Gastrointestinal colonization of extended-spectrum beta-lactamase-producing bacteria among children below five years of age hospitalized with fever in Dar es Salaam, Tanzania

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ABSTRACT

Objectives: Gastrointestinal colonization of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) is of concern because prior colonization increases risk for subsequent infections. To date, the link between ESBL-PE faecal carriage and the risk of subsequent ESBL-PE infection has not been well established, and information on carriage of such pathogens among children with invasive infections such as bloodstream infections (BSI) remains to be explored worldwide.
Methods: This cross-sectional study was conducted among children under the age of 5 years admitted for febrile illness in Dar es Salaam, Tanzania, between March 2017 and July 2018. We used rectal swabs to screen for ESBL-PE using selective media, ChromID ESBL. Bacterial isolates were identified by MALDI-TOF. Blood cultures were drawn from all children. Antimicrobial susceptibility testing was done using a disk diffusion method. ESBL alleles were identified by real-time PCR and sequencing.
Results: The overall prevalence of ESBL-PE carriage was 56% (112/200) and was highest among children 4 to 6 months old (17/21, 81%) ($P = 0.05$). Children with BSI had high ESBL-PE carriage (78.4%) compared to those without BSI (53.1%) ($P = 0.02$; aOR 3.4, 95% confidence interval 1.20–9.58). The most common isolate was *E. coli* (64/112, 45%). Sixteen pairs of ESBL-PE isolates (from the gut and from blood) had a similar antimicrobial susceptibility profile. We detected bla_{CTX-M} gene in 97% of all phenotypically detected ESBL-PE; among those, bla$_{CTX-M-15}$ was dominant (99%).
Conclusion: We report a high prevalence of ESBL-PE faecal carriage among children with BSI in Tanzania. Colonization of ESBL-PE was a risk factor for ESBL-BSI.

1. Introduction

The production of extended-spectrum β-lactamases (ESBLs) among Enterobacteriaceae is an emerging problem and associated with severe infections. There are increasing numbers of reports worldwide of high prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) causing different clinical infections such as bloodstream infections (BSI), urinary tract infections, and meningitis [1–3]. ESBL-PE are frequently co-resistant to non-beta-lactam antimicrobial agents and display multidrug resistance [4]. As a result, infections caused by ESBL-PE have serious consequences including increased morbidity and mortality [5–7].

The prevalence of ESBL-PE is high not only in clinical infections, but also in gastrointestinal colonization [8,9]. Worldwide, gastrointestinal colonization of ESBL-PE is reported to range from 10% to 48% among healthy and hospitalized children [10–13]. Gastrointestinal colonization of ESBL-PE is of concern because previous studies have shown that prior colonization of ESBL-PE increases the risk of infections such as bloodstream infections by the same

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ESBL-producing strains [14,15]. Recently, Falcone and colleagues demonstrated that adult patients colonized by carbapenemase-producing *K. pneumoniae* were at risk of developing bloodstream infection caused by the same pathogens [16].

Although in Tanzania there are some studies that have explored ESBL-PE colonization in healthy children [17,18], there is still limited information about hospitalized children with invasive infections and lack of data linking ESBL carriage and ESBL infection. Therefore, this study was carried out to investigate the magnitude of ESBL-PE carriage in hospitalized children with bloodstream infections, the diversity of ESBL genotypes, and the possible association between ESBL carriage and infection.

2. Materials and methods

2.1. Study site and population

This was part of a prospective cross-sectional study conducted from March 2017 to July 2018 in Dar es Salaam, Tanzania [19]. The study enrolled children below five years of age who were hospitalized because of fever ($>37.5°$C) at three regional hospitals, Amana, Temeke, and Mwananyamala, and one tertiary hospital, Muhimbili National Hospital (MNH). The sites involved are the main public hospitals serving the Dar es Salaam population of about 5 million people. The study [19] included 2226 children hospitalized with fever, with about 10% positive blood cultures. The purpose of this study was to evaluate the association between intestinal colonization and the risk of subsequent BSI caused by the same colonizing organism. We included 200 children. Among these, 83 children had confirmed Gram-negative Enterobacteriaceae BSI, and 117 children had negative blood cultures. A systematic sampling approach was used to select the children, whereby after every 11 children, a child was selected. If the child did not meet the inclusion criteria (complete clinical and social demographic information), the next child was selected. Enrolment alternated between children with positive BSI and without BSI to ensure that each group was well represented. Age and sex were also considered to match the BSI-positive children and those without BSI.

