hu Q, Zhou Y, Wang D, Yu Y: **Dissemination of bla(NDM-5) gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China**. *Antimicrob Resist Infect Control* 2018, **7**:59.

299. Yang P, Xie Y, Feng P, Zong Z: **blaNDM-5 carried by an IncX3 plasmid in *Escherichia coli* sequence type 167**. *Antimicrob Agents Chemother* 2014, **58**(12):7548-7552.

300. Wu M, Tong X, Liu S, Wang D, Wang L, Fan H: **Prevalence of methicillin-resistant *Staphylococcus aureus* in healthy Chinese population: A system review and meta-analysis**. *PLoS One* 2019, **14**(10):e0223599.

301. Choi CS, Yin CS, Bakar AA, Sakewi Z, Naing NN, Jamal F, Othman N: **Nasal carriage of *Staphylococcus aureus* among healthy adults**. *J Microbiol Immunol Infect* 2006, **39**(6):458-464.

302. Kotton CN, Lankowski AJ, Hohmann EL: **Comparison of rectal swabs with fecal cultures for detection of *Salmonella typhimurium* in adult volunteers**. *Diagn Microbiol Infect Dis* 2006, **56**(2):123-126.

303. Kotar T, Pirš M, Steyer A, Cerar T, Šoba B, Skvarc M, Poljšak Prijatelj M, Lejko Zupanc T: **Evaluation of rectal swab use for the determination of enteric pathogens: a prospective study of diarrhoea in adults**. *Clin Microbiol Infect* 2019, **25**(6):733-738.

304. Jazmati N, Hein R, Hamprecht A: **Use of an Enrichment Broth Improves Detection of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae in Clinical Stool Samples**. *J Clin Microbiol* 2016, **54**(2):467-470.

305. Girlich D, Poirel L, Nordmann P: **Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for detection of Enterobacteriaceae with reduced susceptibility to carbapenems**. *Diagn Microbiol Infect Dis* 2013, **75**(2):214-217.

306. Nordmann P, Girlich D, Poirel L: **Detection of carbapenemase producers in Enterobacteriaceae by use of a novel screening medium**. *J Clin Microbiol* 2012, **50**(8):2761-2766.

307. Carrër A, Fortineau N, Nordmann P: **Use of ChromID extended-spectrum beta-lactamase medium for detecting carbapenemase-producing Enterobacteriaceae**. *J Clin Microbiol* 2010, **48**(5):1913-1914.

Appendices

Case report form (CRF) I

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES (MUHAS)

Title: Randomized clinical trial to assess whether the duration of cotrimoxazole preventive therapy in HIV patients with CD4 counts >350 CD4 cells/μL by antiretroviral treatment influences the rate of carriage of multidrug-resistant bacteria

i) Study participation number: _____

ii) Patient registration number: _____

iii) Name of the patient: _____

1.Date of birth ___, ___, _____ Age: _____			**2.Sex** Male ◯: Female ◯		
3. (a)Place of residence……			**(b)District**……		
4. Level of education: Informal ◯: Primary ◯ **:** Secondary ◯ **:** Post-secondary ◯					
5. Height _____cm			**6.Weight** _____ Kg.		
8. (a) Anti-TB treatment: Yes ◯ No ◯ (b) If Yes: Date started ___ ,___,_____(c) Date stopped ___ ,___,_____					
9. (a) Previous antibiotic use last two years				Yes ◯	No ◯
(b) If YES: Fill name of drug and tick (x) when					
Name of drug	Ongoing	Last week	Last 6 months	Last 2 years	
i)	◯	◯	◯	◯	
ii)	◯	◯	◯	◯	
iii)	◯	◯	◯	◯	
10. (a) Previous hospitalization (last two years)				Yes ◯	No ◯
(b) If YES: Fill diagnose and tick (x) when					
Diagnosis	Last week	Last 6 months	Last 2 years		
i)	◯	◯	◯		
ii)	◯	◯	◯		
iii)	◯	◯	◯		
11. (a)History of attending outpatient clinic		Yes ◯: No ◯	(b) If yes when ___, ___, _____		
12. Any travel abroad last two years:		Yes ◯: No ◯			

13.(a) WHO stage ____	(b)Date of staging ___, ___, _____				
14(a) CD4 count _____					
15. Started on which ART					
16.Starting on PJP prophylaxis	Yes ◯	No ◯			

Case report form (CRF) II

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES (MUHAS)

Title: Randomized clinical trial to assess whether the duration of cotrimoxazole preventive therapy in HIV patients with CD4 counts ≤350 CD4 cells/μL by antiretroviral treatment influences the rate of carriage of multidrug-resistant bacteria

i) Study participation number: _____

ii) Patient registration number: _____

iii) Name of the patient: _____

1.Date of birth ___, ___, _____ Age: _____			**2.Sex** Male ◯: Female ◯		
3. (a)Place of residence……			**(b)District**……		
4. Level of education: Informal ◯: Primary ◯ **:** Secondary ◯ **:** Post-secondary ◯					
5. Height _____cm			**6.Weight** _____ Kg.		
8. (a) Anti-TB treatment: Yes ◯ No ◯ (b) If Yes: Date started ___ ,___,_____(c) Date stopped ___ ,___,_____					
9. (a) Previous antibiotic use last two years				Yes ◯	No ◯
(b) If YES: Fill name of drug and tick (x) when					
Name of drug	Ongoing	Last week	Last 6 months	Last 2 years	
i)	◯	◯	◯	◯	
ii)	◯	◯	◯	◯	
iii)	◯	◯	◯	◯	
10. (a) Previous hospitalization (last two years)				Yes ◯	No ◯
(b) If YES: Fill diagnose and tick (x) when					
Diagnosis	Last week	Last 6 months	Last 2 years		
i)	◯	◯	◯		
ii)	◯	◯	◯		
iii)	◯	◯	◯		
11. (a)History of attending outpatient clinic		Yes ◯ : No ◯	(b) If yes when ___, ___, _____		
12. Any travel abroad last two years:		Yes ◯ : No ◯			

13.(a) WHO stage ____	(b)Date of staging ___, ___, _____				
14(a) CD4 count _____					
15. Started on which ART					
16.Starting on PJP prophylaxis	Yes ◯	No ◯			

Paper I

European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-021-04160-2

ORIGINAL ARTICLE

Predominance of PVL-negative community-associated methicillin-resistant *Staphylococcus aureus* sequence type 8 in newly diagnosed HIV-infected adults, Tanzania

Joel Manyahi [1,2,3] · Sabrina J. Moyo [1,3] · Said Aboud [3] · Nina Langeland [1,2] · Bjørn Blomberg [1,2]

Received: 26 August 2020 / Accepted: 11 January 2021

Abstract

Difficult-to-treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are of concern in people living with HIV infection as they are more vulnerable to infection. We aimed to identify molecular characteristics of MRSA colonizing newly diagnosed HIV-infected adults in Tanzania. Individuals newly diagnosed with HIV infection were recruited in Dar es Salaam, Tanzania, from April 2017 to May 2018, as part of the randomized clinical trial CoTrimResist (ClinicalTrials.gov identifier: NCT03087890). Nasal/nasopharyngeal isolates of *Staphylococcus aureus* were susceptibility tested by disk diffusion method, and cefoxitin-resistant isolates were characterized by short-reads whole genome sequencing. Four percent (22/537) of patients carried MRSA in the nose/nasopharynx. MRSA isolates were frequently resistant towards gentamicin (95%), ciprofloxacin (91%), and erythromycin (82%) but less often towards trimethoprim-sulfamethoxazole (9%). Seventy-three percent had inducible clindamycin resistance. Erythromycin-resistant isolates harbored *ermC* (15/18) and *LmrS* (3/18) resistance genes. Ciprofloxacin resistance was mediated by mutations of the quinolone resistance-determining region (QRDR) sequence in the *gyrA* (S84L) and *parC* (S80Y) genes. All isolates belonged to the CC8 and ST8-SCC*mec*IV MRSA clone. Ninety-five percent of the MRSA isolates were *spa*-type t1476, and one exhibited *spa*-type t064. All isolates were negative for Panton-Valentine leucocidin (PVL) and arginine catabolic mobile element (ACME) type 1. All ST8-SCC*mec*IV-*spa*-t1476 MRSA clones from Tanzania were unrelated to the globally successful USA300 clone. Carriage of ST8 MRSA (non-USA300) was common among newly diagnosed HIV-infected adults in Tanzania. Frequent co-resistance to non-beta lactam antibiotics limits therapeutic options when infection occurs.

Keywords Panton-Valentine leukocidin-negative · Sequence type 8 (ST8) · Methicillin-resistant *Staphylococcus aureus* (MRSA) · Human immunodficiency virus · Community · Tanzania

✉ Joel Manyahi
manyahijoel@yahoo.com; joel.manyahi@uib.no

Sabrina J. Moyo
sabrina.moyo@uib.no

Said Aboud
aboudsaid@yahoo.com

Nina Langeland
nina.langeland@uib.no

Bjørn Blomberg
bjorn.blomberg@uib.no

[1] Department of Clinical Science, University of Bergen, Bergen, Norway

[2] National Advisory Unit for Tropical Infectious Diseases, Department of Medicine, Haukeland University Hospital, Bergen, Norway

[3] Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, MUHAS, P.O. Box 65001, Dar es Salaam, Tanzania

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are difficult to treat and MRSA-bacteremia is associated with increased risk of fatal outcome [1]. Nasal colonization with MRSA is a risk factor for invasive disease [2] and a particular threat to HIV-infected individuals who are more susceptible to severe bacterial disease, including staphylococcal infections [3, 4]. Therefore, spread of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is of obvious concern [5] and particularly so in communities with large vulnerable population of HIV-infected people.

Understanding community transmission dynamics and spread of emerging CA-MRSA clonal populations is critical

Published online: 14 February 2021

for the control and prevention of MRSA infection in HIV disease, and sequence typing can help shed light on the molecular epidemiology of circulating CA-MRSA population structures [6, 7].

Despite the fact that nasal colonization with *Staphylococcus aureus*/MRSA is a risk factor for invasive infection in HIV-infected individuals, there are few countries in an African setting, Botswana, Ethiopia, Nigeria, and South Africa, which have reported carriage of MRSA among HIV-infected individuals [8–11]. These reports have focused on prevalence and risk factors of *Staphylococcus aureus* and MRSA using either conventional or PCR-based methods for detection and characterization of MRSA isolates. In Tanzania, no study has reported carriage of MRSA among HIV-infected individuals, and there is only one study from the Northern part of Tanzania which has described MRSA clone populations in clinical isolates, using whole genome sequencing technology [12]. Therefore, in Tanzania and many resource-limited countries in Africa, detailed information on the CA-MRSA population structure among HIV-infected individuals is scarce. As a result, measures for prevention and control of MRSA spread are insufficiently implemented in most health care facilities. This study used whole genome sequencing approach to provide data on nasal/nasopharyngeal carriage of MRSA among newly diagnosed HIV-infected adults in a community setting in Tanzania. Additionally, we report sequence types, virulence genes, and phylogenetic analysis of the MRSA isolates.

Methods and materials

Study participants

A total of 537 individuals newly diagnosed with HIV infection were recruited at six sites: Amana, Mwananyamala, Temeke Regional Referral, PASADA, Mbagala, and Mnazi Mmoja hospitals in Dar es Salaam, from April 2017 to May 2018, as part of the randomized clinical trial CoTrimResist (ClinicalTrials.gov identifier: NCT03087890).

Microbiological procedures

Specimen collection and bacterial culture

Nasopharynx was swabbed first and, while retracting the swab anterior nares were swabbed. Nasopharyngeal/anterior nares swabs were collected by clinicians and transported to the microbiology laboratory (Muhimbili bacteriology research laboratory) immediately in a cool box at 4 °C. Swabs were cultured on sheep blood agar for isolation of *S. aureus*. Isolates were identified by coagulase, mannitol fermentation and Staphaurex agglutination tests (Remel, Europe Ltd., Dartford, UK).

Antimicrobial susceptibility testing and screening for MRSA

Antimicrobial susceptibility testing was performed on Muller Hinton agar, and plates were incubated at 35 °C for 16–18 h. Kirby Bauer disk diffusion method was used for susceptibility testing for penicillin, gentamicin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, and clindamycin; cefoxitin was also included for initial screening of MRSA (Oxoid, UK). Antimicrobial susceptibility testing was interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The minimum inhibitory concentrations (MICs) for vancomycin and linezolid were determined by E-test (BioMérieux, Marcy-l'Etoile, France).

Polymerase chain reaction (PCR) testing

For all cefoxitin-resistant *S. aureus* isolates, we did real-time multiplex PCR targeting the *nuc* and *mecA* genes to confirm the bacterial identity and presence of methicillin resistance, respectively. DNA was extracted by a rapid boiling procedure. PCR was performed using 2× QuantiTect Multiplex PCR NoROX Master Mix (Qiagen), and amplification was carried out on a Light Cycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). Primers used and PCR conditions have been described previously [14].

Whole genome sequencing for MRSA

All 22 MRSA isolates underwent whole genome sequencing (WGS). DNA extraction and whole genome sequencing was performed by MicrobesNG (MicrobesNG, Birmingham, UK). For WGS, Illumina HiSeq technology approach was used (2 × 250 bp paired-end reads protocol) (Illumina, San Diego, CA, USA). Trimming and quality filtering of the sequencing reads were assembled using SPAdes and annotated in GenBank.

Assignment of multilocus sequence typing (MLST) was based on sequencing seven housekeeping genes (*pta*, *arcC*, *tpi*, *aroE*, *gmk*, *yqiL*, and *glpF*). Sequence type (ST) and clonal complex were determined by submission of sequence files to an online MLST database website (https://pubmlst.org/).

For identification of acquired antimicrobial resistance genes, virulence gene, SCCmec and *spa*-type, we used ResFinder v3.2, virulenceFinder 2.0, SCCmecFinder 1.2 and spaTyper 1.0 of the Center for Genomic Epidemiology GEE server (http://www.genomicepidemiology.org/). For analysis of inter-strain whole genome single nucleotide polymorphism (SNPs), we used CSI phylogeny 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny/).

We used Figtree (https://github.com/rambaut/figtree/releases) to construct the phylogenetic tree. The whole genome SNPs tree included 22 ST8 from the present study, *S. aureus* USA300_FPR3757 (accession number CP000255),

and 10 already well-characterized ST8 MRSA [15, 16]. This Whole Genome Shotgun project for the present study has been deposited at DDBJ/ENA/GenBank under BioProject number PRJNA649684.

Results

Bacterial isolates

In this study, overall nasal/nasopharyngeal carriage rate of *S. aureus* was 14% (77/537), and 29% of the *S. aureus* isolates ($n = 22$) were resistant to cefoxitin, corresponding to a four percent MRSA carrier rate among all participants. All 22 isolates carried the *mecA* and *nuc* gene, confirming methicillin-resistant *S. aureus*. Of 22 isolates, five were isolated from participants who reported having visited an outpatient clinic during the 1 month prior to specimen collection, and one had history of hospitalization during the month prior to specimen collection. Sixteen participants had not visited any outpatient clinics nor been hospitalized recently.

Antimicrobial susceptibility patterns and genomic resistance traits

All MRSA isolates were susceptible to vancomycin, linezolid, and clindamycin. The overall MRSA antimicrobial susceptibility profiles to other antibiotics are shown in Table 1. Resistance to gentamicin, erythromycin, ciprofloxacin and trimethoprim-sulfamethoxazole and were 95% (21/22), 82% (18/22), 91% (20/22) and 9% (2/22), respectively. Inducible clindamycin resistance was observed in 73% (16/22) of MRSA isolates. Phenotypic susceptibility patterns of all MRSA were highly concordant with genotypic findings (resistome and mutations in genome) (Table 1). All isolates carried *blaZ* gene mediating resistance to penicillin resistance. Resistance to aminoglycosides was mediated by the *aac6'-aph2"* gene. MRSA isolates resistant to erythromycin harbored *ermC* (15/18) and *LmrS* (3/18) resistance genes (Table 1). MRSA resistance to ciprofloxacin was mediated by mutation of the quinolone resistance-determining region (QRDR) sequence in the *gyrA* (S84L) and *parC* (S80Y) genes. All MRSA isolates carried trimethoprim resistance gene *dfrG*, but resistance was not expressed phenotypically (91%, 20/22 susceptible to TMP-SMX). Fosfomycin resistance gene (*FosB*) and tetracycline resistance gene (*tetK*) were detected in 100% (22/22) and 55% (12/22) of MRSA isolates, respectively.

Virulence factors and immune evasion cluster genes

Staphylococcal complement inhibitor (*scn*) and Staphylokinase (*sak*), which are among immune evasion cluster (IEC) genes, were detected in all MRSA isolates (Table 2). None of the isolates carried other IEC genes, but one carried staphylococcal enterotoxin A.

Gamma-hemolysins (*hlgA*, *hlgB*, and *hlgC*) and leucocidin ED genes were detected in all MRSA isolates. Almost all isolates carried staphylococcal enterotoxin genes, with *sej* and *ser* genes being the most predominant. None of the MRSA harbored exfoliative toxin A/B or leucocidin S/F-PV genes. Only one isolates carried toxic shock syndrome toxin 1(*tst*) gene.

Population genetic structure of MRSA strains

All MRSA isolates were ST8 and belonged to the CC8 lineage (Table 1). All MRSA isolates were genotyped by *spa* type, the most predominant (21/22, 95%) was *spa*-type t1476, and one isolate was *spa*-type t064. On the other hand, genotype ST8-SCC*mec*IV, which is a major CA-MRSA worldwide, were detected in MRSA isolates. All but one of the MRSA isolates belonged to SCC*mec*IV (2B&5) subtype, and the remaining isolate had SCC*mec*IVa subtype.

Overall, genotype ST8-SCC*mec*IV-*spa*-t1476 was the predominant, and all isolates were negative for arginine catabolic mobile element (ACME) type 1 and Panton-Valentine leucocidin (PVL).

Genetic relatedness among CC8/ST8 MRSA isolates

We compared all 22 CC8/ST8 MRSA isolates from this study with already known ST8 MRSA (USA300) and one MRSA isolate from Tanzania (NZ_FMMT01000000), one from Gabon [16], and eight from the USA [15]. The percentage of reference genome (USA300_FPR3757 (accession number CP000255) covered by all isolates was 91.1%, the size of the reference genome was 2,872,769, and 2,652,029 positions were found in all analyzed genomes. SNP analysis of 22 ST8 MRSA from the present study revealed SNPs differences from 0 to 988, showing high genetic diversity.

Phylogenetic analysis showed the presence of two distinct clades (Fig. 1). All MRSA isolates with ST8-SCC*mec*IV-*spa*-t1476 from Tanzania formed one clade, had SNPs difference between 1 and 199, and were unrelated to known ST8 clones. Three MRSA isolates had 2 SNPs differences (TZ6, TZ 380, and T207); these were from patients enrolled at different study sites. In one instance, 2 MRSA isolates had 3 SNPs differences (TZ 4 and TZ127). These two instances indicated recent spread of this isolates from a common source.

Two MRSA isolates with pairwise SNP difference of 102 SNPs, both ST8-*spa*-t064 from Tanzania (1 from present study and 1 from previous study), were clustered in another clade with USA300 isolates from the USA and Gabon, Africa. While these two ST8 MRSA isolates from Tanzania possessed SCC*mec*IVa and grouped with ST8 (USA300) from the USA, they were not closely related and had SNPs distances of more than 600 SNPs.

