

RESEARCH ARTICLE

Colonisation with extended spectrum beta-lactamase-producing and carbapenem-resistant Enterobacterales in children admitted to a paediatric referral hospital in South Africa

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Abstract

Introduction

There are few studies describing colonisation with extended spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) among children in sub-Saharan Africa. Colonisation often precedes infection and multi-drug-resistant Enterobacterales are important causes of invasive infection.

Methods

In this prospective cross-sectional study, conducted between April and June 2017, 200 children in a tertiary academic hospital were screened by rectal swab for ESBL-PE and CRE. The resistance-conferring genes were identified using polymerase chain reaction technology. Risk factors for colonisation were also evaluated.

Results

Overall, 48% (96/200) of the children were colonised with at least one ESBL-PE, 8.3% (8/96) of these with 2 ESBL-PE, and one other child was colonised with a CRE (0.5% (1/200)). Common colonising ESBL-PE were *Klebsiella pneumoniae* (62.5%, 65/104) and *Escherichia coli* (34.6%, 36/104). The most frequent ESBL-conferring gene was blaCTX-M in 95% (76/80) of the isolates. No resistance-conferring gene was identified in the CRE isolate (*Enterobacter cloacae*). Most of the *Klebsiella pneumoniae* isolates were susceptible to piperacillin/tazobactam (86.2%) and amikacin (63.9%). Similarly, 94.4% and 97.2% of the *Escherichia coli* isolates were susceptible to piperacillin/tazobactam and amikacin, respectively. Hospitalisation for more than 7 days before study enrolment was associated with ESBL-PE colonisation.

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Conclusion

Approximately half of the hospitalised children in this study were colonised with ESBL-PE. This highlights the need for improved infection prevention and control practices to limit the dissemination of these microorganisms.

Introduction

Infection with multidrug-resistant organisms is a threat to global health [1, 2]. Notable are the emergence of extended spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) [3]. These organisms are among pathogens categorised as critical on the World Health Organization list of priority antibiotic resistant pathogens [3]. These organisms are notorious for causing healthcare-associated infections, and there are growing concerns about their increasing contribution to community-acquired infections [4]. Invasive infection with CRE is associated with high mortality, high cost of management, few therapeutic options and the absence of effective consensus treatment guidelines [5].

Colonisation refers to the presence of a microorganism on a body surface such as the gastrointestinal tract without causing disease. Colonisation is distinct from infection in which there is invasion of bodily tissues by a disease-causing microorganism, or contamination caused by the accidental introduction of a microorganism during the course of sample collection, transport or processing. Bacterial colonisation often precedes infection, however reports of ESBL-PE and CRE colonisation progressing to infection in children are rare [6, 7]. Most paediatric studies on ESBL-PE colonisation are in neonatal intensive care units (NICUs) with reported prevalence ranging from 4.3% to 75% [8–10]. Outside the neonatal age, ESBL-PE colonisation among hospitalised paediatric patients have recorded prevalence between 18.5% and 57.1% [11–13] while carriage in community settings is lower, ranging from 0.1% to 12.4% [14–18]. In some settings, community carriage has increased over time. For example, surveillance studies in Bolivia and Peru, over a decade, show a steady rise from 0.1% in 2002 to 12.4% in 2011 [14, 18, 19]. Community carriage may be a reservoir for the development of community-acquired ESBL-PE infections, particularly *Escherichia coli* infections [4].

Factors which predispose to ESBL-PE colonisation in the neonatal period include prior carbapenem, penicillin and aminoglycoside exposure, prolonged hospitalisation, early onset pneumonia, prolonged antibiotic use, formula milk feeds, and having an ESBL-PE colonised mother [8, 9, 10, 20–24]. Risk factors for ESBL-PE colonisation in older children include mechanical ventilation, prolonged hospital stay, prior antibiotic use and prior hospitalisation [11, 12]. However, ESBL-producing *E. coli* carriage documented among children in a very remote community in Senegal, where prior antibiotic use was most unlikely, suggesting that other factors may play a role in promoting colonisation [15]. Conversely, breastfeeding confers protection against ESBL-PE colonisation in the newborn period [10, 25]. In early studies, ESBL-PE colonisation was associated with the sulphhydryl variable (SHV) enzyme. More recent studies suggest that the cefotaxime-hydrolyzing beta-lactamase, (CTX-M) enzyme now predominates because of superior transmission efficiency. [4, 9, 21, 22, 25, 26].

Studies in children have reported CRE colonisation rates of 0.5% to 29.5% [27–39]. All the studies on CRE colonisation have documented *Klebsiella pneumoniae* colonisation [30–32, 34, 36, 39] while two studies also documented colonisation with *Serratia* spp., *E. coli* and *Enterobacter* spp. [30, 39]. Though there are likely due to geographical variations, one or more carbapenemases have been reported on all continents [30–32, 34, 39]. Risk factors associated with

CRE colonisation include carbapenem exposure, transfer-in from other health facilities, aminoglycoside exposure, surgical procedures, urinary catheterisation, nasogastric intubation and prolonged antibiotic administration [31, 34, 36, 39]. Infection risk among children previously colonised with CRE have ranged from 3.4 to 28.2% [30, 34, 36, 39]. Risk of children colonised with carbapenem-resistant *K. pneumoniae* progressing to infection is increased by existing metabolic disease, previous carbapenem use, neutropaenia and prior surgical procedures [36].