2.2. Data collection

As previously described [19], the study used REDCap, (Research Electronic Data Capture, Vanderbilt University, Nashville, TN) to gather demographic and clinical information including date of birth; sex; duration of fever; history of convulsions, vomiting, cough, diarrhoea and its duration; HIV status; antibiotic use one month prior to admission; and history of hospitalization in the last six months. Clinical information was recorded by the attending clinician after performing clinical examination. The information recorded was level of consciousness, pulse rate, respiratory rate, neck stiffness, irritability, and jaundice. Information was also recorded about any prescribed antibiotics or antimalarials, and whether the child had received antibiotics or antimalarials before blood culture was drawn. Outcome was recorded as discharge, improved, transfer to another hospital or death.

2.3. Specimen collection

One rectal swab was collected for each child recruited in the study and transported on Cary-Blair transport medium to the Muhimbili University of Health and Allied Sciences bacteriology research laboratory and stored at $-80°$C until time of analysis.

The collection and processing of blood cultures have been described in detail in a previous publication [19].

2.4. Phenotypic screening for ESBL-producing Enterobacteriaceae

Frozen rectal swabs were suspended in brain heart infusion (BHI) media for overnight incubation at 37°C. Two drops (mls) from BHI were inoculated onto CHROMID® ESBL agar (BioMérieux, Marcy l'Etoile, France) and incubated for 24 hours. Positive growth was interpreted according to the manufacturer's instructions.

2.5. Identification of bacteria and antimicrobial susceptibility testing

Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) was used for final identification of isolates using the Microflex 99 LT instrument and MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility testing was done by the disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotics used for antimicrobial susceptibility testing were piperacillin/tazobactam (110 μg), tigecycline (15 μg), ciprofloxacin (5 μg), aztreonam (30 μg), ceftazidime (30 μg), trimethoprim/sulphamethoxazole (1.25/25.75 μg), gentamicin (10 μg), and chloramphenicol (30 μg), all from Oxoid, Basingstoke, UK. Interpretation of results was done based on the CLSI guidelines [20]. Intermediately susceptible isolates were regarded as resistant. Klebsiella pneumoniae ATCC 700603 and *Escherichia* coli ATCC 25922 were used as controls.

2.6. Confirmation and identification of ESBL genotypes

Genomic DNA template was extracted by the rapid boiling method [21]. Real-time PCR detecting bla_{CTX-M} gene as a surrogate marker of ESBL was performed. The primers previously described by Tellevik et al. [17] were used for amplification. The total volume of the PCR reaction mix was 25 μl, which included: 1x SYBR Premix Ex Taq (Tli RNaseH Plus) (TaKaRa, Otsu, Japan), 0.4 μM each of the primers, 2 μl of sample DNA, and water. The assay was performed using a LightCycler 480 Instrument II (Roche Diagnostics, Mannheim, Germany). The cycling conditions are described elsewhere [17]. Sequencing was done using the same reverse primers used in PCR reaction and BigDye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3730 DNA Analyzer (Applied Biosystems). Sequences were analysed using the RipSeq software (Pathogenomix Inc., CA, USA).

2.7. Statistical analysis

Data were analysed using SPSS software version 20.0 (IBM SPSS Statistics 20.0, SPSS, Inc., Chicago, IL, USA). χ^2 test was performed to observe the significance of proportion differences, and $P < 0.05$ was considered to be statistically significant. The association between sociodemographic characteristics and ESBL-BSI was measured using bivariate and multivariate analyses, whereas the association was regarded significant for a P-value <0.05. All factors with a P value of ≤ 0.2 and those that have been documented by others to be associated ESBL-BSI were included in multivariable analysis.

3. Results

3.1. Study population

Of 200 participants screened for ESBL-PE, the majority (60%) were males, and 61% were below 3 months old (Table 1). Almost half of the study participants were from the Temeke hospital (101, 50.5%). Most children had neither a history of previous admission in the last six months (94%) nor antibiotic use in the last one