Table 1 Phenotypic and genotypic characterization of MRSA

Isolate number	Antimicrobial agents					Antimicrobial resistant genes						QRDRs †mutation		ST	CC	Spa-type	SCCmec type
	Gen	Ery	Sxt	Cip	Cli	β-lactam	Aminoglycoside	Macrolide	Trimethoprim	Tetracycline	Fosfomysin	GyrA	ParC				
4	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
6	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
32	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
60	R	S	S	S	S	*blaZ, mecA*	*aac6'-aph2"*	–	*dfrG*	*tetK*	FosB			8	CC8	t1476	IV(2B&5)
84	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
127	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
184	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
201	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
207	R	R	R	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
228	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
238	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
240	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
272	R	S	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	–	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
274	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*LmrS*	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
370	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
380	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
497	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
2003	R	S	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	–	*dfrG*	–	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
2010	S	S	R	S	S	*blaZ, mecA*	–	–	*dfrG*	–	FosB			8	CC8	t064	IVa
2028	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*LmrS*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
2079	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*ermC*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)
2130	R	R	S	R	S	*blaZ, mecA*	*aac6'-aph2"*	*LmrS*	*dfrG*	*tetK*	FosB	S84L	S80Y	8	CC8	t1476	IV(2B&5)

Pen penicillin, *Gen* gentamicin, *Ery* erythromycin, *Sxt* trimethoprim-sulfamethoxazole, *Cip* ciprofloxacin, *Cli* clindamycin, *R* resistant, *S* susceptible

†Quinolone resistance-determining regions (QRDR), *ST* sequence type, *CC* clonal complex, *SCCmec* Staphylococcal cassette chromosomes

Table 2 Virulence Genes of MRSA

PID number	Toxin	Exo-enzyme	IEC
4	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
6	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
32	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
60	*lukD, lukE, hlgA, hlgB, hglC*	*aur, splA, splB, slpE*	*sak, scn*
84	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
127	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB*	*sak, scn*
184	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB*	*sak, scn*
201	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
207	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
228	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
238	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB*	*sak, scn*
240	*lukD, lukE, hlgA, hlgB, hglC, sec sej, sel, ser*	*aur, splA, splB*	*sak, scn*
272	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB*	*sak, scn*
274	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
370	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
380	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
497	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
2003	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*
2010	*lukD, lukE, hlgA, hlgB, hglC, sea, seb, sek, seq*	*aur, splA, splB, slpE*	*sak, scn*
2028	*lukD, lukE, hlgA, hlgB, hglC, sej, ser, tst*	*aur, splA, splB, slpE*	*sak, scn*
2079	*lukD, lukE, hlgA, hlgB, hglC, sej, seq, ser*	*aur, splA, splB, slpE*	*sak, scn*
2130	*lukD, lukE, hlgA, hlgB, hglC, sej, ser*	*aur, splA, splB, slpE*	*sak, scn*

IEC Immune evasion cluster, *luk* leucocidin, *hlgA* gamma-hemolysin chain II precursor, *hlgB* gamma-hemolysin component B precursor, *hlgC* gamma-hemolysin component C precursor, *sea* enterotoxin, *sea* enterotoxin A *seb* enterotoxin B, *sec* enterotoxin C, *sej* enterotoxin J, *sek* enterotoxin K, *ser* enterotoxin R, *seq* enterotoxin Q, *aur* aureolysin, *spl* serine protease, *sak* Staphylokinase, *scn* Staphylococcal complement inhibitor, *tst* toxic shock syndrome toxin-1

Discussion

This is the first description of whole genome sequencing data from MRSA isolates from HIV-infected adults in Tanzania and also the first report of nasal/nasopharyngeal MRSA colonization among newly diagnosed HIV-infected adults in Tanzania. Overall, four percent of all participants were nasal/nasopharyngeal MRSA carriers, and all but one belonged to lineage ST8-SCC*mec*IV-t1476. These isolates were collected in the span of 2 years, from six HIV care and treatment centers located in different municipalities in Dar es Salaam. This finding suggests predominance and wide spread of the ST8 CA-MRSA (non-USA300) clone in the HIV-infected population in Dar es Salaam, Tanzania. Diverse lineages of ST8 CA-MRSA dominate different regions in the world. However, the ST8-SCC*mec*IV (USA300) clone has successfully spread in HIV-infected populations in the developed world [17] and is commonly associated with skin and soft tissue infections [18]. No previous study from East Africa has characterized MRSA isolates from HIV-infected population using whole genome sequencing. The only other study in Tanzania, unveiling characteristics of MRSA clinical isolates by whole genome sequencing, found six out of ten MRSA which were ST8 (non-USA), followed by two ST239 and two had unknown sequence type [12]. However, this study did not describe the SCC*mec* and *spa*-type of those isolates.

The ST8-*spa*-t1476 CA-MRSA has rarely been reported outside the African continent, and the types of infection it causes are not known. It has been reported once in the UK in an outbreak of patients with no evidence of links to the African region [19]. The ST8-SCC*mec*V-t1476 clone has been frequently reported among CA-MRSA in the Democratic Republic of Congo (DRC) [20, 21]. Our ST8-SCC*mec*IV MRSA isolates had close resemblance to those circulating in DRC, despite carrying different staphylococcal cassette chromosomes [20, 21]. There is evidence that MSSA acts as reservoir for MRSA before acquisition of staphylococcal cassette chromosomes [22]. Isolates from Tanzania and DRC may have shared common genetic ancestors before each acquiring different SCC*mec*.

On phylogenetic analysis, all ST8-SCC*mec*IV-t1476 from the present study were clustered into one clade and might have shared a common ancestor and were not related to already known ST8 (USA300) clones from the USA and Africa.

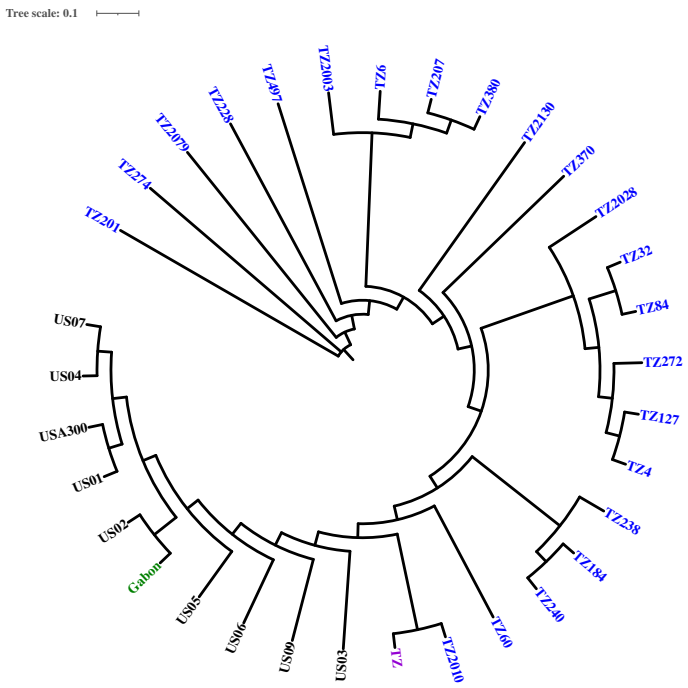


Fig. 1 SNP phylogenetic tree for ST8 MRSA isolates. Blue indicates MRSA isolated from this study, pink indicates MRSA isolated previously from Tanzania, black are MRSA isolated from USA at different point of time, and green is an isolate from Gabon

One isolate from the present study was clustered in a clade with already known ST8 clone, but SNPs analysis showed they were not related. Our findings demonstrate that the epidemiology of the ST8 CA-MRSA clone lineages varies remarkably in different regions of the world. Evolutionary studies have shown that all ST8 clones have a common ancestor but may subsequently acquire certain characteristics like PVL, ACME, and SCC*mec*-type [23]. Repeated introduction of one ST8 CA-MRSA clone in a geographic area may replace existing clones. Our finding and previous data from East and Central Africa confirms predominance of non-USA300 clones [20, 21]. A previous evolutionary study involving ST8 MRSA clones from different continents found that no African ST8 isolates had direct ancestry to the USA300 clades [23]. Further studies are needed to understand the origin and evolution of this clone in our region.

MRSA isolated from newly diagnosed HIV-infected patients were also resistant to gentamicin, erythromycin, and ciprofloxacin but showed low rate of resistance to trimethoprim-sulfamethoxazole phenotypically. However, the presence of the resistance gene *dfrG* in all isolates phenotypically susceptible to trimethoprim-sulfamethoxazole in our study, suggests that this antibiotic could be suboptimal for treatment of MRSA infections. In case it is used, careful clinical monitoring is needed to avoid treatment failure, since in vitro susceptibility might not be matched by susceptibility in vivo.

The present study demonstrates that vancomycin and linezolid could be used for treatment of MRSA infections; however, these drugs are not widely available in East Africa. Macrolides and lincosamides are commonly used in treatment of staphylococcal skin and soft tissue infections. MRSA resistant to macrolides, lincosamides, and streptogramin type B are mainly mediated by *erm*A and *erm*C, which codes for erythromycin ribosomal methylase [24]. We observed predominance of *erm*C from our MRSA isolates (83%), and no isolates carried *erm*A. Previous studies in Tanzania and the DRC reported similar predominance of *erm*C among ST8 CA-MRSA [12, 25]. The observation of 73% inducible clindamycin resistance among MRSA implies that clindamycin may not be a reliable treatment option for infections caused by MRSA. In such cases, treatment with clindamycin may induce clinical resistance in apparently susceptible isolates harboring *erm*C genes [26], leading to clinical treatment failure.

Gentamicin resistance in MRSA was mediated by *aac6'-aph2"* and ciprofloxacin resistance by mutations in the QRDR sequence of the *gyrA* and *parC* genes. This finding is similar to other studies among ST8 CA-MRSA from East Africa and Europe [12, 27, 28]. Although fosfomycin, an older broad spectrum antibiotic [29], has not been commonly used in Tanzania, we still found 100% of MRSA carrying genes conferring resistance to fosfomycin. The high prevalence of FosB

in the CA-MRSA isolates is surprising considering the absence of selective pressure by actual use of fosfomycin. This finding could be accounted for by high consumption of other antibiotics including tetracycline which may select for fosfomycin resistance or by the emergence of spontaneous mutation [30, 31].

The ability of MRSA to cause invasive disease depends on virulence factors. Remarkably, the PVL, which is usually present in ST8 CA-MRSA, was lacking from the MRSA isolates in our study. However, several virulence factors were identified including immune evasion cluster, gamma-hemolysins (*hlgA*, *hlgB*, *hlgC*), enterotoxin, and leucocidin ED. Recently, an animal model has shown that leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo [32]. The immune evasion gene cluster enhances capacity of *S. aureus* to colonize, disseminate and persist in a human host [33]. The present study demonstrates that MRSA colonizing the nose/nasopharynx of HIV-infected individuals may have a significant potential to cause invasive disease due to the variety of virulence factors observed.

One caveat of our study is that, although only one of our participants had a history of recent hospitalization and five had visited outpatient clinics, we cannot rule out that MRSA in these patients could have been hospital acquired. However, the fact that all MRSA had the same SCCmecIV type which is commonly found in community-acquired strains supports the notion that the MRSA isolates were community acquired. The anterior nares are a common place of staphylococcal colonization, and swabbing this site is appropriate to detect the bacteria. We swabbed the nasopharynx first, and the anterior nares were swabbed afterwards while retracting the swab. This procedure may have led to falsely low prevalence of *S. aureus*, which could be a limitation of the study.

Conclusion

ST8 CA-MRSA (non-USA300) was found to be the commonest circulating population structure in newly diagnosed HIV-infected adults in Tanzania. The circulating ST8 CA-MRSA isolates were not related to other common, successful circulating ST8 lineages. The *spa*-type t1476 is predominant in this CA-MRSA. The majority of CA-MRSA was highly resistant to non-beta lactam antibiotics. Screening for colonization of MRSA and intervention in HIV-infected outpatients and inpatients may control the spread of the strain.

Acknowledgments The authors acknowledge members of the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Science, Dar es Salaam, Tanzania, for their support during data collection and initial laboratory procedures.

Authors' contributions BB, NL, and SM conceived the study. All authors contributed to designing the study. JM collected study data. JM and SM performed the microbiological investigations. BB and JM performed statistical analysis. JM drafted the manuscript. SA, BB, SM, and NL revised the manuscript. All authors approved the final version.

Funding This study was funded by the Helse Bergen HF, Haukeland University Hospital, Norway, through project number 912132 and by the National Advisory Unit on Tropical Infectious Diseases, Haukeland University Hospital. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability Data are available on request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval Approvals to conduct this study in Tanzania were obtained from Muhimbili University of Heath and Allied Sciences Senate Research and Publications Committee (Ref. No. 2015-10-27/AEC/Vol.X/54), National Health Research Ethics Committee (Ref. No. NIMRlHQ/R. SaJVol. 1X12144), Tanzania Medicines and Medical Devices Authority (Ref. No. TZ16CT007), and Regional Committee for Medical and Health Research Ethics of Western Norway (Ref. No. REK2015/540).

Informed consent Written informed consent was obtained from each study participant prior to the enrolment in the study.

Consent to publish Consent to publish was obtained from National Health Research Ethics Committee (Ref. No. NIMRlHQ/R. SaJVol. 1X12144).

References

1. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y (2003) Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis 36(1):53–59
2. Stenehjem E, Rimland D (2013) MRSA nasal colonization burden and risk of MRSA infection. Am J Infect Control 41(5):405–410
3. Szumowski JD, Wener KM, Gold HS, Wong M, Venkataraman L, Runde CA, Cohen DE, Mayer KH, Wright SB (2009) Methicillin-resistant *Staphylococcus aureus* colonization, behavioral risk factors, and skin and soft-tissue infection at an ambulatory clinic serving a large population of HIV-infected men who have sex with men. Clin Infect Dis 49(1):118–121

4. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP (2000) Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. Clin Infect Dis 31(1):170–176
5. Naimi TS, LeDell KH, Boxrud DJ, Groom AV, Steward CD, Johnson SK, Besser JM, O'Boyle C, Danila RN, Cheek JE, Osterholm MT, Moore KA, Smith KE (2001) Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996-1998. Clin Infect Dis 33(7):990–996
6. Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD (2010) Evolution of MRSA during hospital transmission and intercontinental spread. Science 327(5964):469–474
7. McAdam PR, Templeton KE, Edwards GF, Holden MT, Feil EJ, Aanensen DM, Bargawi HJ, Spratt BG, Bentley SD, Parkhill J, Enright MC, Holmes A, Girvan EK, Godfrey PA, Feldgarden M, Kearns AM, Rambaut A, Robinson DA, Fitzgerald JR (2012) Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci U S A 109(23):9107–9112
8. Ouko TT, Ngeranwa JN, Orinda GO, Bii CC, Amukoye E, Lucy M, Wamae CN (2010) Oxacillin resistant *Staphylococcus aureus* among HIV infected and non-infected Kenyan patients. East Afr Med J 87(5):179–186
9. Olalekan AO, Schaumburg F, Nurjadi D, Dike AE, Ojurongbe O, Kolawole DO, Kun JF, Zanger P (2012) Clonal expansion accounts for an excess of antimicrobial resistance in *Staphylococcus aureus* colonising HIV-positive individuals in Lagos, Nigeria. Int J Antimicrob Agents 40(3):268–272
10. Lemma MT, Zenebe Y, Tulu B, Mekonnen D, Mekonnen Z (2015) Methicillin resistant *Staphylococcus aureus* among HIV infected pediatric patients in Northwest Ethiopia: carriage rates and antibiotic co-resistance profiles. PLoS One 10(9):e0137254
11. Reid MJA, Steenhoff AP, Mannathoko N, Muthoga C, McHugh E, Brown EL, Fischer RSB (2017) *Staphylococcus aureus* nasal colonization among HIV-infected adults in Botswana: prevalence and risk factors. AIDS Care 29(8):961–965
12. Kumburu HH, Sonda T, Leekitcharoenphon P, van Zwetselaar M, Lukjancenko O, Alifrangis M, Lund O, Mmbaga BT, Kibiki G, Aarestrup FM (2018) Hospital epidemiology of methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital in Moshi, Tanzania, as determined by whole genome sequencing. Biomed Res Int 2018:2087693
13. Clinical and Laboratory Standards Institute (2018) Performance standards for antimicrobial susceptibility testing; twenty-eighth informational supplement CLSI document M100–S20. Clinical and Laboratory Standards Institute, Wayne, PA
14. Moyo SJ, Aboud S, Blomberg B, Mkopi N, Kasubi M, Manji K, Lyamuya EF, Maselle SY, Langeland N (2014) High nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthy Tanzanian under-5 children. Microb Drug Resist 20(1):82–88
15. Uhlemann AC, Dordel J, Knox JR, Raven KE, Parkhill J, Holden MT, Peacock SJ, Lowy FD (2014) Molecular tracing of the emergence, diversification, and transmission of *S. aureus* sequence type 8 in a New York community. Proc Natl Acad Sci U S A 111(18): 6738–6743
16. Schaumburg F, Ngoa UA, Kösters K, Köck R, Adegnika AA, Kremsner PG, Lell B, Peters G, Mellmann A, Becker K (2011) Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. Clin Microbiol Infect 17(10): 1507–1513
17. Popovich KJ, Hota B, Aroutcheva A, Kurien L, Patel J, Lyles-Banks R, Grasso AE, Spec A, Beavis KG, Hayden MK, Weinstein RA (2013) Community-associated methicillin-resistant *Staphylococcus aureus* colonization burden in HIV-infected patients. Clin Infect Dis 56(8):1067–1074
18. Srinivasan A, Seifried S, Zhu L, Bitar W, Srivastava DK, Shenep JL, Bankowski MJ, Flynn PM, Hayden RT (2009) Short communication: methicillin-resistant *Staphylococcus aureus* infections in children and young adults infected with HIV. AIDS Res Hum Retrovir 25(12):1219–1224
19. Utsi L, Pichon B, Arunachalam N, Kerrane A, Batten E, Denton M, Townsend R, Agwuh KN, Hughes GJ, Kearns A (2019) Circulation of a community healthcare-associated multiply-resistant meticillin-resistant *Staphylococcus aureus* lineage in South Yorkshire identified by whole genome sequencing. J Hosp Infect 103(4):454–460
20. De Boeck H, Vandendriessche S, Hallin M, Batoko B, Alworonga JP, Mapendo B, Van Geet C, Dauly N, Denis O, Jacobs J (2015) *Staphylococcus aureus* nasal carriage among healthcare workers in Kisangani, the Democratic Republic of the Congo. Eur J Clin Microbiol Infect Dis 34(8):1567–1572
21. Vandendriessche S, De Boeck H, Deplano A, Phoba MF, Lunguya O, Falay D, Dauly N, Verhaegen J, Denis O, Jacobs J (2017) Characterisation of *Staphylococcus aureus* isolates from bloodstream infections, Democratic Republic of the Congo. Eur J Clin Microbiol Infect Dis 36(7):1163–1171
22. Driebe EM, Sahl JW, Roe C, Bowers JR, Schupp JM, Gillece JD, Kelley E, Price LB, Pearson TR, Hepp CM, Brzoska PM, Cummings CA, Furtado MR, Andersen PS, Stegger M, Engelthaler DM, Keim PS (2015) Using whole genome analysis to examine recombination across diverse sequence types of *Staphylococcus aureus*. PLoS One 10(7):e0130955
23. Strauss L, Stegger M, Akpaka PE, Alabi A, Breurec S, Coombs G, Egyir B, Larsen AR, Laurent F, Monecke S, Peters G, Skov R, Strommenger B, Vandenesch F, Schaumburg F, Mellmann A (2017) Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8. Proc Natl Acad Sci U S A 114(49):E10596–e10604
24. Khodabandeh M, Mohammadi M, Abdolsalehi MR, Alvandimanesh A, Gholami M, Bibalan MH, Pournajaf A, Kafshgari R, Rajabnia R (2019) Analysis of resistance to macrolide-Lincosamide-Streptogramin B among mecA-positive *Staphylococcus aureus* isolates. Osong Public Health Res Perspect 10(1):25–31
25. Phaku P, Lebughe M, Strauss L, Peters G, Herrmann M, Mumba D, Mellmann A, Muyembe-Tamfum JJ, Schaumburg F (2016) Unveiling the molecular basis of antimicrobial resistance in *Staphylococcus aureus* from the Democratic Republic of the Congo using whole genome sequencing. Clin Microbiol Infect 22 (7):644.e641-645
26. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, McGowan JE Jr, Tenover FC (2005) Testing for induction of clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. J Clin Microbiol 43(4):1716–1721
27. Kyany'a C, Nyasinga J, Matano D, Oundo V, Wacira S, Sang W, Musila L (2019) Phenotypic and genotypic characterization of clinical *Staphylococcus aureus* isolates from Kenya. BMC Microbiol 19(1):245
28. Enström J, Fröding I, Giske CG, Ininbergs K, Bai X, Sandh G, Tollström UB, Ullberg M, Fang H (2018) USA300 methicillin-resistant *Staphylococcus aureus* in Stockholm, Sweden, from 2008 to 2016. PLoS One 13(11):e0205761
29. Raz R (2012) Fosfomycin: an old–new antibiotic. Clin Microbiol Infect 18(1):4–7
30. McCaughey G, Diamond P, Elborn JS, McKevitt M, Tunney MM (2013) Resistance development of cystic fibrosis respiratory pathogens when exposed to fosfomycin and tobramycin alone and in combination under aerobic and anaerobic conditions. PLoS One 8(7):e69763

