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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

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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

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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.

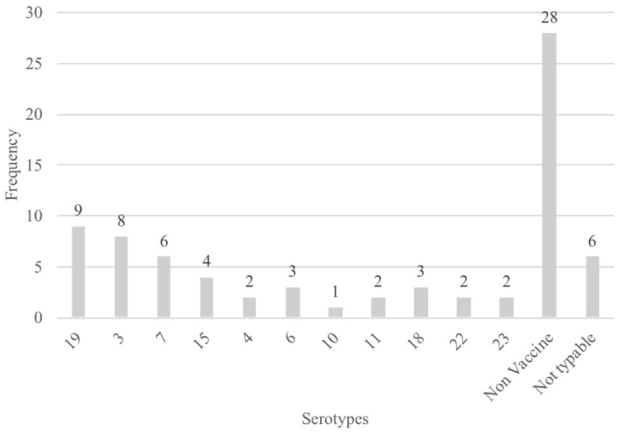

Fig. 1. Serotypes distribution among nasopharyngeal *Streptococcus pneumoniae* isolates from HIV-infected adults in Tanzania ($n = 76$).

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.

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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. NIMRlHQ/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.

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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.