Table 1

Risk factors for faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae

Characteristics (N)	ESBL positive (n = 112) n (%)	ESBL negative (n = 88) n (%)	P; OR (95% CI)
Sex			
Male (120)	63 (52.5)	57 (47.5)	1
Female (80)	49 (61.2)	31 (38.8)	0.22; 1.43 (0.81–2.54)
Age groups (months)			
0–3 (122)	63 (51.6)	59 (48.4)	0.89; 1.07 (0.40–2.87)
4–6 (21)	17 (81.0)	4 (19.0)	0.05; 4.25 (1.02–17.73)
7–12 (17)	9 (52.9)	8 (47.1)	0.86; 1.13 (0.30–4.24)
13–24 (22)	14 (63.6)	8 (36.4)	0.39; 1.75 (0.49–6.22)
>24 (18)	9 (50.0)	9 (50.0)	1
Study site			
Amana (78)	46 (59.0)	32 (41.0)	0.65; 1.28 (0.45–3.67)
MNH (17)	9 (52.9)	8 (47.1)	1
Mwananyamala (4)	3 (75.0)	1 (25.0)	0.43; 2.67 (0.23–31.07)
Temeke (101)	54 (53.5)	47 (46.5)	0.97; 1.02 (0.37–2.86)
Antibiotic use in the last 1 month			
Yes (7)	6 (85.7)	1 (14.3)	0.14; 4.96 (0.59–42.01)
No (189)	104 (54.7)	85 (45.3)	1
Admission in the last 6 months			
Yes (12)	8 (66.7)	4 (33.3)	0.45; 1.62 (0.47–5.55)
No (188)	104 (55.3)	84 (44.7)	1
Mode of delivery[a]			
SVD (132)	76 (57.6)	56 (42.4)	0.73; 1.16 (0.50–2.71)
C-section (26)	14 (53.8)	12 (46.2)	1
Prolonged labour[b]			
Yes (41)	25 (61.0)	16 (39.0)	0.56; 1.25 (0.60–2.58)
No (115)	64 (55.7)	51 (44.3)	1
PROM[a]			
Yes (28)	15 (53.6)	13 (46.4)	1
No (130)	75 (57.7)	55 (42.3)	0.69; 0.85 (0.37–1.92)
HIV status[c]			
Positive (3)	2 (66.7)	1 (33.3)	0.62; 1.87 (0.16–21.75)
Negative (60)	31 (51.7)	29 (48.3)	1
BSI[d]			
Yes (57)	40 (70.2)	17 (29.8)	0.05; 3.77 (1.42–9.96)
No (26)	10 (38.5)	16 (61.5)	1

NOTE: 1 in the final column refers to the reference group

BSI, bloodstream infection; C-section, Caesarean section; HIV, human immunodeficiency virus; MNH, Muhimbili National Hospital; PROM, premature rupture of membranes; SVD, spontaneous vaginal delivery.

[a] Data for 158 children.
[b] Data for 156 children.
[c] Data for 63 children.
[d] Data for 83 children.

month (95%). HIV test results were available for 63 children, of whom three (4.8%) had a positive HIV test.

3.2. Prevalence and factors associated with carriage of ESBL-producing Enterobacteriaceae

The overall prevalence of faecal carriage of ESBL-PE was 56% (112/200). Table 1 shows ESBL-PE faecal carriage and risk factors. ESBL-PE was highest in the age group 4–6 months (17/21, 81%, $P = 0.05$). The majority of children from the Mwananyamala hospital were colonized by ESBL-PE, (75%), which was higher compared to those from other hospitals (Amana 59%, Temeke 53.5%, and MNH 53%). The prevalence of ESBL-PE colonization in children who had used antibiotics in the past one month (85.7%), and those who were admitted in the past 6 months (66.7%), was not significantly higher compared to their counterparts (54.7%, $P = 0.14$ and 55.3%, $P = 0.45$, respectively). The colonization rate was comparable for children who were born through spontaneous vaginal delivery (SVD) (57.6%) compared to those born by caesarean section (CS) (53.8%).

Out of the 200 processed rectal swabs, 83 were from children with laboratory-confirmed BSI. Of those 83, more than half (57/83; 68.7%) had ESBL-positive bloodstream infections. ESBL bloodstream infections (ESBL-BSI) were more prevalent in children who were colonized by ESBL-producing pathogens (78.4% vs. 53.1%, $P = 0.01$).

3.3. ESBL carriage and BSI infection

To determine the risk factors for getting ESBL-BSI, we included 83 children who had Enterobacteriaceae BSI infection in the analysis (Table 2). Children who were colonized by ESBL-PE were three times more likely to have ESBL-BSI compared to non-colonized in both univariate and multivariate models ($P = 0.02$, AOR 3.4, 95% confidence interval 1.20–9.58). Children whose mothers had prolonged labour and premature rupture of membranes were not more prone to get ESBL-BSI compared to other groups. There was no difference in acquisition of ESBL-BSI regarding antibiotic use in the past one month, and whether a child was born through SVD or C-section. Sex was not associated with ESBL-BSI.