31. Sahuquillo Arce JM, Colombo Gainza E, Gil Brusola A, Ortiz Estévez R, Cantón E, Gobernado M (2006) In vitro activity of linezolid in combination with doxycycline, fosfomycin, levofloxacin, rifampicin and vancomycin against methicillin-susceptible *Staphylococcus aureus*. Rev Esp Quimioter 19(3): 252–257
32. Alonzo F 3rd, Benson MA, Chen J, Novick RP, Shopsin B, Torres VJ (2012) *Staphylococcus aureus* leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo. Mol Microbiol 83(2):423–435
33. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA (2006) The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J Bacteriol 188(4):1310–1315

Paper II

MICROBIAL DRUG RESISTANCE
Volume 26, Number 12, 2020
Mary Ann Liebert, Inc.
DOI: 10.1089/mdr.2020.0066

High Prevalence of Fecal Carriage of Extended Spectrum β-Lactamase-Producing Enterobacteriaceae Among Newly HIV-Diagnosed Adults in a Community Setting in Tanzania

Joel Manyahi,[1–3] Sabrina John Moyo,[1,3] Marit Gjerde Tellevik,[2] Nina Langeland,[1,2] and Bjørn Blomberg[1,2]

Colonization in HIV-infected populations with extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) is particularly worrisome in low-income settings. This study describes the prevalence of ESBL-PE carriage and associated risk factors among newly HIV-diagnosed adults in a community setting in Tanzania. A total of 595 newly diagnosed HIV-positive adults with a median age of 35 years with interquartile range (IQR) 29–42 years and a median CD4 count of 492 cells/μL (IQR 390–666 cells/μL) were recruited. Among these, 194/595 (32.6%, 95% confidence interval [CI] 28.9–36.6) were ESBL-PE carriers. Participants with low CD4 count (<350 cells/μL) had significantly higher prevalence of ESBL-PE carriage compared with those with CD4 count ≥350 cells/μL (26/58, 44.8%, vs. 168/537, 31.3%, $p = 0.04$). Antibiotic use in last 4 weeks (odds ratio [OR] 1.55, 95% CI 1.08–2.22, $p = 0.02$) and CD4 count <350 cells/μL (OR 1.78, 95% CI 1.03–3.09, $p = 0.04$) were independent risk factors for fecal carriage of ESBL-PE. In total, 244 isolates of ESBL-PE were isolated from 194 participants. Of these, 238/244 (97.5%) harbored $bla_{\text{CTX-M}}$ genes, with $bla_{\text{CTX-M-15}}$ being predominant (219/238 (92%), followed by $bla_{\text{CTX-M-27}}$ (9/238 (3.8%), $bla_{\text{CTX-M-14}}$ (8/238 (3.4%), $bla_{\text{CTX-M-55}}$ (1/238), and $bla_{\text{CTX-M 211/3}}$ (1/238). $bla_{\text{SHV-2a}}$ genes were detected in four isolates, whereas the $bla_{\text{SHV-12}}$ gene was detected in one isolate. Phenotypic carbapenemase-producing Enterobacteriaceae was detected in one HIV-positive person with CD4 count 132 cells/μL. In conclusion prevalence of ESBL-PE carriage is high among newly diagnosed HIV adults in Dar es Salaam, and is significantly associated antibiotic use and low CD4 count.

Keywords: ESBL, HIV, community, Tanzania

Introduction

Individuals living with HIV are at risk not only of classical HIV-related opportunistic infections such as pneumocystis pneumonia and tuberculosis, but also severe infections caused by common bacterial pathogens such as *Escherichia coli*, salmonella, pneumococci, and staphylococci.[1–3] The World Health Organization (WHO) recognizes antimicrobial resistance (AMR) in such bacterial infections as a major threat to global health. Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) constitute a particular challenge, as almost all are multidrug resistant. Previously, infections due to ESBL producers were mainly a health care-associated problem.[4,5] However, ESBL-PE infections are now increasingly acquired in the community.[6,7]

In resource-constrained settings, ESBL-PE infections are spread more easily due to deficient infection control, whereas expensive reserve antibiotics such as carbapenems and colistin are inaccessible. Consequently, infections caused by ESBL-PE carries high mortality exceeding mortality outcome of bacterial infections in the preantibiotic era.[5,8]

Colonization with ESBL-PE frequently precedes invasive diseases.[9,10] Fecal carriage of ESBL-PE could pose greater risk in people living with HIV due to the increased risk of severe bacterial infections.[1,2] In Tanzania, studies have documented high frequency of infections caused by ESBL-PE

[1]Department of Clinical Science, University of Bergen, Bergen, Norway.
[2]National Advisory Unit on Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway.
[3]Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania.

in hospitalized patients.[5,11–13] ESBL-PE carriage is also common in hospital settings, and carriage rates of 50% have been documented in hospitalized children in Tanzania.[14] Even in community settings, studies have documented ESBL-PE carriage rates of 11–32% in healthy children[15] and 17% in healthy adults[16] in the same country. Although high rates (89.7%) of ESBL-PE carriage have been reported in hospitalized HIV positive children in Tanzania,[14] others documented lower rates (14%) in HIV-infected children in the community in Zimbabwe.[17] To date, no community-based studies from Africa have reported frequencies and risk factors for ESBL-PE carriage among newly diagnosed HIV adults.

HIV is pandemic in sub-Saharan Africa, and Tanzania has an estimated 1.4 million people aged between 15 and 64 years living with HIV.[18] We designed this study aimed to determine prevalence of fecal carriage of ESBL producers in HIV-infected people in the community; and assess any influence of antibiotic use, hospitalization, and CD4 count on fecal carriage of ESBL producers. The findings may help develop strategies for reducing the burden of AMR in a population at high risk of severe infections.

Materials and Methods

Study sites and study participants

Newly diagnosed HIV-infected adults with CD4 count of ≥350 cells/μL who had not yet started antiretroviral treatment (ART) were recruited consecutively as part of a double-blinded randomized clinical trial (RCT), CoTrimResist (ClinicalTrials.gov identifier: NCT03087890) between April 2017 and May 2018. The study was performed in Dar es Salaam, Tanzania, the largest city and economic capital of Tanzania. Participants were residents of the main municipals of Dar es Salaam city, namely Kinondoni, Temeke, Ilala, Ubungo, and Kigamboni, and were recruited through six HIV care and treatment clinics at Mwananyamala Hospital, Amana Hospital, Temeke Hospital, Pastoral Activities and Services for People with AIDS Dar es Salaam Archdiocese (PASADA), Mbagala Hospital, and Mnazimmoja Hospital. In addition, newly diagnosed HIV-infected adults with CD4 counts of <350 cells/μL ($n = 58$) were recruited from the same Hospitals during screening for eligibility to the RCT. This article reports on the baseline data before interventions and treatment.

Data collection

Health workers recorded participants' data such as demographics and clinical parameters on tablet computers using standardized electronic case report forms employing the RedCAP system (Research Electronic Data Capture, Vanderbilt University, Nashville, TN), which automatically synchronizes study data to a central data server.

Screening for ESBL- and carbapenemase-producing Enterobacteriaceae

From each participant, a rectal swab was collected and transported in liquid Cary-Blair medium (Fecal Transwab, MWE Co Bath Ltd., Corsham, United Kingdom) in a cool box and maintained at 4°C with icepack. Rectal swabs were inoculated into brain heart infusion (BHI) broth and incubated overnight at 37°C. Two drops (0.1 mL) from BHI broth were subcultured on CHROMID ESBL (BioMérieux, Marcy l'Etoile, France) for screening of ESBL-PE,[19] and CHROMID CARBA SMART (BioMérieux) for combined screening for carbapenemase-producing Enterobacteriaceae (CPE) and OXA-48-producing Enterobacteriaceae. The plates were incubated at 37°C for 24 hours and bacterial growth interpreted according to manufacturer's instructions. Molecular characterized strains harboring different combinations of β-lactamases were used for quality control as previously described.[14]

Bacterial isolates were identified by MALDI-TOF MS using the Microflex LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany).

PCR and sequencing for detection and identification of ESBL genes

DNA was extracted by a rapid boiling procedure and stored at −20°C. Real-time PCR was used for detection of cefotaxime-Munich (CTX-M) encoding genes using a LightCycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). Forward primer CTXM-F 5′-ATGTGCAGYACCAGTAAR GT-3′, and reverse primers CTXM-R1 5′-TGGGTGAAGTAA GTGACCAGA-3′ and CTXM-R2 5′-TGGGTAAARTAGG TCACCAGA-3′ (TIB Molbiol, Berlin, Germany), which target a 595 bp internal region present in all the five different CTX-M groups, were used as previously described.[14] Each run included positive and negative controls in duplicates. The PCR products were sequenced using the same reverse primers as for PCR. Sequencing was done using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequences were analyzed using the Basic Local Alignment Search Tool program available at the website of the National Center for Biotechnology Information.

For confirmation of ESBL producers, isolates negative for CTX-M PCR were tested for the presence of bla_{SHV} genes as previously described by Kommedal *et al.*[20]

Statistical analysis

We used chi-square test to assess the proportions of fecal carriage of ESBL producers by patient characteristics. We analyzed the association between risk factors of interest (antibiotic use, prior hospitalization, and low CD4 counts) and fecal carriage of ESBL producers, adjusting for age, gender, education status, district of residence, and study sites (individual study sites). For each main exposure variable, we assessed all potential confounders using both logistic regression and Mantel–Haenszel method, defining a confounder as a factor that changes the effect size by ≥10%. Effect modification of factor between different groups were examined by Mantel–Haenszel method. A significance level of 0.05 was used and all p-values refer to two-sided tests. Statistical analysis was performed using STATA version 16 (College Station, TX).

Ethical approval and consent to participate

Ethical approval to conduct the study in Tanzania was obtained from Muhimbili University of Heath and Allied Sciences senate research and publication committee (Ref. No. 2015-10-27/Vol.X/54)—Muhimbili University of Health and Allied Sciences, National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2144)—Tanzania, Ministry of Health,

Community development, Gender, Elderly, and Children. The clinical trial was also registered by Tanzania Food and Drugs Authority (TZ16CT007) and Clinical trial.org (NCT03087890). In Norway, the study was approved by the Regional Committee for Medical and Health Research Ethics of Western Norway (REK2015/540). Informed written consent was obtained from each of the study participants.

Results

Description of study participants

We recruited a total of 595 newly HIV-diagnosed adults with a median age of 35 years with interquartile range (IQR) 29–42 years and a median CD4 count of 492 cells/μL with IQR 390–666 cells/μL. Among these, 537 (90.2%) were newly diagnosed HIV seropositive individuals with CD4 counts ≥350 cells/μL (median 518 IQR 413–687) and 58 (9.8%) were newly diagnosed HIV seropositive individuals with CD4 counts of <350 cells/μL (median 159 IQR 61–259). Out of 595, 451 (75.8%) were female and 233 (39.2%) were aged between 30 and 39 years. The majority, 360/595 (60.5%), of the HIV seropositive individuals had WHO stage 1 HIV disease. Although 64/595 (10.8%) of participants had been admitted to hospital within the last year, 191/595 (31.7%) reported use of antibiotics during the last 4 weeks before enrollment (Table 1).

Table 1. (Clinical Demographics) Characteristics of the Study Participants

Variable	*Frequency*	*Percentage*
Gender		
Female	451	75.8
Male	144	24.2
Age (years)		
Up to 29	165	27.7
30–39	233	39.2
40–49	148	24.9
50–59	33	5.5
60 and above	16	2.7
Education		
Primary school	388	65.2
Secondary	126	21.2
Postsecondary	24	4.0
None/informal	57	9.6
District of residence		
Temeke	289	48.6
Kinondoni	114	19.1
Ubungo	32	5.4
Ilala	139	23.4
Kigamboni	12	2.0
Others	9	1.5
HIV stage		
1	360	60.5
2	173	29.1
3	59	9.9
4	3	0.5
CD4 counts (cells/μL)		
≥350	537	90.2
<350	58	9.8
Study site		
PASADA	168	28.2
Temeke	54	9.1
Amana	30	5.0
Mwananyamala	100	16.8
Mbagala	124	20.9
Mnazimmoja	119	20.0
Hospital admission within last year		
No	531	89.2
Yes	64	10.8
Antibiotic use last 4 weeks		
No	404	67.9
Yes	191	32.1

PASADA, Pastoral Activities and Services for People with AIDS Dar es Salaam Archdiocese.

Prevalence of ESBL carriage and identification of the bacterial isolates

The overall prevalence of fecal carriage of ESBL-PE was 194/595 (32.6%, 95% confidence interval [CI] 28.9–36.6), and the proportions of carriage in subgroups are shown in Table 2. The prevalence of fecal carriage of ESBL-PE varied between study sites (Table 2). Participants with low CD4 counts (<350 cells/μL) had significantly higher prevalence of ESBL-PE carriage than those with higher CD4 counts (44.8%, 26/58 vs. 31.3%, 168/537, $p=0.04$).

A total of 244 isolates of Enterobacteriaceae were isolated from 194 participants harboring ESBL-PE phenotypes. The majority of participants, 74.2%, were colonized by a single ESBL-PE isolate, whereas 50/194 (25.8%) had two or more bacterial isolates. *E. coli* was the predominant microbe, 209/244 (85.7%), followed by *Klebsiella pneumoniae*, 33/244 (13.5%), and *Enterobacter cloacae*, 2/244 (0.8%). Patients with CD4 counts <350 cells/μL were more likely to carry multiple isolates of ESBL-PE than those with higher CD4 counts (15.5%, 9/58 vs. 6.9%, 37/537, $p=0.02$). One *E. coli* isolate was CPE and isolated from an HIV participant with CD4 count 132 cells/μL.

ESBL genotypes

PCR confirmed ESBL genotype for 242/244 (99.2%) of isolates with phenotypic ESBL-PE. The majority, 238 (97.5%), harbored $bla_{\text{CTX-M}}$ encoding genes. The most predominant $bla_{\text{CTX-M}}$ gene was $bla_{\text{CTX-M-15}}$ (219/238, 92%), followed by $bla_{\text{CTX-M-27}}$ 9/238 (3.8%), $bla_{\text{CTX-M-14}}$ 8/238 (3.4%), $bla_{\text{CTX-M-55}}$ (1/238), and $bla_{\text{CTX-M 211/3}}$ (1/238). All *K. pneumoniae* and *E. cloacae* isolates carried the $bla_{\text{CTX-M-15}}$ gene only, whereas *E. coli* displayed a diversity of $bla_{\text{CTX-M}}$ genes, including the ones mentioned earlier. $Bla_{\text{SHV-2a}}$ genes were detected in two *E. coli* and two *K. pneumonia*, whereas the $bla_{\text{SHV-12}}$ gene was detected in one *E. coli*.

Predictors of ESBL producers

Among the main risk factors of interest, only antibiotic use during the last 4 weeks (odds ratio [OR] 1.55, 95% CI 1.08–2.22, $p=0.02$) and CD4 count <350 cells/μL (OR 1.78, 95% CI 1.03–3.09, $p=0.04$) were independently associated with fecal carriage of ESBL producers (Table 3). Prior hospitalization during the last 1 year was not a significant risk factor (OR 0.79, 95% CI 0.44–1.40, $p=0.41$). The effect size of each risk factors did not change significantly

Table 2. Prevalence of Extended-Spectrum β-Lactamase-Producing Bacteria by Clinical-Demographic Characteristics

Variable	Frequency	Prevalence of ESBL, % (n)	Prevalence of ESBL, % (n)
Gender			
Female	451	32.6 (147)	
Male	144	32.6 (47)	1.000
Age (years)			
Up to 29	165	35.8 (59)	
30–39	233	30.0 (70)	
40–49	148	32.8 (50)	
50–59	33	18.2 (6)	
60 and above	16	56.3 (9)	0.068
Education			
Primary school	388	30.2 (117)	
Secondary	126	31.7 (40)	
Postsecondary	24	50.0 (12)	
None/informal	57	43.9 (25)	0.053
District of residence			
Temeke	289	29.4 (85)	
Kinondoni	114	38.6 (44)	
Ubungo	32	34.4 (11)	
Ilala	139	36.7 (51)	
Kigamboni	12	0.0 (0)	
Others	9	33.3 (3)	0.072
HIV stage			
1	360	31.9 (115)	
2	173	31.8 (55)	
3	59	40.7 (24)	
4	3	0.0 (0)	0.344
CD4 counts (cells/μL)			
≥350	537	31.3 (168)	
<350	58	44.8 (26)	0.037*
Study site			
PASADA	168	22.0 (37)	
Temeke	54	20.4 (11)	
Amana	30	33.3 (10)	
Mwananyamala	100	40.0 (40)	
Mbagala	124	39.5 (49)	
Mnazimmoja	119	39.5 (47)	0.001*
Hospital admission within last year			
No	531	33.1 (176)	
Yes	64	28.1 (18)	0.418
Antibiotic use last 4 weeks			
No	404	29.5 (119)	
Yes	191	39.3 (75)	0.017*

*p-Values calculated by chi-square test with 0.05 as cutoff for statistical significance.
ESBL, extended-spectrum β-lactamase.

Table 3. Risk Factors for Fecal Carriage of Extended-Spectrum β-Lactamase Producers in HIV Patients

Variable	Frequency	ESBL carriage, % (n)	OR	95% CI	p
CD4 counts (cells/μL)*					
≥350	537	31.3 (168)	1		
<350	58	44.8 (26)	1.78	1.03–3.09	0.04
Antibiotic use last 4 weeks†					
No	404	29.5 (119)			
Yes	191	39.3 (75)	1.55	1.08–2.22	0.02
Hospital admission within last year					
No	531	33.1 (176)	1		
Yes	64	28.1 (18)	0.79	0.44–1.40	0.41

*Adjusted for antibiotic use in last 4 weeks, study sites, hospital admission with last year, age, gender, and education status.
†Adjusting for age, gender, education status, hospital admission within last year, CD4 count and study sites.
CI, confidence interval; OR, odds ratio.

(>10%), whereas adjusting for the other main risk factors and potential confounders and effect modifiers, that is, age, gender, education status, district of residence, and study sites (individual study site). Thus, we did not find any significant confounders or effect modifiers.

Discussion

This first community-based study reports prevalence of fecal carriage of ESBL-PE in almost one third (194/595, 32.6%) of individuals newly diagnosed with HIV in Dar es Salaam. This rate of ESBL-PE fecal carriage is much higher than found among adults with unknown HIV status in community-based studies in Northern Tanzania (55/334, 16.5%, $p<0.001$),[16] Burkina Faso (22/101, 21.8%, $p=0.03$),[21] Gambia (28/565, 5.0%, $p<0.001$),[22] and Morocco (4/93, 4.3%, $p<0.001$).[23] Although we are not aware of other community-based studies from HIV-infected adults in Africa, there was significantly lower ESBL-PE carriage rate (24/175 13.7%, $p<0.001$) in a community-based studies of HIV-infected children in Zimbabwe.[17] The high carriage rate observed in our study could be attributed to several factors, including the choice of screening technique employed. Our use of broth enrichment of rectal swabs may have increased the yield in detecting ESBL-PE colonization.[24,25] In contrast, our use of rectal swabs may have inferior sensitivity compared with stool culture used in other studies.[19]

We observed that antibiotic use during the last 4 weeks was a strong risk factor for fecal carriage of ESBL producers in this cohort. In Tanzania, almost all antibiotics can be accessed over the counter. Overuse of antibiotics and misuse of broad-spectrum antibiotics, such as cephalosporins for trivial infections, are known to contribute to increasing AMR, including ESBL-PE. In this setting, people with an undiagnosed HIV infection are likely to opt for self-medication with antibiotics for intercurrent infections and symptoms related to an, as yet, unrecognized immunodeficiency. Such overuse of antibiotics may partially explain the high prevalence of ESBL-PE carriage in our study. This study confirms our hypothesis that antibiotic use affects carriage of resistant bacteria in HIV populations, and our finding is consistent with earlier observations from community and hospital settings in Africa.[14,16,21,22]

Previous hospitalization is a well-known risk factor for fecal carriage of ESBL producers,[12,21] contributing both to increased likelihood of antibiotics uses and acquisition of ESBL producers in gastrointestinal tract. However, this study could not add support this observation. Tanzania's current implementation of the ''test and treat'' strategy for HIV is expected to result in more people getting diagnosed before actually experiencing symptomatic disease. Despite this, it is

plausible that many patients will have consulted health care services, without actually having been admitted, for unknown HIV-related ailments before getting diagnosed with HIV, and that this may have led to increased risks of gastrointestinal colonization with ESBL-PE.