Despite the global distribution of these organisms and the association of their emergence due to widespread and indiscriminate use of broad-spectrum antibiotics, little is known about the epidemiology of ESBL-PE and CRE colonisation and infection on the African continent, especially in children. There have been reports of ESBL-PE infections in South Africa from the early 1990s, and some more recent studies describing ESBL-PE infections, but few colonisation studies [40–43]. One of the first reports of CRE infection and colonisation in children in South Africa described the treatment and outcome of a case series of invasive CRE infections [44]. To address the dearth of ESBL-PE and CRE colonisation research in children in South Africa, we describe ESBL-PE and CRE colonisation at a children's hospital including prevalence, factors associated with ESBL-PE colonisation, and the distribution of ESBL genes among ESBL-PE isolates.

Methods

Study design and setting

This prospective, cross-sectional study was conducted at Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, South Africa, a 273-bedded tertiary hospital affiliated to the University of Cape Town academic complex and dedicated to the care of children aged 13 years and below. The hospital is a referral centre for sick children from the Western Cape Province, but also receives referrals from surrounding provinces.

Enrolment and sampling

Recruitment took place in four wards at RCWMCH, including two general medical wards and two surgical wards. All children admitted to the four wards were eligible for enrolment, except those who experienced colonisation or infection with ESBL-PE and/or CRE in the previous 1-year period.

A systematic sampling approach was employed for selecting study participants. From the daily ward list of children resident in the four wards, every third patient was approached for enrolment. If the inclusion criteria were not met, the next patient on the ward list was approached for enrolment. Enrolment took place from Monday to Friday. Between 3 and 8 participants were enrolled on any one day. Enrolment alternated between the medical and surgical participants to ensure that each participant type constituted a minimum of 45% of all enrolled study participants. Participant enrolment was completed between 3 April and 7 June 2017.

Data collection

Demographic and clinical information of the enrolled participants was entered on a study-specific structured data sheet. The information included age at enrolment, gender, source of admission—either from a health care facility or convalescence home, current diagnosis, surgical procedures, and intensive care unit (ICU) treatment in the current admission or the preceding 12 months. Information on antibiotic use was also collected such as the type and duration of antibiotic therapy in the 12 months preceding this admission or in the current admission. Information on out of hospital antibiotics exposure was mainly as reported by the care giver,

while information on in-hospital antibiotics used was collected from the participant's clinical records.

Stool specimen collection

A soft tipped Transystem™ sterile transport swab (COPAN Italia S.a.A via Perotti 10, 25125 Brescia Italy) was used to collect rectal stool specimens in a quiet, comfortable room. Swabbing was performed by an experienced research nurse assisted by a chaperone and usually in the presence of the child's parent or legal guardian. All measures were taken to minimise participant discomfort. The specimens were transported to the National Health Laboratory Service (NHLS) medical microbiology laboratory, Groote Schuur Hospital (GSH), Cape Town, for processing within 6 hours of collection.

Microbiological procedures

All microbiological procedures were performed at the medical microbiology laboratory, GSH, located 4.3 km from RCWMCH. Stool specimens were plated onto ChromID ESBL media (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C for 24 hours. Suspected colonies were subcultured onto blood agar plates for pure growth. Purified isolates were identified using the Vitek 2® Gram negative card with susceptibility testing performed using the AST GN-N255 card (bioMérieux, Marcy l'Etoile, France). Where necessary, this was supplemented with E-test (bioMérieux, Marcy l'Etoile, France) gradient diffusion to confirm minimal inhibitory concentrations (MICs) of ertapenem, imipenem and meropenem. MICs were interpreted according to the 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines [45] while ESBLs and CRE were reported based on the Advanced Expert System (AES) interpretation of the Vitek 2® system. The susceptibility of Enterobacterales isolated was evaluated for the following antibiotics: ampicillin, co-amoxiclav, piperacillin-tazobactam, cefuroxime, cefoxitin, ceftriaxone, ceftazidime, cefepime, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, cotrimoxazole, tigecycline and colistin. All isolates were stored at -70°C on microbeads for further molecular testing for ESBL and carbapenemase-producing genes.

Molecular testing for common ESBL and carbapenemase genes

Stored isolates were grown overnight (18–24 hours) on McConkey agar plates, before sub-culturing onto 2% Blood agar. A loop-full of each culture was suspended in 750 µl BashingBead™ Buffer and pre-lysed on the Tissue Lyser in a ZR BashingBead™ Lysis Tube (Zymo Research Corporation), at 50Hz for 5 min. The Lysis tube was centrifuged at 10 000 rpm, and 200 µl of the supernatant extracted and purified using the QIA Symphony DSP Virus/Pathogen Kit (Qiagen), according to the manufacturer's recommendations.