3.4. Bacterial species and antimicrobial resistance pattern in ESBL faecal isolates

Overall, 142 Enterobacteriaceae faecal isolates were isolated from 112 out of 200 children. In these 112 children, 82 (72%) had a single bacterial isolate and 31 (28%) had more than one isolate. *Escherichia coli* (*E. coli*) were the most frequently isolated ESBL-PE (45%), followed by *Klebsiella pneumoniae* (*K. pneumoniae*) 32.3%, *Enterobacter* spp. (19.0%), and *Citrobacter* spp. (3.5%). Both *E. coli* and *K. pneumoniae* isolates were mostly detected in children aged between 0 and 3 months, and the presence of *K. pneumoniae* was

Table 2
Univariate and multivariate analysis for factors associated with ESBL-BSI (N = 83)

Characteristics (N)	ESBL-BSI POS (n = 57) n (%)	ESBL-BSI NEG (n = 26) n (%)	Univariate P; cOR (95% CI)	Multivariate P; aOR (95% CI)
Sex				
Male (120)	33 (68.8)	15 (31.2)	1	
Female (80)	24 (68.6)	11 (31.4)	0.99; 0.99 (0.39–2.54)	
Age groups (months)				
0–3 (122)	41 (68.3)	19 (31.7)	0.59; 2.17 (0.13–36.37)	
4–6 (21)	10 (90.9)	1 (9.1)	0.19; 10.00 (0.32–31.27)	1.0; 0.00 (0.00)
7–12 (17)	0 (0.0)	3 (100.0))	0.99; 0.00 (0.00)	
13–24 (22)	1 (50.0)	1 (100.0)	0.58; 2.50 (0.10–62.60)	
>24 (18)			1	
Antibiotic use in the last 1 month				
Yes (7)	7 (100.0)	0 (0.0)	0.81; 1.33 (0.13–13.48)	
No (189)	50 (65.8)	26 (34.2)	1	
Mode of delivery				
SVD (132)	43 (69.4)	19 (30.6)	0.96; 1.03 (0.31–3.37)	
C-section (26)	11 (68.8)	5 (31.2)	1	
Prolonged labour				
Yes (41)	16 (88.9)	2 (11.1)	0.06; 4.4 (0.93–21.12)	0.06; 0.22 (0.44–1.07)
No (115)	38 (64.4)	21 (35.6)	1	
PROM				
Yes (28)	7 (77.8)	2 (22.2)	0.56; 1.64 (0.31–8.54)	
No (130)	47 (68.1)	22 (31.9)	1	
ESBL faecal carriage				
Yes (112)	40 (78.4)	11 (21.6)	0.02; 3.21 (1.23–8.41)	0.02; 3.40 (1.20–9.58)
No (88)	17 (53.1)	15 (46.9)	1	

NOTE: 1 in the final column refers to the reference group
aOR, adjusted odds ratio; cOR, crude odds ratio; C-section, caesarean section; HIV, human immunodeficiency virus; PROM, premature rapture of membrane; SVD, spontaneous vaginal delivery.

similar for other age groups. *Citrobacter* species were not isolated from children above the age of 6 months. Fig. 1 summarizes these results.

Almost all isolated Enterobacteriaceae expressed co-resistance towards amoxicillin/clavulanic acid, ciprofloxacin, and trimethoprim/sulfamethoxazole. *K. pneumoniae* (84.8%), *Enterobacter* spp. (92.6%), and *Citrobacter* spp. (80%) were highly resistant to gentamicin. *Citrobacter* spp. were resistant to most antibiotics tested, whereas *E. coli* displayed low rates of resistance towards piperacillin/tazobactam, tigecycline, and chloramphenicol (Table 3).

Multidrug resistance (MDR), defined as resistance to three or more classes of antibiotics [19], was observed in 93.06% of isolates. Except for *E. coli* isolates, of which some were non-MDR (14.1%), all other isolated pathogens were resistant to more than three classes of antibiotics.

3.5. Comparison of antimicrobial resistance pattern between ESBL-BSI and faecal bacterial isolates

We compared the antimicrobial susceptibility patterns of ESBL-BSI isolates and faecal Gram-negative bacterial isolates as shown in
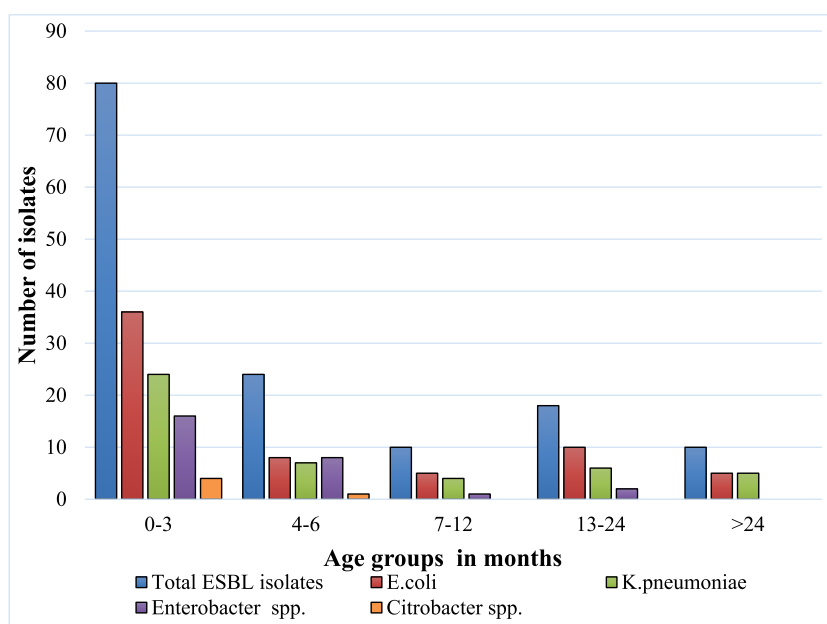
Fig. 1. Distribution of isolated faecal Enterobacteriaceae in different age groups. The figure illustrates the distribution ESBL-PE isolates (n = 142) from rectal swabs among children in different age groups.