This study found low CD4 count (<350 cells/μL) was an independent risk factor for fecal carriage of ESBL. Low immunity predisposes to risk of opportunistic infections increasing the likelihood of antimicrobial use and hospitalization. However, adjustment for both antibiotic use and hospitalization did not alter the independent association between low CD count and fecal carriage of ESBL producers. Furthermore, Wilmore et al.[17] found no association between low CD count and fecal carriage of ESBL producers in children in Zimbabwe.[17] The differences could be explained by inclusion criteria, this study recruited newly diagnosed HIV-infected adults not yet on ART, whereas Wilmore *et al.* enrolled children who were stable on ART.[17]

Our study confirmed $bla_{\text{CTX-M 15}}$ as the predominant ESBL gene type accounting 92% of all isolates, supporting other reports on the extraordinary dissemination of the $bla_{\text{CTX-M-15}}$ genotype worldwide. In the first ever report of ESBL-PE from Tanzania,[5,13] we showed that $bla_{\text{CTX-M-15}}$, alongside TEM-63, was the dominant cause of ESBL-PE bloodstream infections in Tanzania as early as 2001–2002. Later on, we found predominance of $bla_{\text{CTX-M-15}}$ in clinical isolates from community-acquired urinary tract infections from 2004 (Ref.[7]). Reports from elsewhere[26,27] indicate that when $bla_{\text{CTX-M-15}}$ genotype penetrates in a new landscape where other ESBL genes persist, $bla_{\text{CTX-M-15}}$ tend to displace other genes and becomes predominant. All recent studies from community and hospital settings in Tanzania have supported the predominance of $bla_{\text{CTX-M 15}}$ genes among Enterobacteriaceae isolated from feces.[12,15,16] The CTX-M group 9, $bla_{\text{CTX-M-27}}$, and $bla_{\text{CTX-14}}$ were also documented in this study and were only isolated from *E. coli*. This correlates with a recent study among HIV-infected children in Zimbabwe.[17] Our previous study on uropathogenic isolates did not detect any $bla_{\text{CTX-M}}$ group 9 genes,[7] whereas a pediatric study reported only two *E. coli* isolates carrying $bla_{\text{CTX-M-14}}$ genes and none with $bla_{\text{CTX-M-27}}$.[14] In conclusion, the study found a high prevalence of fecal ESBL-PE carriage among people newly diagnosed with HIV. Antibiotic use during the last 4 weeks and low CD4 count were risk factors for fecal carriage of ESBL producers. Further studies should investigate transmission dynamics of ESBL-PE in the community, complex factors driving the emergence of multidrug-resistant bacteria, and the relationship between ESBL-PE carriage and invasive disease. Social behavior and policies on AMR need to be crafted and regulation on antimicrobial use must be enforced.

Acknowledgments

We thank the Helse Bergen HF, Haukeland University Hospital, Norway, through project number 912132, which funded the study (funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article). We also thank members of the Department of Clinical Science, University of Bergen, Norway, for their technical support during the molecular study.

Disclosure Statement

All authors disclose no commercial associations that may create a conflict of interest in connection with the study.

Funding Information

The study was funded by Western Norway Regional Health Authority (Helse Vest Regionalt Helseforetak) through PhD grant no. 912132 and project grant no 911971, and by the National Advisory Unit for Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway.

References

1. Hirschtick, R.E., J. Glassroth, M.C. Jordan, T.C. Wilcosky, J.M. Wallace, P.A. Kvale, N. Markowitz, M.J. Rosen, B.T. Mangura, and P.C. Hopewell. 1995. Bacterial pneumonia in persons infected with the human immunodeficiency virus. Pulmonary Complications of HIV Infection Study Group. N. Engl. J. Med. 333:845–851.
2. Madhi, S.A., A. Madhi, K. Petersen, M. Khoosal, and K.P. Klugman. 2001. Impact of human immunodeficiency virus type 1 infection on the epidemiology and outcome of bacterial Meningitis in South African children. Int. J. Infect. Dis. 5:119–125.
3. O'Connor, J., M.J. Vjecha, A.N. Phillips, B. Angus, D. Cooper, B. Grinsztejn, G. Lopardo, S. Das, R. Wood, A. Wilkin, H. Klinker, P. Kantipong, K.L. Klingman, D. Jilich, E. Herieka, E. Denning, I. Abubakar, F. Gordin, J.D. Lundgren, and INSIGHT START study group. 2017. Effect of immediate initiation of antiretroviral therapy on risk of severe bacterial infections in HIV-positive people with CD4 cell counts of more than 500 cells per muL: secondary outcome results from a randomised controlled trial. Lancet HIV. 4:e105–e112.
4. Moyo, S.J., S. Aboud, M. Kasubi, E.F. Lyamuya, and S.Y. Maselle. 2010. Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. BMC Res. Notes. 3:348.
5. Blomberg, B., R. Jureen, K.P. Manji, B.S. Tamim, D.S. Mwakagile, W.K. Urassa, M. Fataki, V. Msangi, M.G. Tellevik, S.Y. Maselle, and N. Langeland. 2005. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J. Clin. Microbiol. 43:745–749.
6. Blanco, V.M., J.J. Maya, A. Correa, M. Perenguez, J.S. Munoz, G. Motoa, C.J. Pallares, F. Rosso, L. Matta, Y. Celis, M. Garzon, and M.V. Villegas. 2016. [Prevalence and risk factors for extended-spectrum beta-lactamase-producing *Escherichia coli* causing community-onset urinary tract infections in Colombia]. Enferm. Infecc. Microbiol. Clin. 34:559–565.
7. Manyahi, J., S.J. Moyo, M.G. Tellevik, F. Ndugulile, W. Urassa, B. Blomberg, and N. Langeland. 2017. Detection of CTX-M-15 beta-lactamases in Enterobacteriaceae causing hospital- and community-acquired urinary tract infections as early as 2004, in Dar es Salaam, Tanzania. BMC Infect. Dis. 17:282.
8. Trecarichi, E.M., R. Cauda, and M. Tumbarello. 2012. Detecting risk and predicting patient mortality in patients with extended-spectrum beta-lactamase-producing Enterobacteriaceae bloodstream infections. Fut. Microbiol. 7: 1173–1189.
9. Reddy, P., M. Malczynski, A. Obias, S. Reiner, N. Jin, J. Huang, G.A. Noskin, and T. Zembower. 2007. Screening

for extended-spectrum beta-lactamase-producing Enterobacteriaceae among high-risk patients and rates of subsequent bacteremia. Clin. Infect. Dis. 45:846–852.
10. Kiddee, A., K. Assawatheptawee, A. Na-Udom, P. Boonsawang, P. Treebupachatsakul, T.R. Walsh, and P.R. Niumsup. 2019. Risk factors for extended-spectrum β-lactamase-producing enterobacteriaceae carriage in patients admitted to intensive care unit in a tertiary care hospital in Thailand. Microb. Drug Resist. 25:1182–1190.
11. Sonda, T., H. Kumburu, M. van Zwetselaar, M. Alifrangis, B.T. Mmbaga, O. Lund, F.M. Aarestrup, and G. Kibiki. 2018. Prevalence and risk factors for CTX-M gram-negative bacteria in hospitalized patients at a tertiary care hospital in Kilimanjaro, Tanzania. Eur. J. Clin. Microbiol. Infect. Dis. 37:897–906.
12. Moremi, N., H. Claus, L. Rutta, M. Frosch, U. Vogel, and S.E. Mshana. 2018. High carriage rate of extended-spectrum beta-lactamase-producing Enterobacteriaceae among patients admitted for surgery in Tanzanian hospitals with a low rate of endogenous surgical site infections. J. Hosp. Infect. 100:47–53.
13. Blomberg, B., K.P. Manji, W.K. Urassa, B.S. Tamim, D.S. Mwakagile, R. Jureen, V. Msangi, M.G. Tellevik, M. Holberg-Petersen, S. Harthug, S.Y. Maselle, and N. Langeland. 2007. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. BMC Infect. Dis. 7:43.
14. Tellevik, M.G., B. Blomberg, O. Kommedal, S.Y. Maselle, N. Langeland, and S.J. Moyo. 2016. High prevalence of faecal carriage of ESBL-producing Enterobacteriaceae among children in Dar es Salaam, Tanzania. PLoS One. 11: e0168024.
15. Moremi, N., H. Claus, U. Vogel, and S.E. Mshana. 2017. Faecal carriage of CTX-M extended-spectrum beta-lactamase-producing Enterobacteriaceae among street children dwelling in Mwanza city, Tanzania. PLoS One. 12:e0184592.
16. Mshana, S.E., L. Falgenhauer, M.M. Mirambo, M.F. Mushi, N. Moremi, R. Julius, J. Seni, C. Imirzalioglu, M. Matee, and T. Chakraborty. 2016. Predictors of blaCTX-M-15 in varieties of *Escherichia coli* genotypes from humans in community settings in Mwanza, Tanzania. BMC Infect. Dis. 16:187.
17. Wilmore, S.M.S., K. Kranzer, A. Williams, B. Makamure, A.F. Nhidza, J. Mayini, T. Bandason, J. Metcalfe, M.P. Nicol, I. Balakrishnan, M.J. Ellington, N. Woodford, S. Hopkins, T.D. McHugh, and R.A. Ferrand. 2017. Carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in HIV-infected children in Zimbabwe. J. Med. Microbiol. 66:609–615.
18. Tanzania Commission for AIDS (TACAIDS), Zanzibar AIDS Commission (ZAC). Tanzania HIV Impact Survey (THIS) 2016-2017: Final Report. Dar es Salaam, Tanzania. December 2018. https://phia.icap.columbia.edu/wp-content/uploads/2019/06/FINAL_THIS-2016-2017_Final-Report__06.21.19_for-web_TS.pdf
19. Jazmati, N., T. Jazmati, and A. Hamprecht. 2017. Importance of pre-enrichment for detection of third-generation cephalosporin-resistant Enterobacteriaceae (3GCREB) from rectal swabs. Eur. J. Clin. Microbiol. Infect. Dis. 36:1847–1851.
20. Kommedal, O., J.L. Aasen, and P.C. Lindemann. 2016. Genetic antimicrobial susceptibility testing in Gram-negative sepsis—impact on time to results in a routine laboratory. APMIS. 124:603–610.
21. Ouedraogo, A.S., S. Sanou, A. Kissou, A. Poda, S. Aberkane, N. Bouzinbi, B. Nacro, R. Ouedraogo, P. Van De Perre, C. Carriere, D. Decré, H. Jean-Pierre, and S. Godreuil. 2017. Fecal carriage of Enterobacteriaceae producing extended-spectrum beta-lactamases in hospitalized patients and healthy community volunteers in Burkina Faso. Microb. Drug Resist. 23:63–70.
22. Sanneh, B., A. Kebbeh, H.S. Jallow, Y. Camara, L.W. Mwamakamba, I.F. Ceesay, E. Barrow, F.O. Sowe, S.M. Sambou, I. Baldeh, A. Jallow, M.A.J. Raul, and A. Andremont. 2018. Prevalence and risk factors for faecal carriage of Extended Spectrum beta-lactamase producing Enterobacteriaceae among food handlers in lower basic schools in West Coast Region of The Gambia. PLoS One. 13:e0200894.
23. Barguigua, A., H. Ouair, F. El Otmani, R. Saile, N. El Mdaghri, M. El Azhari, and M. Timinouni. 2015. Fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in community setting in Casablanca. Infect. Dis. (Lond.). 47:27–32.
24. Kluytmans-van den Bergh, M.F., C. Verhulst, L.E. Willemsen, E. Verkade, M.J. Bonten, and J.A. Kluytmans. 2015. Rectal carriage of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in hospitalized patients: selective preenrichment increases yield of screening. J. Clin. Microbiol. 53:2709–2712.
25. Murk, J.-L.A.N., E.R. Heddema, D.L.J. Hess, J.A. Bogaards, C.M.J.E. Vandenbroucke-Grauls, and Y.J. Debets-Ossenkopp. 2009. Enrichment broth improved detection of extended-spectrum-beta-lactamase-producing bacteria in throat and rectal surveillance cultures of samples from patients in intensive care units. J. Clin. Microbiol. 47:1885–1887.
26. Urban, C., N. Mariano, P.A. Bradford, M. Tuckman, S. Segal-Maurer, W. Wehbeh, L. Grenner, R. Colon-Urban, B. Johnston, J.R. Johnson, and J.J. Rahal. 2010. Identification of CTX-M beta-lactamases in *Escherichia coli* from hospitalized patients and residents of long-term care facilities. Diagn. Microbiol. Infect. Dis. 66:402–406.
27. Diaz, M.A., J.R. Hernandez-Bello, J. Rodriguez-Bano, L. Martinez-Martinez, J. Calvo, J. Blanco, A. Pascual, and Spanish Group for Nosocomial Infections (GEIH). 2010. Diversity of *Escherichia coli* strains producing extended-spectrum beta-lactamases in Spain: second nationwide study. J. Clin. Microbiol. 48:2840–2845.

Address correspondence to:
Joel Manyahi, MD
Department of Microbiology and Immunology
Muhimbili University of Health and Allied Sciences
P.O. Box 65001
Dar es Salaam
Tanzania

E-mail: joel.manyahi@uib.no, manyahijoel@yahoo.com

Paper III

JOURNAL OF MEDICAL MICROBIOLOGY

RESEARCH ARTICLE

Manyahi *et al.*, *Journal of Medical Microbiology* 2022;71:001513
DOI 10.1099/jmm.0.001513

MICROBIOLOGY SOCIETY

OPEN ACCESS

First identification of *bla*$_{NDM-5}$ producing *Escherichia coli* from neonates and a HIV infected adult in Tanzania

Joel Manyahi[1,2,3,*], Sabrina J. Moyo[1,2,4], Upendo Kibwana[2], Richard N. Goodman[4], Ellie Allman[4], Alasdair T. M. Hubbard[4,5], Bjørn Blomberg[1,3], Nina Langeland[1,3] and Adam P. Roberts[4]

Abstract

Introduction. Carbapenem-resistant members of the family Enterobacteriaceae are emerging as a global public-health threat and cause substantial challenges in clinical practice.

Gap Statement. There is a need for increased and continued genomic surveillance of antimicrobial resistance genes globally in order to detect outbreaks and dissemination of clinically important resistance genes and their associated mobile genetic elements in human pathogens.

Aim. To describe the resistance mechanisms of carbapenem-resistant *Escherichia coli*.

Methods. Rectal swabs from neonates and newly diagnosed human immunodeficiency virus (HIV) infected adults were collected between April 2017 and May 2018 and screened for faecal carriage of carbapenamases and OXA-48 producing members of the family Enterobacteriaceae. Bacterial isolates were identified using matrix assisted laser desorption ionization time of flight mass spectrometry. Antimicrobial susceptibility testing was performed by E-test. Whole genomes of carbapenem-resistant *E. coli* were investigated using a hybrid assembly of Illumina and Oxford Nanopore Technologies sequencing reads.

Results. Three carbapenem-resistant *E. coli* were detected, two from neonates and one from an HIV infected adult. All three isolates carried *bla*$_{NDM-5}$. Two *E. coli* from neonates belonged to ST167 and *bla*$_{NDM-5}$ co-existed with *bla*$_{CTX-M-15}$ and *bla*$_{OXA-01}$, and all were carried on IncFIA type plasmids. The *E. coli* from the HIV infected adult belonged to ST2083, and carried *bla*$_{NDM-5}$ on an IncX3 type plasmid and *bla*$_{CMY-42}$ on an IncI type plasmid. All *bla*$_{NDM-5}$ carrying plasmids contained conjugation related genes. In addition, *E. coli* from the HIV infected adult carried three more plasmid types; IncFIA, IncFIB and Col(BS512). One *E. coli* from a neonate also carried one extra plasmid Col(BS512). All three *E. coli* harboured resistance genes to fluoroquinolone, aminoglycosides, sulfamethoxazole, trimethoprim, macrolides and tetracycline, carried on the IncFIA type plasmid. Furthermore, *E. coli* from the neonates carried a chloramphenicol resistance gene (*catB3*), also on the IncFIA plasmid. All three isolates were susceptible to colistin.

Conclusion. This is the first report, to our knowledge, from Tanzania detecting *bla*$_{NDM-5}$ producing *E. coli.* The carbapenemase gene was carried on an IncFIA and IncX3 type plasmids. Our findings highlight the urgent need for a robust antimicrobial resistance (AMR) surveillance system to monitor and rapidly report on the incidence and spread of emerging resistant bacteria in Tanzania.

Received 27 September 2021; Accepted 18 January 2022; Published 28 February 2022
Author affiliations: [1]Department of Clinical Science, University of Bergen, Bergen, Norway; [2]Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, MUHAS, Dar es Salaam, Tanzania; [3]Norwegian National Advisory Unit for Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway; [4]Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK; [5]Department of Biosciences, School of Science and Technology, Nottingham Trent University, Nottingham NG11 8NS, UK.
***Correspondence:** Joel Manyahi, manyahijoel@yahoo.com
Keywords: blaNDM-5; *E. coli*; neonates; HIV; Tanzania.
Abbreviations: AMR, antimicrobial resistance; HIV, human immunodeficiency virus; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; SNPs, single nucleotide polymorphisms.
Repositories: PRJNA756167 (strain PC-NDM34), PRJNA756168 (strain NDM_12_14482) and PRJNA756169 (strain NDM_11.16372).
Supplementary material is available with the online version of this article.

BACKGROUND

Infections caused by carbapenem-resistant members of the family Enterobacteriaceae are emerging as a global public health concern. These infections cause substantial challenges in clinical practice as they are associated with increased morbidity and mortality as well as health care costs [1]. Carbapenems are considered a last resort for treatment of infections with multidrug-resistant members of the family Enterobacteriaceae. The remaining alternative treatment options for infections by carbapenem-resistant members of the family Enterobacteriaceae are colistin and tigecycline [2–5], which are expensive, poorly tolerated and often unavailable in low-and middle-income countries.

Resistance to carbapenems in Enterobacteriaceae is mainly mediated by the production of carbapenemase enzymes, which hydrolyze carbapenems and all other β-lactam antibiotics. Currently, three classes of carbapenemase enzymes of clinical importance have been identified in pathogenic bacteria. These are Ambler class A (KPC, IMI), class B (NDM, IMP, VIM) and class D (OXA-48) [2, 6].

Previously, carbapenem resistance in Gram-negative bacteria was mainly mediated by production of KPC, IMP, VIM and OXA-48 carbapenemases [6, 7]. In recent years, New Delhi metallo-β-lactamase has received global attention due to its world-wide dissemination, rapid evolution, and high levels of resistance to β-lactam antibiotics [5]. Since the first report of $bla_{\text{NDM-1}}$ in 2008 [8], 24 variants of NDM have been discovered in Gram-negative bacteria [5]. Of the NDM variants, $bla_{\text{NDM-5,}}$ which was first detected in *Escherichia coli* in the United Kingdom in 2011 [9], is notable for its elevated resistance to carbapenems compared with other variants [10]. NDM-5 differs from NDM-1 by substitution of two amino acids (Val88Leu and Met154Leu) [11].

The $bla_{\text{NDM-5}}$ is mostly carried on plasmids, which also may carry resistance genes to other antibiotics [12, 13] and catalyse their transfer between bacteria. Several plasmids have been reported to carry $bla_{\text{NDM-5}}$ [5], with IncX3 type plasmid being the most commonly reported [4, 14, 15]. Since its discovery, $bla_{\text{NDM-5}}$ producing *E. coli* has been reported in Asia, mainly PR China [16, 17], Europe [18, 19] and Africa [20, 21].