Table 3

Antimicrobial resistance pattern for isolated Enterobacteriaceae from faecal samples

Antimicrobial agent	Bacteria, n (%)			
	E. coli (n = 64)	*K. pneumoniae* (n = 46)	*Enterobacter* species (n = 27)	*Citrobacter* species (n = 5)
TZP	10 (15.6)	32 (69.6)	6 (22.2)	4 (80.0)
TGC	1 (1.6)	21 (45.7)	9 (33.3)	5 (100.0)
AMC	47 (73.4)	40 (87.0)	27(100.0)	5 (100.0)
CIP	42 (65.6)	34 (73.9)	17 (62.9)	5 (100.0)
SXT	58 (90.6)	43 (93.5)	25 (92.6)	5 (100.0)
CN	35 (54.7)	39 (84.8)	25 (92.6)	4 (80.0)
C	10 (15.6)	20 (43.5)	21 (77.8)	5 (100.0)

NOTE: *Enterobacter* species: *E. bugandensis* (1), *E. cloacae* (18) and *E. xiangfangensis* (8); *Citrobacter* species: *C. freundii* (2) and *C. sedlakii* (3).

AMC, amoxicillin/clavulanic acid; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamycin; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline; TZP, peperacillin/tazobactam.

Table 4

Comparison of antimicrobial susceptibility pattern between ESBL-BSI and fecal bacterial isolates from the same child