Meanwhile, in Tanzania, unpublished data shows increased use of carbapenems, but it is unknown whether this has led to an increased incidence of carbapenem resistance. Three Tanzanian studies have described resistance to carbapenems in clinical isolates, documenting the presence of VIM, IMP, NDM-1 and KPC type carbapenemases [22–24], but only one of these studies found NDM-1 carbapenemases in members of the family Enterobacteriaceae [24]. We performed this study to assess the faecal carriage of carbapenem-resistant members of the family Enterobacteriaceae among newly diagnosed HIV infected adults and children hospitalized with fever in Dar es Salaam, Tanzania. We also identified resistance mechanisms to carbapenems and their genetic context using a whole genome sequencing approach.

METHODS

Study sites and study participants

Newly diagnosed HIV infected adults were recruited consecutively from six HIV care and treatment centres in Dar es Salaam, Tanzania, namely Amana hospital, Mwananyamala hospital, Temeke hospital, PASADA, Mbagala hospital and Mnazi Mmoja hospital, as part of the double blinded randomized clinical trial, CoTrimResist (ClinicalTrials.gov identifier: NCT03087890) between April 2017 to May 2018. At the same time, children admitted with fever were enrolled in a febrile illness study at four hospitals in Dar es Salaam (Amana, Temeke and Mwananyamala Regional hospitals and Muhimbili National Hospital).

Screening for carbapenemase producing *Enterobacteriaceae*

Rectal swabs were collected from each participant and transported in liquid Cary–Blair medium (Faecal transwab, MWE). Screening for faecal carriage of carbapenamases and OXA-48 producing members of the family Enterobacteriaceae was carried out on CHROMID CARBA SMART (BioMérieux) using two drops (0.1 ml) from an overnight culture in brain–heart infusion broth.

Bacterial identification and antimicrobial susceptibility testing

Carbapenem resistant bacterial isolates which grew on the selective media were identified by matrix assisted laser desorption ionization time of flight mass spectrometry using the Microflex LT instrument and matrix assisted laser desorption ionization Biotyper 3.1 software (Bruker Daltonics).

Antibiotic susceptibility testing was determined by E-test (when available), following guidelines from the Clinical and Laboratory Standards Institute [25]. The antimicrobial agents tested were cefotaxime, imipenem, tetracycline, ciprofloxacin, gentamicin and colistin. When E-tests were not available disc diffusion was used to determine meropenem susceptibility according to Clinical and Laboratory Standards Institute guidelines.

Whole genome sequencing

Genomic DNA isolation for short read sequencing was carried out at MicrobesNG (Birmingham, UK). Short read whole genome sequencing was performed using HiSeq X10 (Illumina) by MicrobesNG, which also performed quality filtering and sequencing read trimming.

Genomic DNA for long read sequencing was extracted using a Fire Monkey High Molecular Weight DNA Extraction Kit (RevoluGen). Long read sequencing was carried out using a R9.4.1 flow cell (Oxford Nanopore Technologies) on a MinION sequencer. Base calling of the reads was performed with MinKNOW software (v20.06.4) using the Guppy algorithm (v4.0.9). The long-read sequences were trimmed using Porechop (https://github.com/rrwick/Porechop) and filtered using Filtlong (https://github.com/rrwick/Filtlong) with a minimum length threshold of 1000 bp, keeping 90% of the best reads up to a total of 500000000 bp.

Long and short read sequences were assembled using Unicycler (v0.4.8.0) [26], running in 'normal' mode and the genome was annotated with Prokka (v1.14.6) [27] and the RAST annotation server [28]. Genomes were aligned using both Clinker (v0.0.12) [29] and BRIG (v0.95) [30]. The genomes were visualized using Snapgene (v3.3.4) (https://www.snapgene.com/). All genome assemblies from this study have been deposited in GenBank under the BioProject numbers PRJNA756167 (strain PC-NDM34), PRJNA756168 (strain NDM_12_14482) and PRJNA756169 (strain NDM_11.16372).

Identification of resistance genes and sequence types

For identification of acquired antimicrobial resistance genes, and detection of virulence genes, we used ResFinder v4.1 [31] and virulence Finder 2.0 [32], respectively, from the Centre for Genomic Epidemiology GEE server (http://www.genomicepidemiology.org/). For identification of mobile genetic elements and their relation to antimicrobial resistance genes and virulence factors we used Center of Genomic Epidemiology Mobile Element Finder v1.0.3 [33]. For assignment of multilocus sequence typing (MLST) and clonal complexes we used an online MLST database website (https://pubmlst.org/).

Phylogenetic analysis

Phylogenetic analysis of *E. coli* ST167 whole genomic sequence single nucleotide polymorphisms (SNPs) was performed using CSI phylogeny 1.4 [34]. For comparison, two *E. coli* ST167 from this study were compared with 22 globally available complete whole genome sequences of *E. coli* ST167 (bla_{NDM-5}-positive and bla_{NDM-5}-negative) downloaded from NCBI nucleotide GenBank and the European nucleotide archive (NEA). The phylogenetic tree was visualized using the Fig Tree programme version 1.4.4 (https://github.com/rambaut/figtree/releases).

Comparative analysis of IncFIA and IncX3 carrying bla_{NDM-5} plasmids

For comparison of the genetic environments surrounding bla_{NDM-5} on IncFIA and IncX3 plasmids, approximately 20 kb segments were selected including the bla_{NDM-5} gene from each strain, and annotated using Prokka (v1.14.6) [27] and aligned using Clinker (v0.0.12) [29].

IncFIA and IncX3 plasmids were compared with other available plasmids. A total of 17 IncX3 and 25 IncFI carrying bla_{NDM-5} plasmids' genome sequences available globally were downloaded from the NCBI 'nuccore' database using Entrez Direct as assembled genomes in fasta file format. BRIG v0.95 [30] was used for genomic comparison and to produce the visualisations. For the IncF comparisons, the 1446 NDM-5 plasmid sequence was used as reference and for the IncX3 comparisons the PC34 NDM-5 plasmid sequence was used as a reference. ResFinder v4 [31]. was used to annotate the reference genomes with acquired antimicrobial resistance (AMR) genes.

RESULTS

Bacterial isolates

Three carbapenem resistant *E. coli* designated as PC-NDM34 (from a HIV infected adult), NDM_12_14482, and NDM_11.16372 (both from neonates) were isolated from screening of 737 rectal swabs (537 HIV infected outpatient adults and 200 under five years old inpatient children). Isolate PC-NDM34 was from a 49-year-old individual, an outpatient newly diagnosed with HIV at PASADA HIV care and treatment center in May 2018. The patient had a CD4 count of 132 cell μl^{-1} with no recent history of hospitalization or antibiotic use. Isolates NDM_12_14482 (22 Jan, 2018) and NDM_11.16372 (05 Feb 2018) were isolated from two neonate patients aged three days from the same ward (Temeke Hospital) two weeks apart in February 2018.

Antimicrobial susceptibility pattern

Table 1 shows the minimum inhibitory concentrations (MICs) of different antimicrobial agents tested against the three isolates. The MICs for all the three isolates to cefotaxime, gentamicin, ciprofloxacin and tetracycline were more than 256 μg ml^{-1}. Meropenem susceptibility was checked for isolates NDM_11.16372 and NDM_12_14482 by disc diffusion, revealing

Table 1. Susceptibility and MIC of the three carbapenem resistant isolates

Antimicrobial agent	MIC		
	PC-NDM34	NDM_11.16372	NDM_12_14482
Meropenem	6 μg ml^{-1} †	13 mm*	12 mm*
Imipenem	3 μg ml^{-1} †	3 μg ml^{-1}†	1.5 μg ml^{-1}†
Colistin	0.125 μg ml^{-1} †	0.25 μg ml^{-1} †	0.19 μg ml^{-1} †
Cefotaxime	>256 μg ml^{-1} †	>256 μg ml^{-1} †	>256 μg ml^{-1} †
Tetracycline	>256 μg ml^{-1} †	>256 μg ml^{-1} †	>256 μg ml^{-1} †
Ciprofloxacin	>256 μg ml^{-1} †	>256 μg ml^{-1} †	>256 μg ml^{-1} †
Gentamicin	>256 μg ml^{-1} †	>256 μg ml^{-1} †	>256 μg ml^{-1} †

*Meropenem E-tests were unavailable, therefore meropenem disc diffusion assays were used for isolates NDM_11.16372 and NDM_12_14482.
†, AST by E-tests.

zones of inhibition of 13 mm and 12 mm respectively. For isolate PC-NDM34 meropenem susceptibility was determined by E-test and shown to be 6 μg ml^{-1}. Subsequently, the MICs of these three isolates PC-NDM34, NDM_11.16372 and NDM_12_14482 to imipenem were determined to be 3 μg ml^{-1}, 3 μg ml^{-1} and 1.5 μg ml^{-1} respectively. The three isolates were susceptible to colistin at different MIC values as follows; 0.125 μg ml^{-1} for isolate PC-NDM34, 0.25 μg ml^{-1} for isolate NDM_11.16372 and 0.19 μg ml^{-1} for isolate NDM_12_14482.

Resistance determinant genes and association with plasmids

Isolate PC-NDM34 contained six plasmids, IncFIA (109 kb), IncFIB (108 kb), IncI (49 kb), IncX3 (46 kb), Col (BS512) (2 kb), and another unknown plasmid with an approximate size of 5 kb. Isolate NDM_11.16372 contained three plasmids, IncFIA (137 kb), Col (BS512) (2 kb), and another of about 4 kb, and isolate NDM_12_14482 contained one IncFIA plasmid of approximately 137 kb. All three carbapenem resistant *E. coli* carried plasmid-located bla_{NDM-5}. For isolate PC-NDM34, bla_{NDM-5} was located on an IncX3 type plasmid, while for both isolates NDM_11.16372 and NDM_12_14482 it was located on the IncFIA plasmid. All the bla_{NDM-5} carrying plasmids also carried genes whose products are predicted to be involved in conjugation.

Other *β*-lactam resistance genes carried by PC-NDM34 were bla_{TEM-1b} and bla_{CMY-42}, located on IncI and IncFIA type plasmids, respectively. The IncFIA type plasmid from P34 harboured other resistance genes including fluoroquinolone-aminoglycosides (*aac(6')-Ib-cr*); sulfamethoxazole (*sul*2); trimethoprim (*dfr*17); aminoglycoside [*aac*(3)-IId, *aph*(3')-lb, *aadA*5]; tetracycline (*tetB*); and macrolides *mph*(A). While bla_{NDM-5} on IncFIA plasmid from both NDM_11.16372 and NDM_12_14482 co-existed with other *β*-lactam resistance genes (bla_{TEM-1b}, bla_{OXA-01} and $bla_{CTX-M-15}$), other resistance genes present on the IncFIA plasmid from both NDM_11.16372 and NDM_12_14482 conferred resistance to fluoroquinolone-aminoglycosides [*aac(6')-Ib-cr*]; aminoglycosides (*aadA*2, *rmtB*); sulfamethoxazole (*sul*1); trimethoprim (*dfr*12); chloramphenicol (*catB*3); tetracycline (*tet*A); and macrolides *mph*(A). Table 2 shows resistance genes present in the three isolates.

Multi-locus sequence typing (MLST)

MLST analysis revealed that both NDM_12_14482 and NDM_11.16372, the isolates from neonates, harbouring bla_{NDM-5} belonged to ST167 (ST10 clonal complex). In contrast, PC-NDM34 bla_{NDM-5}, isolated from an adult HIV infected patient, belonged to ST2083.

Phylogenetic analysis

A total of 22 *E. coli* ST167 (bla_{NDM-5}-positive and bla_{NDM-5}-negative) globally available from GenBank and the European nucleotide archive (see Supplementary file 1, available with the online version of this article, for accession numbers) were compared with two ST167 strains from this study. The SNP analysis of all *E. coli* ST167 differed by between 2 and 3410 SNPs, revealing the high genetic diversity of this high-risk clone. The phylogenetic analysis revealed that *E. coli* ST167 was clustered into two distinct clades with multiple subclades (Fig. 1). The two *E. coli* ST167 from this study had a SNP difference of two SNPs and were clustered in clades with other ST167 from PR China, Italy and Myanmar and two with undetermined origins. In this clade, the SNP difference between this study's ST167 and other ST 167 ranged between 880 and 1312 SNPs.

This analysis reveals that the two isolates (NDM_12_14482 and NDM_11.16372 *E. coli*) which were isolated from neonates, were closely related and probably arose from the same source. Furthermore, this revealed that the isolates from neonates were more distantly related to the ST167 isolates from other part of the world.

Table 2. Genotypic characteristics of the three bla_{NDM-5} containing *E. coli*

Genotypic characteristics	PC-NDM34	NDM_11.16372	NDM_12_14482
Resistance genes			
Beta-lactams	bla_{TEM-1B}	bla_{TEM-1B}	bla_{TEM-1B}
	bla_{NDM-5}	bla_{NDM-5}	bla_{NDM-5}
	bla_{CMY-42}	–	–
	–	$bla_{CTX-M-15}$	$bla_{CTX-M-15}$
	–	bla_{OXA-1}	bla_{OXA-1}
Fluoroquinolone-aminoglycosides	*aac*(6')-*Ib-cr*	*aac*(6')-*Ib-cr*	*aac*(6')-*Ib-cr*
Sulfamethoxazole	*sul*2	*sul*1	*sul*1
Trimethoprim	*dfr17*	*dfr12*	*dfr12*
Aminoglycosides	*aac*(3)-*IId, aph*(3')-*Ib, aadA5*	*aadA2, rmtB*	*aadA2, rmtB*
Chloramphenicol		*catB3*	*catB3*
Tetracycline gene	tetB	*tetA*	*tetA*
Mdf	*MdfA*	*MdfA*	*MdfA*
Macrolide	*mph*(A)	*mph*(A)	*mph*(A)
Sequence type (ST)	2083	167	167
Plasmid replicon types	IncFIA	IncFIA,	IncFIA
	Col (BS512)	Col (BS512)	
	IncI	–	–
	IncIB	–	–
	IncX3	–	–
	Unknown	Unknown	–
Virulence gene			
Fimbrial protein	yfcV	–	–
EAST-1 heat-stable toxin	astA	–	–
Long polar fimbriae	lpfA	–	–
Glutamate decarboxylase	gad	gad	gad
Increased serum survival	–	iss	iss
OMP complement resistance	trat	trat	trat
Heat resistant agglutinin	–	hra	hra
Hexosyltransferase homology	–	capU	capU
Tellurium ion resistance protein	terC	terC	terC

Genetic environment for the bla_{NDM-5} carrying plasmids

Comparative analysis of plasmids carrying bla_{NDM-5}, revealed the IncFIA type plasmids from NDM_12_14482 and NDM_11.16372 harboured 100% identical sequences and bla_{NDM-5} co-existed with several resistance determinant genes (Fig. 2).

Comparative analysis of the genetic environments of bla_{NDM-5} in IncX3 and IncFIA type plasmids revealed similar genetic context downstream of bla_{NDM-5} flanked by conserved ble_{MBL}–*trpF–DsbD*. In the IncX3 plasmid bla_{NDM-5} was flanked upstream by IS5, and in the IncFIA type plasmid it was flanked upstream by IS*26* interrupted by a gene predicted to encode a conserved hypothetical protein (Fig. 3). In the IncX3 type plasmid, bla_{NDM5}–ble_{MBL}–*trpF–DsbD* was flanked by *IS*5 upstream and IS*26* downstream (Fig. 4).

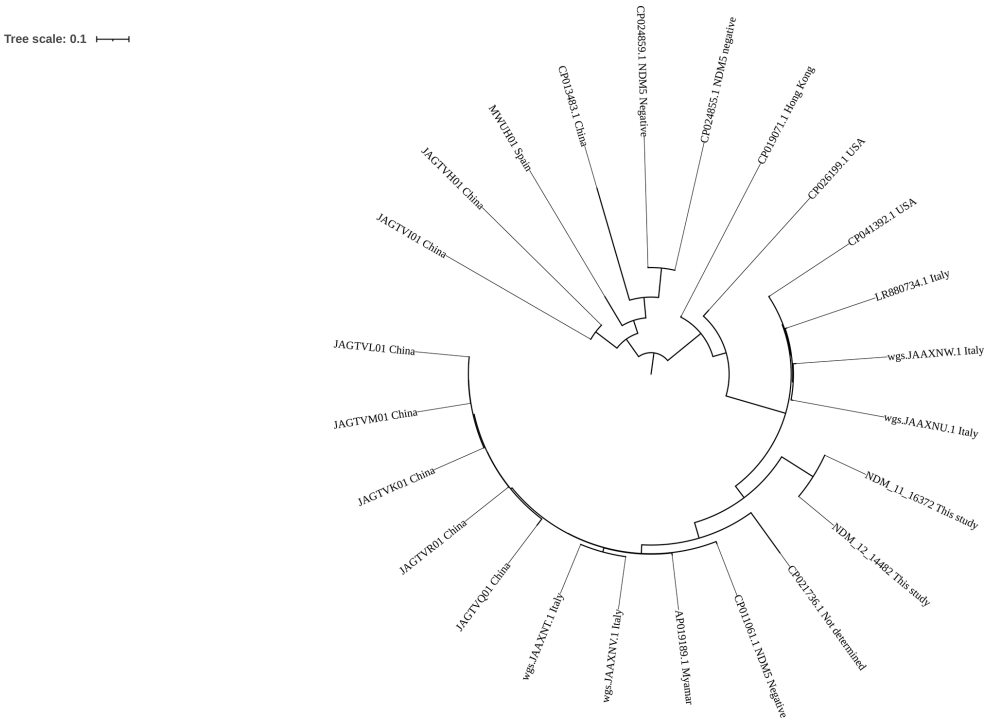


Fig. 1. Phylogenetic tree *E. coli* ST167 (bla_{NDM-5}-positive and bla_{NDM-5}-negative). Countries of origin of each *E. coli* ST167 have been highlighted in the tree, not determined indicates that the origin is not known.

In the IncFIA plasmid, further downstream of bla_{NDM5}–ble_{MBL}–*trpF–DsbD* were a set of several genes including *sul*-2, *ant*(3′)-Ia, *dfrA*12 and *IntI*1. The bla_{NDM-5} –*IntI*1 genetic complex was flanked on both ends by IS*26* (Fig. 3).

Fig. 5 shows the results of the comparative analysis of the fully sequenced bla_{NDM-5} carrying IncFIA plasmids from this study (NDM_12_14482 and NDM_11.16372) and the IncF plasmids (bla_{NDM-5}-positive and bla_{NDM-5}-negative) of global representatives (see Supplementary file 1 for accession numbers). Structural similarities (downstream) were observed within the genetic environment surrounding bla_{NDM5} (Fig. 5). The NDM_12_14482 and NDM_11.16372 plasmid sequences differ slightly compared with other global IncF plasmids.

Comparative analysis of bla_{NDM-5} carrying IncX3 plasmids is depicted in Fig. 6, comparing PC-NDM34 from this study with globally identified IncX3 plasmids (see Supplementary file 1 for accession numbers). The genetic context of bla_{NDM-5} on IncX3 plasmids was similar downstream. The genetic sequence of PC-NDM34 IncX3 was almost identical to those of most other global IncX3 plasmids, with slight differences observed (Fig. 6).

DISCUSSION

This is the first report from Tanzania and East Africa, to our knowledge, on the detection of bla_{NDM-5} producing *E. coli*. We found three *E. coli* carrying bla_{NDM-5}, one from a newly diagnosed HIV infected patient and two from admitted neonates.

The comparative genomic analysis of bla_{NDM-5} producing *E. coli* revealed that the carbapenemase gene was plasmid located and that these plasmids also carried resistance gene determinants to other antibiotics, including aminoglycosides, fluoroquinolones, macrolides, tetracycline, trimethoprim, sulfamethoxazole and chloramphenicol. The finding of carbapenem resistance conferred by the bla_{NDM-5} gene in Tanzania is of the great concern, since these isolates also were resistant to virtually all other antibiotics commonly used in Tanzania. Carbapenems have been used as the last alternative for treatment of severe infections caused by multi-drug resistant Gram-negative bacteria infection, the emergence of carbapenem resistance severely

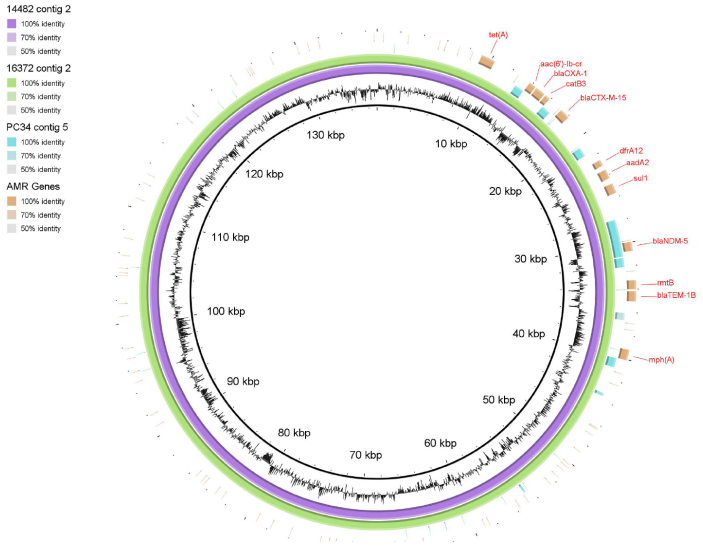


Fig. 2. Comparative analysis of the three plasmids carrying bla_{NDM-5}, image produced using BRIG. A comparison between the three bla_{NDM-5} positive plasmids across the three strains, each coloured accordingly. The reference genome was the plasmid of the isolate 14482. The inner black ring represents GC content of the reference sequence. AMR genes are labelled. 14482= NDM_12_14482, 16372= NDM_11.16372 and PC34= PC-NDM34.

limits treatment options for these patients. Lately, the use of carbapenems in Tanzania has been frequent [35], and increased use is anticipated in the future due to increased extended spectrum beta-lactamase (ESBL) producing bacterial infections [36]. Increasing carbapenem use will increase selection for horizontal gene transfer events and, therefore, the movement of the gene and human movement will increase dissemination of carbapenem resistant bacteria around the country.

To date, reports on bla_{NDM-5} producing Gram-negative bacteria from Africa have been uncommon. In Africa, bla_{NDM-5} was first reported in Algeria in three *E. coli* recovered from urine and blood in 2012 [20]. Since then, bla_{NDM-5} in humans has been reported in Egypt [37], Angola [38], South Africa [39], Nigeria [40], Chad [15] and Malawi [41, 42]. Recently, bla_{NDM-5} producing *E. coli* have been detected in Mali from one outpatient [21] and in Mozambique in a patient with a bloodstream

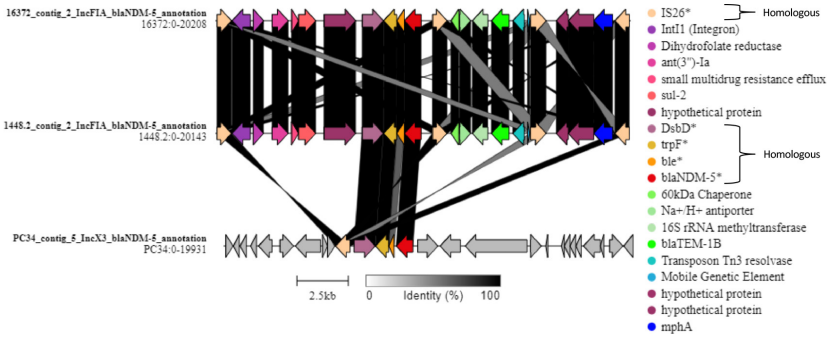


Fig. 3. Comparison of bla_{NDM-5} genetic context on 16372 (IncFIA), 14482 (IncFIA) and PC34 (IncX3) plasmids. A sequence comparison between the genetic environments surrounding bla_{NDM-5} in the three strains. Approximately 20 kb segments were selected including the bla_{NDM-5} gene from each strain, these were annotated using Prokka and aligned using clinker. Unique sequences are coloured according to feature. Those features which are homologous across all three strains are highlighted with asterisks. 14482= NDM_12_14482, 16372= NDM_11.16372 and PC34= PC-NDM34.

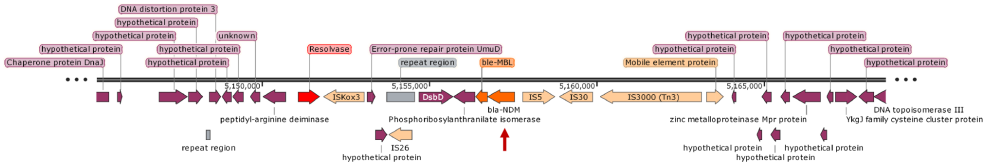


Fig. 4. Genetic context of *bla*$_{NDM-5}$ on the IncX3 plasmid (PC-NDM34).

infection [12]. The reason for increased detection of *bla*$_{NDM-5}$-producing Gram-negative bacteria in Africa is not known, it could be independent introduction from the rest of the world. Our three *bla*$_{NDM-5}$ *E. coli* were isolated from patients with no history of travel beyond Tanzania. It is therefore likely that the patients have acquired the *bla*$_{NDM-5}$ producing *E. coli* in Tanzania or their resident *E. coli* had acquired *bla*$_{NDM-5}$ from a transient donor also in Tanzania.

In analysing mobile genetic elements and their relationship with resistance genes, we found that the *bla*$_{NDM-5}$ producing *E. coli* ST2085 from the HIV infected patient was located on an IncX3 type plasmid. Similarly, a recent published study from Malawi reported detection of blaNDM-5 contained in an IncX3 plasmid from *E. coli* ST2085 isolated from the stool of an HIV-infected adult [42]. The results of previous studies have demonstrated that *bla*$_{NDM-5}$ is commonly carried on IncX3 type plasmid [13, 15, 43–45]. In addition, the results of previous studies have indicated that the IncX3 type plasmid plays an important role in dissemination of *bla*$_{NDM-5}$ in members of the family *Enterobacteriaceae* [13, 14, 21]. This hypothesis has been supported by the results of several experimental conjugation studies, where *bla*$_{NDM-5}$ *E. coli* carrying IncX3 type plasmids were able to be successfully transferred amongst, and between, different species of the family *Enterobacteriaceae* [14, 43]. The *bla*$_{NDM-5}$ carrying IncX3 plasmid from this study revealed almost identical plasmid sequence to globally identified IncX3 plasmid. Our finding of an IncX3 type plasmid associated with *bla*$_{NDM-5}$ in a newly diagnosed HIV patient from the community setting in Tanzania is concerning, since this plasmid is epidemic and has been shown to carry multiple carbapenemase genes,

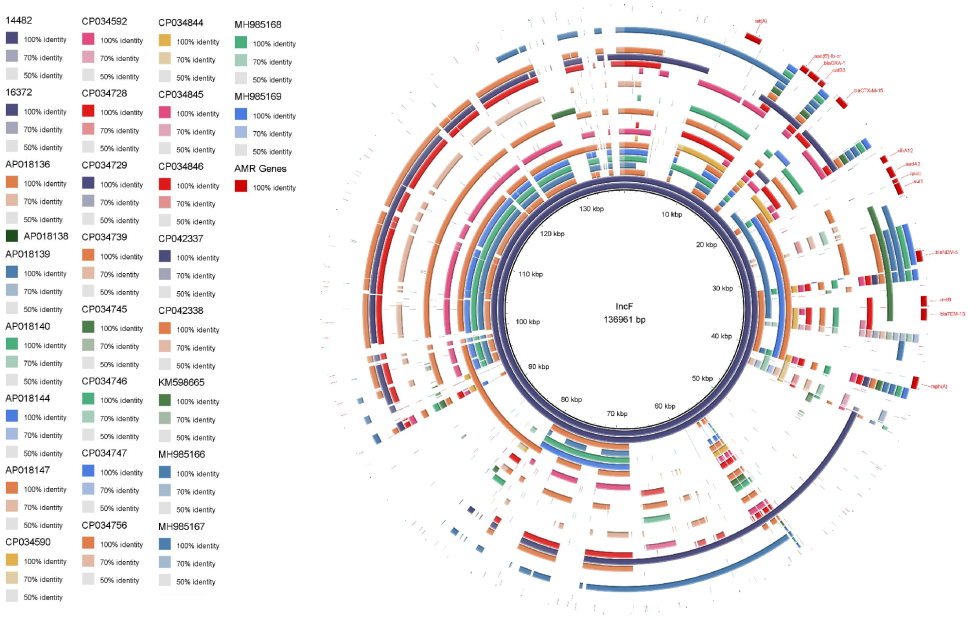


Fig. 5. A comparative analysis of the two IncFIA plasmids (16372 and 14482) containing *bla*$_{NDM-5}$ against IncF plasmids identified globally. BRIG was used for genomic comparison and visualization. Plasmid (137 Kb) from isolate NDM_12.14482 containing *bla*$_{NDM-5}$ was used as the reference. Sequences are named using their accession numbers. (16372=NDM_11.16372 and 14482=NDM_12.14482).

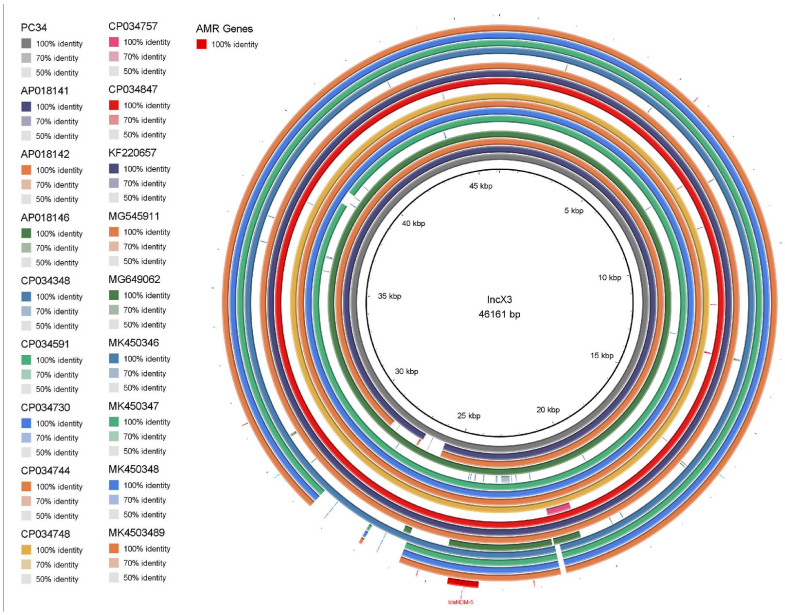


Fig. 6. A comparative analysis of the IncX3 (PC34) plasmid of the isolate PC-NDM34 against IncX3 plasmids identified globally. BRIG was used for genomic comparison and visualization. The plasmid (46 Kb) from isolate PC-NDM34 containing bla_{NDM-5} was used as a reference. All sequences were of plasmids containing IncX3 except for CP034348 which contained an IncX1 plasmid. Sequences are named using their accession numbers.

including bla_{NDM-5}, and has a high potential to efficiently disseminate globally. Spread of bla_{NDM-5} in the community has serious implication since bla_{NDM-5} carrying isolates also display multidrug resistance [13].

IncF type plasmids have also been shown to contribute to the dissemination of bla_{NDM-5} among *E. coli* [17, 18, 46]. In this study, we observed that the bla_{NDM-5} in *E. coli* from neonates was carried on IncFIA type plasmids. The bla_{NDM-5} from these isolates co-existed with $bla_{CTX-M-15}$, bla_{TEM-1B} and bla_{OXA-1} located on the IncFIA plasmid. Other recent studies in Africa and elsewhere have observed co-harbouring of bla_{NDM-5} and bla_{CTX-15}, bla_{TEM-1B}, bla_{OXA-1} in the same plasmid type in *E. coli* [3, 12, 46]. Co-localization of bla_{NDM-5} and extended spectrum β-lactamase ($bla_{CTX-M-15}$) plus other resistance genes in the same plasmid could increase the dissemination of multiple resistances in a single gene transfer event.

Furthermore, the two bla_{NDM-5} producing *E. coli* from neonates had similar resistance gene determinants, virulence genes and ST167, indicating that the isolates were likely to be clonal. Chromosomal DNA SNP analysis of the two isolates also revealed a pairwise distance of two SNPs. This finding indicates that these two isolates were closely related and the source of spread was likely to be the same. It implies there was probably local transmission of bla_{NDM-5} producing *E. coli*. *E. coli* ST167 carrying bla_{NDM-5} has been reported in South Africa in one inpatient with nosocomial infection [39]. Recently, *E. coli* ST167 has been regarded as a high-risk and successful epidemic clone involved in transmission of the bla_{NDM-5} gene [19, 43]. Furthermore, bla_{NDM-5} producing *E. coli* ST167 has the potential for global dissemination due its combination of resistance and virulence genes [43]. The bla_{NDM-5} producing *E. coli* ST167 has been reported in neonatal and adult infections in PR China [3, 16, 17, 47], the USA [48], Europe [18] and Africa [39]. Similar to our finding, an *E. coli* ST167 strain co-producing bla_{NDM-5}, $bla_{CTX-M-15}$ and bla_{OXA-1} has been reported in bacteria causing infection in PR China [3]. Phylogenetic analysis revealed the *E. coli* ST167 strains are clonally diverse and our study isolates were phylogenetically distant from global circulating *E. coli* ST167, (Fig. 1).

Our study revealed in all three *E. coli*, that the bla_{NDM-5} on each plasmid was flanked by highly a conserved region (ble_{MBL}–*trpF–DsbD*) downstream, indicating that the region is very probably transferring between replicons (plasmids) and between bacteria as a single unit. The upstream genetic environment of bla_{NDM5} in two plasmids were different. Previous in-depth analysis of the genetic environment of bla_{NDM-5} genes revealed that the bla_{NDM-5} is flanked upstream by IS*Aba125* and downstream by ble_{MBL}–*trpF–DsbD* (IS*3000*–IS*5*–ΔIS*Aba125*–bla_{NDM-5}–ble_{MBL}–*trpT–DsbD*) [21, 49, 50]. In this study we found complete deletion of IS*3000*–IS*5*–ΔIS*Aba125* upstream

of bla_{NDM-5} in the IncFIA plasmid from the neonates. In the IncX3 plasmid, complete deletion of Δ*ISAba125* was observed and the IS*3000*–IS*5* element was interrupted by IS*30*.

CONCLUSION

This is the first detection of bla_{NDM-5} producing *E. coli* in Tanzania, to our knowledge. We found bla_{NDM-5} in *E. coli* ST167 located on an IncFIA plasmid colonizing the gut of two neonates and bla_{NDM-5} producing *E. coli* ST2083 located on an IncX3 plasmid colonizing the gut of an outpatient newly diagnosed with HIV. Our findings highlight the urgent need for a robust AMR surveillance system to monitor and rapidly report on the incidence and spread of emerging resistant bacteria in Tanzania. In addition, long-term infection prevention and control procedures and antimicrobial stewardship policies need to be introduced, optimized and maintained to curb the spread of resistant bacteria within healthcare environments.

Funding information
This research was supported by the Helse Bergen HF, Haukeland University Hospital, Norway, under project number 912132, as well as the Haukeland University Hospital's National Advisory Unit on Tropical Infectious Diseases. The funders had no say in the study's design, data collection and analysis, publication decision, or manuscript preparation. R. N. G. is supported by the Medical Research Council via the LSTM-Lancaster Doctoral Training Partnership (Grant number MR/N013514/1).

Acknowledgements
A. P. R. would like to acknowledge funding from the AMR Cross-Council Initiative through a grant from the Medical Research Council, a Council of UK Research and Innovation, and the National Institute for Health Research (Grant numbers MR/S004793/1 and NIHR200632).

Author contributions
B. B., N. L. and S. J. M., conceived the study. J. M., collected study data. J. M., S. M. and U. K., performed the microbiological investigations. S. J. M., R. N. G., E. A., A. T. M. H. and A. P. R., carried out further experimentation and bioinformatics analysis. J. M., drafted the manuscript. S. J. M., B. B., A. T. M. H., N. L. and A. P. R., revised the manuscript. All authors approved the final version.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
Approvals to conduct this study were obtained from Muhimbili University of Heath and Allied Sciences Senate Research and Publications Committee (reference number 2015-10-27/AEC/Vol.X/54), National Health Research Ethics Committee (reference number. NIMRIHQ/R. SaJVol. 1X12144), Tanzania Medicines and Medical Devices Authority (reference number TZ16CT007) in Tanzania and Regional Committee for Medical and Health Research Ethics of Western Norway (Ref. No. REK2015/540). Written informed consent were obtained from the patients or parents/guardians or legally authorized persons for participation in the study.

References

1. Bartsch SM, McKinnell JA, Mueller LE, Miller LG, Gohil SK, *et al*. Potential economic burden of carbapenem-resistant *Enterobacteriaceae* (CRE) in the United States. *Clin Microbiol Infect* 2017;23:48.
2. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *Biomed Res Int* 2014;2014:249856.
3. Sun L, Xu J, He F. Draft genome sequence of an NDM-5, CTX-M-15 and OXA-1 co-producing *Escherichia coli* ST167 clinical strain isolated from a urine sample. *J Glob Antimicrob Resist* 2018;14:284–286.
4. Sun P, Xia W, Liu G, Huang X, Tang C, *et al*. Characterization of bla_{NDM-5}-positive *Escherichia coli* prevalent in a university hospital in eastern China. *Infect Drug Resist* 2019;12:3029–3038.
5. Wu W, Feng Y, Tang G, Qiao F, McNally A, *et al*. NDM metallo-β-lactamases and their bacterial producers in health care settings. *Clin Microbiol Rev* 2019;32:e00115-18.
6. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 2017;8:460–469.
7. Partridge SR, Ginn AN, Wiklendt AM, Ellem J, Wong JSJ, *et al*. Emergence of bla_{KPC} carbapenemase genes in Australia. *Int J Antimicrob Agents* 2015;45:130–136.
8. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, *et al*. Characterization of a new metallo-beta-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 2009;53:5046–5054.
9. Hornsey M, Phee L, Wareham DW. A novel variant, NDM-5, of the New Delhi metallo-β-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. *Antimicrob Agents Chemother* 2011;55:5952–5954.
10. Tian X, Zheng X, Sun Y, Fang R, Zhang S, *et al*. Molecular mechanisms and epidemiology of carbapenem-resistant *Escherichia coli* isolated from Chinese patients during 2002-2017. *Infect Drug Resist* 2020;13:501–512.
11. Soliman AM, Khalifa HO, Ahmed AM, Shimamoto T, Shimamoto T. Emergence of an NDM-5-producing clinical *Escherichia coli* isolate in Egypt. *Int J Infect Dis* 2016;48:46–48.
12. Sumbana JJ, Santona A, Fiamma M, Taviani E, Deligios M, *et al*. Extraintestinal pathogenic *Escherichia coli* ST405 isolate coharboring blaNDM-5 and blaCTXM-15: a new threat in Mozambique. *Microb Drug Resist* 2021;27:1633–1640.
13. Yang P, Xie Y, Feng P, Zong Z. bla_{NDM-5} carried by an IncX3 plasmid in *Escherichia coli* sequence type 167. *Antimicrob Agents Chemother* 2014;58:7548–7552.
14. Li X, Fu Y, Shen M, Huang D, Du X, *et al*. Dissemination of bla_{NDM-5} gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. *Antimicrob Resist Infect Control* 2018;7:59.
15. Ouchar Mahamat O, Lounnas M, Hide M, Tidjani A, Benavides J, *et al*. Spread of NDM-5 and OXA-181 carbapenemase-producing *Escherichia coli* in Chad. *Antimicrob Agents Chemother* 2019;63:11.
16. Zhang LP, Xue WC, Meng DY. First report of New Delhi metallo-β-lactamase 5 (NDM-5)-producing *Escherichia coli* from blood cultures of three leukemia patients. *Int J Infect Dis* 2016;42:45–46.
17. Xu L, Wang P, Cheng J, Qin S, Xie W. Characterization of a novel bla_{ndm-5}-harboring IncFII plasmid and an mcr-1-bearing IncI2

plasmid in a single *Escherichia coli* st167 clinical isolate. *Infect Drug Resist* 2019;12:511–519.