SN. ID	Bacteria isolate Blood (BL)/Faecal (FC)	CIP BL/FC	ATM BL/FC	CAZ BL/FC	GEN BL/FC
1. FS0558	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	32 (S)/32;26 (S; S)	12 (R)/12;13 (R; R)	15 (R)/15;16 (R; R)	6 (R)/6;6 (R; R)
2. FS1386	BL: *K. pneumoniae*/FC: *K. pneumoniae*	31 (S)/31 (S)	14 (R)/14 (R)	17 (R)/17 (R)	6 (R)/6 (R)
3. FS1448	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	28 (S)/28;9(S; R)	15 (R)/15;12(R; R)	18 (R)/18;13 (R; R)	6 (R)/6;9 (R; R)
4. FS1462	BL: *K. pneumoniae*/FC; *K. pneumoniae*	6 (R)/6 (R)	12 (R)/12 (R)	14 (R)/14 (R)	6 (R)/6 (R)
5. FS1654	BL: *E. coli*/FC: *E. coli*	6 (R)/6 (R)	16 (R)/16 (R)	20 (R)/20 (R)	9 (R)/9 (R)
6. FS1866	BL: *K. pneumoniae*/FC: *K. pneumoniae*	28 (S)/28 (S)	14 (R)/14 (R)	13 (R)/13 (R)	6 (R)/6 (R)
7. FS1925	BL: *K. pneumoniae*/FC: *K. pneumoniae*	22 (R)/22 (R)	10 (R)/ 10 (R)	8 (R)/8 (R)	6 (R)/6 (R)
8. FS2071	BL: *E. coli*/FC: *E. coli*	26 (S)/26 (S)	11 (R)/11 (R)	7 (R)/7 (R)	19 (S)/19 (S)
9. FS2111	BL: *K. pneumoniae*/FC: *K. pneumoniae*	17 (R)/17 (R)	13 (R) /13 (R)	16 (R)/16 (R)	6 (R)/6 (R)
10. FS2112	BL: *K. pneumoniae*/FC: *K. pneumoniae*	28 (S)/28 (S)	15 (R)/15 (R)	17 (R)/17 (R)	6 (R)/6 (R)
11. FS2130	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	19 (R)/19;6 (R; R)	13 (R)/13;17 (R; R)	15 (R)/15;20 (R; R)	6 (R)/6;9 (R; R)
12. FS2155	BL: *K. pneumoniae*/FC: *K. pneumoniae*	20 (R)/20 (R)	15 (R)/15 (R)	19 (R)/19 (R)	7 (R)/7 (R)
13. FS2166	BL: *K. pneumoniae*/FC: *K. pneumoniae*	15 (R)/15 (R)	13 (R)/13 (R)	20 (R)/20 (R)	8 (R)/8 (R)
14. FS2240	BL: *E. coli*/FC: *E. coli*	6 (R)/6 (R)	16 (R)/16 (R)	19 (S)/19 (R)	9 (R)/9 (R)
15. FS2258	BL: *E. coli*/FC: *E. coli*	6 (R)/6 (R)	14 (R)/14 (R)	20 (R)/20 (R)	9 (R)/ 9 (R)
16. FS2265	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	26 (S)/26;27 (S; S)	9 (R)/9;15 (R; R)	12 (R)/12;18 (R; R)	6 (R)/6;20 (R; S)
17. FS1431	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	30 (S)/30;6 (S; R)	19 (R)/12;17 (R; R)	15 (R)/15;17(R; R)	6 (R)/6;9 (R; R)
18. FS2261	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. xiangfangensis*	29 (S)/25;27 (S; S)	6 (R)/6;6 (R; R)	12 (R)/12;10 (R; R)	6 (R)/6;6 (R; R)
19. FS1637	BL: *E. coli*/FC: *E. coli; C. freundii*	6 (R)/6;21 (R; S)	13 (R)/16;13 (R; R)	17 (R)/20;17 (R; R)	9 (R)/9;6 (R; R)
20. FS1701	BL: *K. pneumoniae*/FC: *E. coli*	17 (R)/6 (R)	8 (R)/8 (R)	15 (R)/6 (R)	6 (R)/6 (R)
21. FS1809	BL: *Acinetobacter*/FC: *K. pneumoniae*	6 (R)/26 (R)	13 (R)/ 13 (R)	16 (R)/17 (R)	6 (R)/6 (R)
22. FS1912	BL: *K. pneumoniae*/FC: *K. pneumoniae*	24 (R)/20 (R)	9 (R)/9 (R)	8 (R)/13 (R)	8 (R)/8 (R)
23. FS2013	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	28 (S)/21;6 (R; R)	15 (R)/15;15(R; R)	12 (R)/16;18 (R; R)	6 (R)/6;8 (R; R)
24. FS2202	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	26 (S)/22;6 (R; R)	10 (R)/10;15 (R; R)	18 (R)/15;18 (R; R)	6 (R)/6;11 (R; R)
25. FS0544	BL: *Acinetobacter*/FC: *E. cloacae*	23 (S)/17 (R)	10 (R)/13 (R)	6 (R)/15 (R)	8 (R)/6 (R)
26. FS0906	BL: *K. pneumoniae*/FC: *K. pneumoniae*	28 (S)/18 (R)	6 (R)/14 (R)	13 (R)/17 (R)	6 (R)/19 (S)
27. FS0987	BL: *K. pneumoniae*/FC: *E. cloacae*	27 (S)/33 (R)	14 (R)/14 (R)	17 (R)/13(R)	6 (R)/ 10(R)
28. FS1139	BL: *Acinetobacter*/FC: *E. cloacae*	28 (S)/25 (R)	16 (R)/15 (R)	6 (R)/17 (R)	6 (R)/19 (S)
29. FS1185	BL: *K. pneumoniae*/FC: *E. coli*	21 (R)/6 (R)	15 (R)/15 (R)	20 (R)/17 (R)	20 (S)/18 (S)
30. FS1252	BL: *K. pneumoniae*/FC: *C. sedlakii*	15 (R)/20 (R)	12 (R)/12 (R)	19 (R)/15 (R)	7 (R)/9 (R)
31. FS1425	BL: *K. pneumoniae*/FC: *E. xiangfangensis*	6 (R)/29 (S)	11(R)/6 (R)	19 (R)/6 (R)	6 (R) /19 (S)
32. FS1432	BL: *K. pneumoniae*/FC: *E. xiangfangensis*	30 (S)/21 (R)	16 (R)/18 (R)	20 (R)/20 (R)	6 (R)/9 (R)
33. FS1775	BL: *K. pneumoniae*/FC: *E. coli*	16 (R)/21 (R)	7 (R)/12 (R)	10 (R)/17 (R)	7 (R)/6 (R)
34. FS1865	BL: *K. pneumoniae*/FC: *E. coli*	30 (S)/6 (R)	15 (R)/15 (R)	13 (R)/18 (R)	6 (R)/9 (R)
35. FS1895	BL: *K. pneumoniae*/FC: *E. coli*	17(R)/6 (R)	15 (R)/16 (R)	6 (R)/19 (R)	18 (R)/9 (R)
36. FS2067	BL: *E. coli* /FC: *E. bugandensis*	24 (R)/27 (S)	26 (S)/13 (R)	20 (R)/17 (R)	18 (S)/6 (R)
37. FS2124	BL: *K. pneumoniae*/FC: *E. coli*	31 (S)/6 (R)	14 (R)/9 (R)	15 (R)/14 (R)	6 (R)/18 (S)
38. FS2163	BL: *K. pneumoniae*/FC: *E. coli*	16 (R)/6 (R)	12 (R)/9 (R)	18 (R)/13 (R)	7 (R)/6 (R)
39. FS2171	BL: *K. pneumoniae*/FC: *K. pneumoniae; E. coli*	17(R)/6;6 (R; R)	10 (R)/6;6 (R; R)	20 (R)/13;6 (R; R)	6 (R)/6;6 (R; R)
40. FS2255	BL: *K. pneumoniae*/FC: *E. coli; E. cloacae*	30 (S)/6;30 (R; S)	6 (R)/16;14 (R; R)	16 (R)/19;17 (R; R)	6 (R) /9;6 (R; R)

ATM, aztreonam; BL, blood bacteria isolate; CAZ, ceftazidime; CIP, ciprofloxacin; FC, faecal bacteria isolate; GEN, gentamycin; ID, identification number; R, resistant; S, sensitive; SN, serial number.

Table 4. Sixteen out of 40 blood-faecal pairs of ESBL isolates (40%) displayed identical phenotypic antimicrobial susceptibility profile (same zone size). Out of these pairs, 12 were *K. pneumoniae: K. pneumoniae* pairs and four were *E. coli: E. coli* pairs. Furthermore, eight out of 40 pairs (20%) showed a similar resistance pattern except for one or two antimicrobial agents regardless of species identity. The rest of the Gram-negative bacteria pairs (40%) from ESBL-BSI and faecal samples differed in their phenotypic antimicrobial resistance pattern.

3.6. ESBL genotypes

Of 142 Enterobacteriaceae isolated, almost all (97%, 138/ 142) carried bla_{CTX-M}. The gene was detected in 100% of *K. pneumoniae, Citrobacter* spp., *E. cloacae,* and *E. bugandensis* isolates. In *E. coli* and *E. xiangfangensis, bla*$_{CTX-M}$ was detected in 92.2% (59/64) and 87.5% of isolates, respectively (7/8). None of the isolates carried more than one CTX-M allele. Among those who carried CTX-M gene, $bla_{CTX-M-15}$ was most prevalent (98.6%; 136/138). All *K. pneumoniae,*

Citrobacter, and *Enterobacter* isolates carried bla$_{CTX-M-15}$. Two *E. coli* isolates carried genes other than *bla*$_{CTX-M-15}$, which were *bla*$_{CTX-M-9}$ and *bla*$_{CTX-M-3}$.

4. Discussion

The current study demonstrates a high prevalence of faecal carriage of ESBL-PE in children below five years of age hospitalized with fever. The study further indicates that the majority of children (78%) with BSI due to ESBL-PE were colonized by ESBL-PE. This is the first study in Tanzania that links colonization with infection among young children.

Several studies have assessed the prevalence of colonization with ESBL-PE among hospitalized children [13,22,23]. The prevalence of ESBL-PE in this study is consistent with a previous study conducted in Tanzania [17] and Bangui, Central African Republic [24]. However, our finding shows slightly higher prevalence compared to that reported in a study from north-western Tanzania [18]. Different study populations, in that our study included hospitalized children with fever, whereas the study conducted in Mwanza included healthy street children, can account for this difference. A lower prevalence has also been reported in other African settings such as South Africa. Babatunde et al. reported an ESBL-PE colonization rate of 48% among hospitalized children [13]. Geographical variation may account for the difference, emphasizing the need for generating local data.

Many previous studies reported ESBL-PE colonization to be high among children less than 12 months [13,17,25]. Similarly, children aged 4–6 months had the highest prevalence of ESBL-PE in the present study. These findings support the idea that the presence of antimicrobial resistance genes in infants' intestines is due to an unstable microbiome, which makes younger children prone to higher carriage rates [26–28]. Contrary to our findings, febrile children below five years of age at the emergency department in Guinea Bissau National hospital showed the same colonization rate of ESBL-PE in different age groups as reported by Isendal et al. [10].

Among the ESBL-PE isolated, we report *K. pneumoniae* and *E. coli* as predominant isolates, findings that are consistent with previous paediatric colonization studies conducted among hospitalized children in Madagascar and Niger [22,23]. The predominance of *E. coli* isolates among ESBL producers has also been reported in a meta-analysis review conducted across Eastern, Central, and South African countries [29].