18. Giufrè M, Errico G, Accogli M, Monaco M, Villa L, *et al*. Emergence of NDM-5-producing *Escherichia coli* sequence type 167 clone in Italy. *Int J Antimicrob Agents* 2018;52:76–81.
19. Garcia-Fernandez A, Villa L, Bibbolino G, Bressan A, Trancassini M, *et al*. Novel insights and features of the NDM-5-producing *Escherichia coli* sequence type 167 high-risk clone. *mSphere* 2020;5:e00269-20.
20. Sassi A, Loucif L, Gupta SK, Dekhil M, Chettibi H, *et al*. NDM-5 carbapenemase-encoding gene in multidrug-resistant clinical isolates of *Escherichia coli* from Algeria. *Antimicrob Agents Chemother* 2014;58:5606–5608.
21. Muggeo A, Maiga A, Maiga I, Brasme L, Dicko OA, *et al*. First description of IncX3 NDM-5-producing plasmid within *Escherichia coli* ST448 in Mali. *J Med Microbiol* 2020;69:685–688.
22. Moyo S, Haldorsen B, Aboud S, Blomberg B, Maselle SY, *et al*. Identification of VIM-2-producing *Pseudomonas aeruginosa* from Tanzania is associated with sequence types 244 and 640 and the location of bla_{VIM-2} in a TniC integron. *Antimicrob Agents Chemother* 2015;59:682–685.
23. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant Gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. *Biomed Res Int* 2014;2014:303104.
24. Moyo SJ, Manyahi J, Hubbard ATM, Byrne RL, Masoud NS, *et al*. Molecular characterisation of the first New Delhi metallo-β-lactamase 1-producing *Acinetobacter baumannii* from Tanzania. *Trans R Soc Trop Med Hyg* 2021;115:1080–1085.
25. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement CLSI Document M100–S20*. Wayne PA: Clinical and Laboratory Standards Institute; 2019.
26. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13:e1005595.
27. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
28. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, *et al*. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
29. Gilchrist CLM, Chooi YH. Clinker & clustermap.js: Automatic generation of gene cluster comparison figures. *Bioinformatics* 2021:btab007.
30. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* 2011;12:402.
31. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, *et al*. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020;75:3491–3500.
32. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, *et al*. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 2014;52:1501–1510.
33. Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, *et al*. Detection of mobile genetic elements associated with antibiotic resistance in *Salmonella enterica* using a newly developed web tool: MobileElementFinder. *J Antimicrob Chemother* 2021;76:101–109.
34. Kaas RS, Leekitcharoenphon P, Aarestrup FM, Lund O. Solving the problem of comparing whole bacterial genomes across different sequencing platforms. *PLoS One* 2014;9:e104984.
35. Manyahi J, Kibwana U, Mgimba E, Majigo M. Multi-drug resistant bacteria predict mortality in bloodstream infection in a tertiary setting in Tanzania. *PLoS One* 2020;15:e0220424.
36. Horumpende PG, Mshana SE, Mouw EF, Mmbaga BT, Chilongola JO, *et al*. Point prevalence survey of antimicrobial use in three hospitals in North-Eastern Tanzania. *Antimicrob Resist Infect Control* 2020;9:149.
37. Gamal D, Fernández-Martínez M, El-Defrawy I, Ocampo-Sosa AA, Martínez-Martínez L. First identification of NDM-5 associated with OXA-181 in *Escherichia coli* from Egypt. *Emerg Microbes Infect* 2016;5:e30.
38. Poirel L, Goutines J, Aires-de-Sousa M, Nordmann P. High rate of association of 16S rRNA methylases and carbapenemases in *Enterobacteriaceae* recovered from hospitalized children in Angola. *Antimicrob Agents Chemother* 2018;62:e00021-18.
39. Pedersen T, Sekyere JO, Govinden U, Moodley K, Sivertsen A, *et al*. Spread of plasmid-encoded NDM-1 and GES-5 carbapenemases among extensively drug-resistant and pandrug-resistant clinical *Enterobacteriaceae* in Durban, South Africa. *Antimicrob Agents Chemother* 2018;62:e02178-17.
40. Brinkac LM, White R, D'Souza R, Nguyen K, Obaro SK, *et al*. Emergence of New Delhi metallo-β-lactamase (NDM-5) in *Klebsiella quasipneumoniae* from neonates in a Nigerian hospital. *mSphere* 2019;4:e00685-18.
41. Kumwenda GP, Sugawara Y, Abe R, Akeda Y, Kasambara W, *et al*. First Identification and genomic characterization of multidrug-resistant carbapenemase-producing *Enterobacteriaceae* clinical isolates in Malawi, Africa. *J Med Microbiol* 2019;68:1707–1715.
42. Lewis JM, Lester R, Mphasa M, Banda R, Edwards T, *et al*. Emergence of carbapenemase-producing Enterobacteriaceae in Malawi. *J Glob Antimicrob Resist* 2020;20:225–227.
43. Huang Y, Yu X, Xie M, Wang X, Liao K, *et al*. Widespread dissemination of carbapenem-resistant *Escherichia coli* sequence type 167 strains harboring bla_{NDM-5} in clinical settings in China. *Antimicrob Agents Chemother* 2016;60:4364–4368.
44. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, *et al*. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. *Plasmid* 2012;68:43–50.
45. Mouftah SF, Pál T, Darwish D, Ghazawi A, Villa L, *et al*. Epidemic IncX3 plasmids spreading carbapenemase genes in the United Arab Emirates and worldwide. *Infect Drug Resist* 2019;12:1729–1742.
46. Zou H, Jia X, Liu H, Li S, Wu X, *et al*. Emergence of NDM-5-producing *Escherichia coli* in a teaching hteaching hospital in Chongqing, China: IncF-type plasmids may contribute to the prevalence of bla_{ndm-5}. *Front Microbiol* 2020;11:11–334.
47. Zhu Y-Q, Zhao J-Y, Xu C, Zhao H, Jia N, *et al*. Identification of an NDM-5-producing *Escherichia coli* sequence type 167 in a neonatal patient in China. *Sci Rep* 2016;6:29934.
48. Flerlage T, Brazelton de Cardenas JN, Garner CD, Hasan NA, Karathia H, *et al*. Multiple NDM-5-expressing *Escherichia coli* isolates from an immunocompromised pediatric host. *Open Forum Infect Dis* 2020;7:ofaa018.
49. Nakano R, Nakano A, Hikosaka K, Kawakami S, Matsunaga N, *et al*. First report of metallo-β-lactamase NDM-5-producing *Escherichia coli* in Japan. *Antimicrob Agents Chemother* 2014;58:7611–7612.
50. Krishnaraju M, Kamatchi C, Jha AK, Devasena N, Vennila R, *et al*. Complete sequencing of an IncX3 plasmid carrying bla_{NDM-5} allele reveals an early stage in the dissemination of the bla_{NDM} gene. *Indian J Med Microbiol* 2015;33:30–38.

Paper IV

Journal of Global Antimicrobial Resistance 22 (2020) 749–753

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

High rate of antimicrobial resistance and multiple mutations in the dihydrofolate reductase gene among *Streptococcus pneumoniae* isolated from HIV-infected adults in a community setting in Tanzania

Joel Manyahi[a,b,c,*], Sabrina Moyo[a,c], Said Aboud[c], Nina Langeland[a,b], Bjørn Blomberg[a,b]

[a] Department of Clinical Science, University of Bergen, Bergen, Norway
[b] National Centre for Tropical Infectious Diseases, Department of Medicine, Haukeland University Hospital, Bergen, Norway
[c] Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania

ARTICLE INFO

Article history:
Received 21 April 2020
Received in revised form 3 June 2020
Accepted 28 June 2020
Available online 9 July 2020

Keywords:
Antimicrobial resistance
Streptococcus pneumoniae
HIV
Tanzania
Dihydrofolate reductase

ABSTRACT

Objectives: The aim of this study was to characterize molecular mechanisms of resistance to trimethoprim and other antibiotics in *Streptococcus pneumoniae* isolates from HIV-infected adults in Dar es Salaam, Tanzania.

Methods: A total of 1877 nasopharyngeal swabs were collected and screened for pneumococcal colonization from 537 newly diagnosed individuals with HIV at four clinic visits during a 1-year follow-up from 2017–2018 as part of the randomized clinical trial CoTrimResist (ClinicalTrials.gov ID: NCT03087890).

Results: A total of 76 pneumococcal isolates were obtained. Of the 70 isolates that could be serotyped, 42 (60.0%) were vaccine serotypes included in pneumococcal conjugate vaccine 23 (PCV23). The majority of isolates (73.7%; 56/76) were non-susceptible to penicillin (MICs of 0.06–2 μg/mL). Isolates were frequently resistant to co-trimoxazole (trimethoprim/sulfamethoxazole) (71.1%) but less so to azithromycin (22.4%), erythromycin (21.1%), chloramphenicol (18.4%), tetracycline (14.5%), clindamycin (10.5%) and levofloxacin (0%). Moreover, 26.3% were multidrug-resistant (resistant to ≥3 antibiotic classes). Vaccine-type pneumococci were resistant to more classes of antibiotics, were more frequently resistant to erythromycin, azithromycin, clindamycin and tetracycline, and had higher MICs to penicillin (median, 0.19 μg/mL; range, 0.002–1.5 μg/mL) compared with non-vaccine serotypes (median, 0.125 μg/mL; range, 0.012–0.25 μg/mL) ($P = 0.003$). Co-trimoxazole-resistant isolates carried from 1 to 11 different mutations in the dihydrofolate reductase (DHFR) gene, most commonly Ile100Leu (100%), Glu20Asp (91.8%), Glu94Asp (61.2%), Leu135Phe (57.1%), His26Tyr (53.1%), Asp92Ala (53.1%) and His120Gln (53.1%).

Conclusion: Streptococcus pneumoniae isolated from HIV-diagnosed patients were frequently non-susceptible to penicillin and co-trimoxazole. Most isolates carried multiple mutations in DHFR.

1. Introduction

Streptococcus pneumoniae is a common cause of invasive and non-invasive diseases. Unfortunately, pneumococcal disease remains a primary cause of morbidity and mortality in immunocompetent and immunodeficient populations [1,2]. Nasopharyngeal colonization with *S. pneumoniae* is considered a prerequisite both for invasive and non-invasive pneumococcal diseases [3,4]. In human immunodeficiency virus (HIV) infection, widespread use of co-trimoxazole (trimethoprim/sulfamethoxazole) and other antibiotics has been associated with increased carriage of multidrug-resistant (MDR) bacteria, including MDR *S. pneumoniae* [5–8]. Infections with penicillin-resistant strains are difficult to treat and are associated with increased morbidity and mortality as well as increased healthcare costs [2].

Few studies have been carried out in Tanzania on pneumococcal nasopharyngeal carriage. Among these, some have found that HIV-exposed and non-exposed children have high rates of *S. pneumoniae* resistant to commonly prescribed antibiotics, including co-trimoxazole and penicillin [9–11]. Likewise, nasopharyngeal *S. pneumoniae* isolates with non-susceptibility to commonly prescribed antibiotics such as penicillin, macrolides and tetracycline have been reported previously in healthy children in Democratic

* Corresponding author at: Muhimbili University of Health and Allied Sciences, P. O. Box 65001, Dar es Salaam, Tanzania.
E-mail addresses: manyahijoel@yahoo.com, joel.manyahi@uib.no (J. Manyahi).

http://dx.doi.org/10.1016/j.jgar.2020.06.026

Republic of Congo [12] and HIV-infected patients in Cameroon [13].

In recent years, trimethoprim has rarely been used alone in the treatment of bacterial infections, with the exception of urinary tract infections. The combination of trimethoprim and sulfamethoxazole (co-trimoxazole) has been used extensively instead in the treatment of respiratory tract infections, urinary tract infections and gastrointestinal tract infections [14]. Resistance to either trimethoprim or sulfamethoxazole renders bacteria resistant to co-trimoxazole as well. Resistance to trimethoprim and sulfamethoxazole is conferred by acquisition of mutations in the dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genes, respectively. Studies have shown that in *S. pneumoniae*, a single substitution of amino acid isoleucine at position 100 with leucine in DHFR is sufficient to confer resistance to trimethoprim [15,16]. A recent report from Malawi found that substitution of amino acid at position 92 of DHFR without Ile100Leu could also confer resistance to trimethoprim [17]. However, in previous studies multiple mutations have been observed in the DHFR gene in *S. pneumoniae*, although their role in conferring trimethoprim resistance is not well known.

In Tanzania, no previous study has assessed the molecular basis of co-trimoxazole resistance in *S. pneumoniae* isolated from HIV-infected adults. Moreover, there are limited data on *S. pneumoniae* colonization and antimicrobial resistance among HIV-infected adults from community settings in Tanzania. The aim of this study was to determine the molecular mechanisms conferring trimethoprim resistance in *S. pneumoniae* as well as to understand the antimicrobial resistance patterns of *S. pneumoniae* colonizing the nasopharynx of HIV-infected adults from a community setting in Tanzania.

2. Materials and methods

2.1. Study participants

Newly diagnosed adults with HIV were recruited from six HIV care and treatment clinics at Amana, Mwananyamala, Temeke Regional Referral, PASADA, Mbagala and Mnazi Mmoja hospitals in Dar es Salaam (Tanzania) as part of the randomized clinical trial CoTrimResist (ClinicalTrials.gov ID: NCT03087890) to assess any effect of prolonged co-trimoxazole prophylaxis on emerging antimicrobial resistance in HIV patients (data not yet analyzed). A total of 537 participants were recruited at baseline between April 2017 and May 2018 and were followed-up for 1 year.

2.2. Microbiological methods

2.2.1. Specimen collection, isolation and identification of Streptococcus pneumoniae

A total of 1877 nasopharyngeal swabs were collected at baseline, at Day 14 and at Weeks 24 and 48. Nasopharyngeal swabs were collected by a trained clinician from each healthcare facility using Sigma Transwab® and were transported immediately in liquid Amies transport medium [Sigma Transwab® PF with Liquid Amies; MWE Co (Bath) Ltd., Corsham, UK] in a cool box at 4 °C. Upon receipt in the laboratory, nasopharyngeal samples were immediately inoculated onto 5% sheep blood agar and were incubated at 37 °C in 5% CO_2 for 24 h. Identification of *S. pneumoniae* was made by colonial morphology, presence of α-haemolysis, optochin susceptibility and bile salt solubility.

2.2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller–Hinton agar supplemented with 5% sheep blood and plates were incubated at 35 °C in 5% CO_2 for 20–24 h. Minimum inhibitory concentrations (MICs) for penicillin, azithromycin and trimethoprim/sulfamethoxazole were determined by Etest (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing for chloramphenicol, tetracycline, erythromycin, clindamycin and levofloxacin was performed by the Kirby–Bauer disk diffusion method. Antimicrobial susceptibility test results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. MDR was defined as bacteria resistant to three or more classes/categories of antibiotics [18].

2.2.3. Nucleic acid extraction

From pure growth, 5–10 colonies were suspended in 500 μL of phosphate-buffered saline (PBS). DNA was extracted using a MagNA Pure LC instrument (Roche Diagnostics, Mannheim, Germany) using a Total Nucleic Acid Isolation Kit (Roche Diagnostics). Extracted DNA was eluted in 100 μL of elution buffer. DNA templates were stored at −20 °C until further analysis.

2.2.4. PCR

PCR for detection of the DHFR gene was performed using 2 × QuantiTect® Multiplex PCR NoROX Master Mix (QIAGEN) and amplification was carried out on a GeneAmp™ 9700 Thermocycler (Applied Biosystems, Foster City, CA, USA). The following set of primers was used as previously described [16]: 5′-TGT AAG CTA TTC CAA ACC AG-3′ and 5′-CTA CGT TCC ATT AGA CTT CC-3′ (PCR product, 760 bp). PCR conditions consisted of initial denaturation at 95 °C for 15 min, followed by denaturation at 94 °C for 60 s, annealing at 45 °C for 30 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. A final reaction volume of 25 μL consisted of the following: 12.5 μL of 2× QuantiTect® Multiplex PCR NoROX Master Mix, 1 μL of each primer (0.4 μM), 8.5 μL of RNase-free water and 2 μL of DNA template. Amplified PCR products were analyzed by gel electrophoresis.

2.2.5. DNA sequencing

Amplified PCR products were purified and both strands were sequenced using the same primers as for PCR. Sequencing was performed using an ABI PRISM 3730 DNA Analyzer (Applied Biosystems) with a BigDye™ Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems). SnapGene® v.5.0.7 software (GSL Biotech LLC, Chicago, IL, USA) was used to assemble, edit and analyze the sequences.

2.2.6. Serotyping of Streptococcus pneumoniae isolates

Serotyping was performed from an overnight growth of *S. pneumoniae* on 5% sheep blood agar using a commercial kit for latex agglutination (Immulex™ Pneumotest Kit; SSI Diagnostica A/S, Hillerød, Denmark). The agglutination kit contains latex particles coated with rabbit antibodies that react with specific pneumococcal capsular polysaccharides. Performance and interpretation of the test results followed the manufacturer's instructions.

2.3. Statistical analysis

Categorical variables were presented as the proportion, and continuous variables were presented using the median and range. Proportions of resistant bacteria between vaccine- and non-vaccine serotype isolates were compared by χ^2 test, and the medians of MICs were compared by Wilcoxon rank-sum test using STATA v.16.0 (StataCorp LLC, College Station, TX, USA). A *P*-value of <0.05 was defined as the cut-off for statistical significance.

3. Results

3.1. Streptococcus pneumoniae *isolates*

A total of 76 *S. pneumoniae* were isolated from 1887 nasopharyngeal swabs. The number of *S. pneumoniae* isolates obtained at different time points was as follows: 20 at baseline ($n = 537$ swabs); 13 at Day 14 ($n = 509$ swabs); 17 at Week 24 ($n = 436$ swabs); and 26 at Week 48 ($n = 395$ swabs).

3.2. Serotyping of Streptococcus pneumoniae *isolates*

The majority of isolates (55.3%, 42/76) were serotypes present in the pneumococcal conjugate vaccine 23 (PCV23), whilst 36.8% (28/76) were non-vaccine serotypes and 7.9% (6/76) could not be typed by the method used. The most frequent conjugate vaccine serotypes were 19 (9/42), 3 (8/42), 7 (6/42) and 15 (4/42) (Fig. 1).

3.3. Antimicrobial susceptibility testing

Table 1 shows the number and percentage of *S. pneumoniae* resistant to different antibiotics. The majority of isolates (73.7%; 56/76) were penicillin-non-susceptible (MICs of 0.06–2 μg/mL), but no isolate was fully penicillin-resistant. Most isolates were also resistant to co-trimoxazole (71.1%; 54/76). In addition, co-trimoxazole resistance was significantly more frequent in pneumococci with non-susceptibility to penicillin (82.1%; 46/56) than in fully penicillin-susceptible isolates (40.0%; 8/20) ($P < 0.001$).

Rates of resistance to azithromycin, erythromycin, chloramphenicol, tetracycline and clindamycin were 22.4% (17/76), 21.1% (16/76), 18.4% (14/76), 14.5% (11/76) and 10.5% (8/76), respectively. All isolates were susceptible to levofloxacin. Approximately one-quarter of the isolates (26.3%; 20/76) were MDR. Vaccine-type *S. pneumoniae* were resistant to more classes of antibiotics compared with non-vaccine serotype *S. pneumoniae* [median of 3 (range 3–5) vs. 2.5 (range 0–5); $P = 0.03$] and were more frequently resistant to erythromycin (33.3% vs. 7.1%; $P = 0.011$), azithromycin (33.3% vs. 10.7%; $P = 0.031$), clindamycin (19.0% vs. 0.0%; $P = 0.014$) and tetracycline (23.8% vs. 0.0%; $P = 0.005$). Although vaccine serotype isolates displayed significantly higher MICs to penicillin (median, 0.19 μg/mL; range, 0.002–1.5 μg/mL) compared with non-vaccine serotype isolates (median, 0.125 μg/mL; range, 0.012–0.25 μg/mL) ($P = 0.003$), there were no significant differences in the proportions of isolates with non-susceptibility to penicillin. Neither co-trimoxazole MICs nor the proportion of co-trimoxazole resistance was significantly different between vaccine and non-vaccine serotypes.

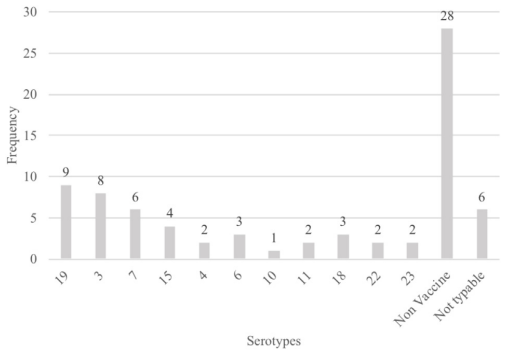


Fig. 1. Serotypes distribution among nasopharyngeal *Streptococcus pneumoniae* isolates from HIV-infected adults in Tanzania ($n = 76$).

3.4. Mutations in dihydrofolate reductase (DHFR)

Among the 61 *S. pneumoniae* isolates with a co-trimoxazole MIC > 2 μg/mL, 49 were successfully sequenced and had nucleotide sequences available for analysis.

Co-trimoxazole-resistant isolates carried from 1 to 11 different mutations in the DHFR gene, with the majority (71.4%; 35/49) having 5 to 9 mutations (Table 2). The most common mutations conferring resistance to trimethoprim were substitution of Ile100Leu (100%), Glu20Asp (91.8%), Glu94Asp (61.2%), Leu135Phe (57.1%), His26Tyr (53.1%), Asp92Ala (53.1%) and His120Gln (53.1%) (Table 3). There was no difference in the number of mutations in the DHFR gene between vaccine and non-vaccine serotype pneumococci (median, 5.5; range, 0–11 for both; $P = 0.4$). There was no significant association between co-trimoxazole MICs and the number or types of mutations observed.

4. Discussion

This study demonstrated that *S. pneumoniae* isolated from the nasopharynx of HIV-infected adults were frequently resistant to commonly prescribed antibiotics in resource-limited settings. Approximately one-quarter of the isolates were MDR bacteria. The non-susceptibility of *S. pneumoniae* to penicillin and co-trimoxazole is worrisome as these antibiotics are commonly used as first-line treatment for pneumococcal pneumonia in resource-constrained countries.

The high rate of co-trimoxazole-resistant *S. pneumoniae* colonizing the nasopharynx observed in this study is in line with findings from HIV-infected populations in Tanzania [9] and other resource-limited settings [19,20]. Co-trimoxazole is widely available over the counter in resource-constrained counties. Irrational use of co-trimoxazole could possibly explain the observed finding. Previous studies have indicated that co-trimoxazole use increases the risk of carriage of co-trimoxazole-resistant *S. pneumoniae* [21–23].

Previous studies have found that trimethoprim resistance mutations, more than sulfamethoxazole resistance mutations, are correlated with resistance to trimethoprim/sulfamethoxazole (co-trimoxazole) [15]. In the current study, the DHFR genes of co-trimoxazole-resistant *S. pneumoniae* ($n = 49$) were sequenced to determine alterations in the chromosomal DHFR gene conferring pneumococcal resistance to trimethoprim. Overall, substitutions were detected in up to 11 amino acid positions; these substitutions were far fewer than those reported previously among 68 trimethoprim-resistant *S. pneumoniae* in North America [16]. Previous studies have demonstrated that substitution of Ile100Leu is critical for development of trimethoprim resistance in *S. pneumoniae* [15,17,24]. In the current study, it was also found that all sequenced co-trimoxazole-resistant pneumococcal isolates had the same mutation of Ile100Leu. Mutations at other locations are thought to increase the MIC of trimethoprim [17]. Mutations of Glu20Asp (91.8%), Glu94Asp (61.2%) and Leu135Phe (57.1%) were also frequently found as documented previously [16,25]. A recent study in Malawi [17] reported that mutation at residue 92 without substitution of Ile100Leu was associated with an increase MIC of trimethoprim-resistant pneumococci. In the current study, a number of isolates with substitution at residue 92 were found, but all of them also had the Ile100Leu mutation. This study did not investigate the mechanism of resistance to sulfamethoxazole, the other ingredient of trimethoprim/sulfamethoxazole (co-trimoxazole). However, the study confirmed the high prevalence of known resistance mutations in the DHFR gene associated with

Table 1
Distribution of *Streptococcus pneumoniae* resistance to various antibiotics in HIV-infected adult patients.

Antibiotic	% (*n*)				*P*-value*
	All (*n* = 76)	Vaccine serotype (*n* = 42)	Non-vaccine serotype (*n* = 28)	Non-typeable (*n* = 6)	
Penicillin-non-susceptible[a]	73.7 (56)	78.6 (33)	64.3 (18)	83.3 (5)	0.188
Co-trimoxazole-resistant[a]	71.1 (54)	76.2 (32)	67.9 (19)	50.0 (3)	0.442
Azithromycin-resistant[a]	22.4 (17)	33.3 (14)	10.7 (3)	0.0 (0)	0.031
Erythromycin-resistant[b]	21.1 (16)	33.3 (14)	7.1 (2)	0.0 (0)	0.011
Clindamycin-resistant[b]	10.5 (8)	19.0 (8)	0.0 (0)	0.0 (0)	0.014
Tetracycline-resistant [b]	14.5 (11)	23.8 (10)	0.0 (0)	16.7 (1)	0.005
Chloramphenicol-resistant[b]	18.4 (14)	21.4 (9)	7.1 (2)	50.0 (3)	0.108
Levofloxacin-resistant[b]	0.0 (0)	0 (0)	0 (0)	0 (0)	–

[a] Determined by Etest.
[b] Determined by the Kirby–Bauer disk diffusion method.
* *P*-value for difference between vaccine and non-vaccine serotype isolates (χ^2 test).

Table 2
Prevalence of mutations in and median co-trimoxazole minimum inhibitory concentrations (MICs) of co-trimoxazole-resistant *Streptococcus pneumonia* isolates (*n* = 49) from HIV-infected adult patients in Tanzania.

No. of mutations	*n* (%)	Median MIC (range)
1	2 (4.1)	3 (3–3)
2	2 (4.1)	19 (6–32)
3	2 (4.1)	4 (4–4)
4	2 (4.1)	5 (2–8)
5	5 (10.2)	8 (4–32)
6	9 (18.4)	16 (4–32)
7	8 (16.3)	5 (2–16)
8	9 (18.4)	3 (2–32)
9	4 (8.2)	3.5 (2–7)
10	2 (4.1)	10 (4–16)
11	4 (8.2)	7 (4–8)

Table 3
Types of mutation and median co-trimoxazole minimum inhibitory concentrations (MICs) of co-trimoxazole-resistant *Streptococcus pneumoniae* isolates (*n* = 49) from HIV-infected adult patients in Tanzania.

Mutation	Prevalence [*n* (%)]	Median MIC (range) (μg/mL)		*P*-value*
		Mutation present	Mutation absent	
E20D	45 (91.8)	6 (2–32)	3 (3–32)	0.4
H26Y	26 (53.1)	6 (2–32)	4 (2–32)	0.7
P70L	5 (10.2)	4 (3–32)	6 (2–32)	0.5
P70S	17 (34.7)	4 (2–32)	6 (2–32)	0.2
A78T	16 (32.7)	4 (2–16)	6 (2–32)	0.06
Q81H	12 (24.5)	4 (2–32)	7 (2–32)	0.1
Q81Y	11 (22.4)	6 (2–8)	5 (2–32)	0.5
V83I	15 (30.6)	4 (2–32)	6 (2–32)	0.7
Q91H	5 (10.2)	8 (4–16)	4 (2–32)	0.3
D92A	26 (53.1)	6 (2–32)	4 (2–32)	0.9
D92V	3 (6.1)	4 (4–32)	5 (2–32)	0.7
D92G	13 (26.5)	7 (2–32)	4 (2–32)	0.9
E94D	30 (61.2)	6.5 (2–32)	4 (2–32)	0.2
I100L	49 (100.0)	6 (2–32)	N/A	–
H120Q	26 (53.1)	6 (2–32)	4 (2–32)	0.7
L135F	28 (57.1)	6.5 (2–32)	4 (2–32)	0.4

N/A, not applicable.
* Wilcoxon rank-sum test (Mann–Whitney).

trimethoprim resistance in *S. pneumoniae* isolates. Hence, co-trimoxazole might not be effective to treat pneumococcal infection in HIV-infected individuals.

The rate of penicillin-non-susceptibility in isolates of *S. pneumoniae* (73.7%) in the current study is comparable with that found among children in the pre-PCV era (67.8–69.2%) [9,10] and post-PCV era (31–53%) [11] in Tanzania. However, in the current study a much higher rate was found than in a recent study in Ghana that reported only 25.9% of penicillin-non-susceptible *S. pneumoniae* from the nasopharynx among HIV-infected individuals in the PCV era [19]. Although none of the *S. pneumoniae* isolates had a high level of resistance to penicillin (>2 μg/mL), the current finding questions the appropriateness of using penicillin for the treatment of severe pneumococcal infections such as meningitis in Tanzania. However, at high intravenous doses, it can still be used to treat non-meningeal pneumococcal infections. Fully penicillin-resistant *S. pneumoniae* have been reported elsewhere in Africa [26,27]. Although they are currently uncommon in Tanzania, there is need for continuous surveillance to monitor the emergence of fully penicillin-resistant strains.

Interestingly, vaccine serotype isolates of *S. pneumoniae* showed higher rates of resistance to erythromycin, azithromycin, clindamycin and tetracycline. The background for this may be that vaccine serotype were selected for use in vaccines because they were quite virulent. With a history of such virulence, the ancestor bacterium of the vaccine serotypes may have caused much illness and elicited more antibiotic use, which in turn may have selected for re-emerging drug resistance. Both tetracycline and macrolides, particularly erythromycin, have been used extensively as they are oral medicines with broad-spectrum activity [28]. What we see now may thus be the result of antibiotic use in the pre-vaccine era. Our observation is similar to a previous study on clinical isolates which found that vaccine serotypes displayed more multidrug resistance compared with non-vaccine serotypes [29].

The current findings are in line with other studies from different populations of children in Tanzania [9–11] as well as studies from Ghana and Cameroon of nasopharyngeal carriage in HIV-infected adults [13,19] and children [26], which have reported low rates of *S. pneumoniae* resistant to erythromycin in the PCV era.

The relatively low rates of resistance to azithromycin documented in this study are also comparable with the findings reported previously in semi-urban settings in Tanzania [30,31]. In a previous study conducted in Tanzania, mass administration of azithromycin was found to correlate with an increased risk for nasopharyngeal carriage of azithromycin-resistant *S. pneumoniae* [32]. Based on the current findings, both azithromycin and erythromycin could still be an option for non-severe *S. pneumoniae* infections in HIV-infected patients. However, rational use of macrolides needs to be advocated in the country, as observed in a previous 6-month cohort study in central Tanzania [32].

5. Conclusions

Streptococcus pneumoniae isolated from HIV-infected adult patients were frequently non-susceptible to penicillin and resistant to co-trimoxazole. The majority of these isolates displayed MDR traits. Most isolates carried multiple mutations in the DHFR gene and all carried the Ile100Leu substitution.

Funding

This study was supported by Helse Bergen HF, Haukeland University Hospital, Norway through project number 912132. The funders had no role in the study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

Competing interests

None declared.

Ethical approval

Ethical approval to conduct the study in Tanzania was obtained from the Muhimbili University of Heath and Allied Sciences Senate Research and Publications Committee [Ref. No. 2015-10-27/AEC/Vol.X/54], the National Ethics Health Research Ethics Committee [Ref. No. NIMRIHQ/R. SaJVol. 1X12144], the Tanzania Food and Drug Authority [Ref. No. TZ16CT007] and the Regional Committee for Medical and Health Research Ethics of Western Norway [Ref. No. REK2015/540]. Written informed consent was obtained from each study participant prior to enrolment in the study.

Acknowledgments

The authors acknowledge members of the Department of Clinical Science, University of Bergen (Bergen, Norway) for their technical support during the molecular study.

References

[1] Grau I, Ardanuy C, Liñares J, Podzamczer D, Schulze MH, Pallares R. Trends in mortality and antibiotic resistance among HIV-infected patients with invasive pneumococcal disease. HIV Med 2009;10:488–95.
[2] Tleyjeh IM, Tlaygeh HM, Hejal R, Montori VM, Baddour LM. The impact of penicillin resistance on short-term mortality in hospitalized adults with pneumococcal pneumonia: a systematic review and meta-analysis. Clin Infect Dis 2006;42:788–97.
[3] Albrich WC, Madhi SA, Adrian PV, van Niekerk N, Telles JN, Ebrahim N, et al. Pneumococcal colonisation density: a new marker for disease severity in HIV-infected adults with pneumonia. BMJ Open 2014;4:e005953.
[4] Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004;4:144–54.
[5] Gill CJ, Mwanakasale V, Fox MP, Chilengi R, Tembo M, Nsofwa M, et al. Effect of presumptive co-trimoxazole prophylaxis on pneumococcal colonization rates, seroepidemiology and antibiotic resistance in Zambian infants: a longitudinal cohort study. Bull World Health Organ 2008;86:929–38.
[6] Polack FP, Flayhart DC, Zahurak ML, Dick JD, Willoughby RE. Colonization by *Streptococcus pneumoniae* in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2000;19:608–12.
[7] Morpeth SC, Thielman NM, Ramadhani HO, Hamilton JD, Ostermann J, Kisenge PR, et al. Effect of trimethoprim–sulfamethoxazole prophylaxis on antimicrobial resistance of fecal *Escherichia coli* in HIV-infected patients in Tanzania. J Acquir Immune Defic Syndr 2008;47:585–91.
[8] Chiller TM, Polyak CS, Brooks JT, Williamson J, Ochieng B, Shi YP, et al. Daily trimethoprim–sulfamethoxazole prophylaxis rapidly induces corresponding resistance among intestinal Escherichia coli of HIV-infected adults in Kenya. J Int Assoc Physicians AIDS Care (Chic) 2009;8:165–9.
[9] Bles P, de Mast Q, van der Gaast-de Jongh CE, Kinabo GD, Kibiki G, van de Ven A, et al. Antibiotic resistance of *Streptococcus pneumoniae* colonising the nasopharynx of HIV-exposed Tanzanian infants. Trop Med Int Health 2015;20:1559–63.
[10] Moyo SJ, Steinbakk M, Aboud S, Mkopi N, Kasubi M, Blomberg B, et al. Penicillin resistance and serotype distribution of *Streptococcus pneumoniae* in nasopharyngeal carrier children under 5 years of age in Dar es Salaam, Tanzania. J Med Microbiol 2012;61:952–9.
[11] Emgard M, Msuya SE, Nyombi BM, Mosha D, Gonzales-Siles L, Norden R, et al. Carriage of penicillin-non-susceptible pneumococci among children in northern Tanzania in the 13-valent pneumococcal vaccine era. Int J Infect Dis 2019;81:156–66.
[12] Birindwa AM, Emgard M, Norden R, Samuelsson E, Geravandi S, Gonzales-Siles L, et al. High rate of antibiotic resistance among pneumococci carried by healthy children in the eastern part of the Democratic Republic of the Congo. BMC Pediatr 2018;18:361.
[13] Kengne M, Lebogo MBB, Nwobegahay JM, Ondigui BE. Antibiotics susceptibility pattern of *Streptococcus pneumoniae* isolated from sputum cultures of human immunodeficiency virus infected patients in Yaoundé, Cameroon. Pan Afr Med J 2018;31:16.
[14] Fehintola FA. Cotrimoxazole, clinical uses and malaria chemotherapy. Afr J Med Med Sci 2010;39:63–8.
[15] Adrian PV, Klugman KP. Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1997;41:2406–13.
[16] Zhanel GG, Wang X, Nichol K, Nikulin A, Wierzbowski AK, Mulvey M, et al. Molecular characterisation of Canadian paediatric multidrug-resistant *Streptococcus pneumoniae* from 1998–2004. Int J Antimicrob Agents 2006;28:465–71.
[17] Cornick JE, Harris SR, Parry CM, Moore MJ, Jassi C, Kamng'ona A, et al. Genomic identification of a novel co-trimoxazole resistance genotype and its prevalence amongst *Streptococcus pneumoniae* in Malawi. J Antimicrob Chemother 2014;69:368–74.
[18] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
[19] Dayie NT, Baffuor-Asare M, Labi AK, Obeng-Nkrumah N, Olayemi E, Lartey M, et al. Epidemiology of pneumococcal carriage among HIV-infected individuals in the conjugate vaccine era: a study in Southern Ghana. Biomed Res Int 2019;2019:3427174.
[20] Verani JR, Massora S, Acacio S, Dos Santos RT, Vubil D, Pimenta F, et al. Nasopharyngeal carriage of Streptococcus pneumoniae among HIV-infected and -uninfected children < 5 years of age before introduction of pneumococcal conjugate vaccine in Mozambique. PLoS ONE 2018;13:e0191113.
[21] Mwenya DM, Charalambous BM, Phillips PP, Mwansa JC, Batt SL, Nunn AJ, et al. Impact of cotrimoxazole on carriage and antibiotic resistance of *Streptococcus pneumoniae* and *Haemophilus influenzae* in HIV-infected children in Zambia. Antimicrob Agents Chemother 2010;54:3756–62.
[22] Pemba L, Charalambous S, von Gottberg A, Magadla B, Moloi V, Seabi O, et al. Impact of cotrimoxazole on non-susceptibility to antibiotics in *Streptococcus pneumoniae* carriage isolates among HIV-infected mineworkers in South Africa. J Infect 2008;56:171–8.
[23] Karpanoja P, Nyberg ST, Bergman M, Voipio T, Paakkari P, Huovinen P, et al. Connection between trimethoprim–sulfamethoxazole use and resistance in *Streptococcus pneumoniae*,*Haemophilus influenzae*, and *Moraxella catarrhalis*. Antimicrob Agents Chemother 2008;52:2480–5.
[24] He M, Yao K, Shi W, Gao W, Yuan L, Yu S, et al. Dynamics of serotype 14 *Streptococcus pneumoniae* population causing acute respiratory infections among children in China (1997–2012). BMC Infect Dis 2015;15:266.
[25] Wilen M, Buwembo W, Sendagire H, Kironde F, Swedberg G. Cotrimoxazole resistance of *Streptococcus pneumoniae* and commensal streptococci from Kampala, Uganda. Scand J Infect Dis 2009;41:113–21.
[26] Dayie N, Tettey EY, Newman MJ, Bannerman E, Donkor ES, Labi AK, et al. Pneumococcal carriage among children under five in Accra, Ghana, five years after the introduction of pneumococcal conjugate vaccine. BMC Pediatr 2019;19:316.
[27] Manenzhe RI, Moodley C, Abdulgader SM, Robberts FJL, Zar HJ, Nicol MP, et al. Nasopharyngeal carriage of antimicrobial-resistant pneumococci in an intensively sampled South African birth cohort. Front Microbiol 2019;10:610.
[28] Mhalu FS, Mmari PW, Ijumba J. Rapid emergence of El Tor *Vibrio cholerae* resistant to antimicrobial agents during first six months of fourth cholera epidemic in Tanzania. Lancet 1979;1:345–7.
[29] Mayanskiy N, Alyabieva N, Ponomarenko O, Lazareva A, Katosova L, Ivanenko A, et al. Serotypes and antibiotic resistance of non-invasive *Streptococcus pneumoniae* circulating in pediatric hospitals in Moscow, Russia. Int J Infect Dis 2014;20:58–62.
[30] Ansah D, Weaver J, Munoz B, Bloch EM, Coles CL, Lietman T, et al. A cross-sectional study of the availability of azithromycin in local pharmacies and associated antibiotic resistance in communities in Kilosa District, Tanzania. Am J Trop Med Hyg 2019;100:1105–9.
[31] Bloch EM, West SK, Mabula K, Weaver J, Mrango Z, Munoz B, et al. Antibiotic resistance in young children in Kilosa District, Tanzania 4 years after mass distribution of azithromycin for trachoma control. Am J Trop Med Hyg 2017;97:815–8.
[32] Coles CL, Mabula K, Seidman JC, Levens J, Mkocha H, Munoz B, et al. Mass distribution of azithromycin for trachoma control is associated with increased risk of azithromycin-resistant *Streptococcus pneumoniae* carriage in young children 6 months after treatment. Clin Infect Dis 2013;56:1519–26.

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

uib.no

ISBN: 9788230861820 (print)
9788230857427 (PDF)