It has been hypothesized that many infections, including BSI, emerge from an intestinal reservoir [30,31]. In the present study, BSI due to ESBL-PE was more prevalent in children who were colonized by ESBL-PE. Although a detailed genetic comparison was not done to compare colonization and infection isolates, the association observed can be used to stipulate that colonization is likely a risk factor for infection. Similar to our finding, Smith et al. reported an association between colonization and BSI in neonates with very low birth weight in Columbia [32]. Further studies to explore the link between colonization and infection are of importance since both carriage and ESBL-PE infections are on the rise.

Appiah-Korang Labi and co-workers in Ghana reported higher colonization rates in children who were born through vaginal delivery [33]. Though not statistically significant, a trend towards similar findings is observed in our study. Delivery through caesarean section has been reported to be a significant risk factor for prolonged faecal colonization with ESBL-producing bacteria among neonates [34]. It is a common practice to subject mothers undergoing caesarean delivery to antibiotic prophylaxis, which can result in emergence of resistant enteric bacteria through selective pressure and thus put the child at risk of acquiring resistant pathogens during delivery [25]. Larger studies need to be conducted to further explore on this.

Other predictive factors such as past admission, previous antimicrobial use, prolonged labour, and HIV status shown to be risk factors in other studies [22,35,36] were not found to be associated with ESBL carriage in the present study; this may be due to low numbers of children with history of previous admission or antibiotic use. The small sample size may account for this lack of association. Most ESBL-positive isolates in our study were *E. coli* and the predominant CTX-M allele was *bla*$_{CTX-M-15}$, and some strains were identified as CTX-M-3 and CTXM-9. The high prevalence of CTX-M-15 is in concordance with reports from Tanzania and globally [11,17]. CTX-M-15 has been associated with community origin, indicating the possibility of acquisition from such surroundings [37]. Contrary to our findings, the most common CTX-M allele reported among HIV-infected children in Zimbabwe was CTX-M-27 [38]. Whether the different genotypes reported are associated with the HIV status is unknown and needs further investigation.

The antimicrobial resistance profile reported in this study is comparable to what has been previously reported [22,39]. High rates of resistance towards amoxicillin/clavulanic acid and trimethoprim have been reported in Tanzania, Kenya, and Ethiopia [39–41]. However, the rate of resistance towards ciprofloxacin, especially among *K. pneumoniae* isolates observed in this study, compared to what was reported in the same setting 10 years back, is of concern [17]. The percentage of ciprofloxacin resistance reported in our study is four times higher (73.9 vs. 17.3) than what was reported by Tellevik et al. in 2011 [17]. The reason could be the wide usage of this antibiotic in the community.

Although this study found that the majority of children with BSI due to ESBL-PE were colonized by ESBL-PE, our study design cannot confirm the direct association of ESBL carriage as the cause of ESBL-BSI infection. This is because it is difficult to know which came first: BSI-ESBL or intestinal colonization of ESBL. Apart from the data showing the highest prevalence of ESBL-BSI among colonized children, this study has also demonstrated that in 40% of ESBL-BSI blood-faecal isolates, pairs had identical AST patterns with the same zone of diameters, and 20% differed in only one or two AST patterns for the agents tested regardless of species identity.

Inclusion of only children with fever is a limitation of this study. This might have underestimated the reported prevalence of BSI. ESBL colonization data from children without signs of BSI would be the best for comparison analysis. A prospective cohort study involving both groups with and without BSI signs would have been more suitable.

In conclusion, rectal colonization with ESBL-PE is high in children admitted for febrile illness, and the high percentage of infection among colonized children indicates that colonization may be a risk factor for subsequent infection. We have further demonstrated resistance towards multiple antimicrobials in the isolates, even for antimicrobial agents that were not commonly resistant 10 years back in the same region. Based on the findings from the present study, we recommend regular microbiological surveillance and introduction of routine screening of ESBL-producing pathogens among children with BSI to avoid cross-transmission to other patients. This would in turn curb the emergence of multidrug-resistant organisms through intervention, prevention, and control programs and decrease morbidity and mortality attributed to the infection. We also advocate the use of culture and sensitivity test results to assist in appropriate selection of treatment choice and review of the current antibiotic prescription to be in line with the current local antibiogram data.

Ethical approval

Ethical approval was obtained from the Senate Research and Publications Committee of Muhimbili University of Health and Al-

lied Sciences in Dar es Salaam, Tanzania and from the Regional Committee for Medical and Health Research Ethics (REK) in western Norway. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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Declaration of Competing Interest

None declared.

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