Using gene-specific primers, selected commonly encountered ESBL and carbapenemase genes were detected and amplified by PCR reaction. The primers used are shown in Table in [S1 File](#) and detailed methods are detailed in a recent publication [46]. The amplification products were analysed by agarose gel electrophoresis with 1.5%, and visualised with ethidium bromide and using ultraviolet light. Positive amplicons were confirmed with Sanger sequencing, and BLAST analysis.

Definitions

HIV-exposed but uninfected child. A child <18 months old in whom a positive HIV serological test was documented in either the mother or the child, but the HIV DNA PCR test was negative in the child who was not on antiretroviral therapy (ART).

HIV infection. A positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test in any child < 18 months old, or 2 positive serological test results (HIV ELISA or HIV Rapid test) or a positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test in a child > 18 months old were considered HIV-infected [47].

Extended Spectrum Beta-Lactamase Producing Enterobacterales (ESBL-PE). Enterobacterales were categorised as ESBL-PE according to the Vitek 2® Advanced Expert System (AES) interpretation of the AST GN-N255 card. This categorisation is based solely on the pattern of susceptibility and resistance to different cephalosporins as the card lacks wells containing cephalosporins combined with a beta-lactamase inhibitor [48].

Carbapenem-Resistant Enterobacterales (CRE). Enterobacterales which are resistant to any carbapenem antibiotic (minimum inhibitory concentration of ≥ 4 mcg/ml for doripenem, meropenem or imipenem, or ≥ 2 mcg/ml for ertapenem) according to 2017 CLSI breakpoints [45]. Resistance to carbapenems may result from several mechanisms including alteration of outer membrane permeability or the production of carbapenemases. Common carbapenemase genes include *bla_{NDM}*, *bla_{KPC}*, *bla_{GES}*, *bla_{VIM}*, *bla_{OXA-48-like}* and *bla_{IMP}* [45].

Co-morbidity. An underlying chronic medical condition for which the participant was receiving care at the time of enrolment.

Major surgery. An invasive operative procedure in which extensive resection is performed, e.g. a body cavity is entered, organs are removed, or normal anatomy is altered. In general, if a mesenchymal barrier is opened (pleural cavity, peritoneum, meninges), the surgery is considered major [49].

Statistical analysis

Data were entered in SPSS Statistics Version 24.0 Software (IBM, Armonk, New York, USA) and analysed. Descriptive statistics, for continuous variables, was reported as medians with interquartile ranges or, where applicable as means and standard deviations. Categorical variables were reported as proportions and percentages. The 95% confidence interval (CI) for binomial proportions were estimated for mid-point prevalence estimates. Categorical variables were compared using either the uncorrected chi square test or Fisher's exact test while continuous variables were analysed using the Student's t test or analysis of variance (ANOVA). Non-normally distributed data were compared using Mann-Whitney U test. Two-tail p values <0.05 were considered statistically significant.

Univariate analyses were used to identify potential risk factors associated with rectal colonisation by ESBL-PE. All factors with a p value <0.2 on univariate analysis, and those biologically plausible or reported in literature, were then analysed in a binomial logistic regression model to identify factors independently associated with ESBL-PE colonisation. The binomial logistic regression model was built using a stepwise backward selection. The univariate results were reported using unadjusted odds ratios (ORs) and 95% confidence interval (95% CI), and the logistic regression results expressed as adjusted odds ratios (aORs) and 95% CI.

Ethical consideration

The study was conducted in accordance with the Helsinki Declaration. The study protocol was approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, reference number: HREC REF: 898/2016. The Research Committee at the RCWMCH also approved the study.

Written informed consent was obtained in the preferred language (English, Afrikaans or isiXhosa) from the parents or legal guardian, and children aged 12 years and above before

enrolment. Children aged between 7 years and above provided written informed assent before being enrolled into the study. The services of translators were employed where necessary during consenting, assenting and enrolment procedures.

Children and their legal guardian were informed of their ESBL-PE and/or CRE colonisation status. When colonisation was documented, the implications for care management, and infection control practices were explained. The result was also provided to the attending physicians and other health care providers involved in their care. Parents or legal guardian of children who had been discharged at the time of receipt of the results were informed telephonically.

Results

Study participants

Of a total of 299 children who were selected for enrolment, 99 were excluded for various reasons. Thus, a total of 200 children were enrolled and completed the study. Study participant enrolment, phenotypic and genotypic results are depicted in [Fig 1](#).

Characteristics of participants

Of the 200 enrolled children, 60.5% were male, the median age was 12 months (range 4 days–7 years and 4 months), and 59.6% (119/200) were less than 24 months of age. The HIV status was known in 153 participants; 3.5% (7/200) were HIV-infected, 19.5% (39/200) were HIV-exposed but uninfected and 53.5% (107/200) were HIV-unexposed. The HIV status in 23.5% (47/200) participants was unknown, as HIV status is not determined routinely in all patients admitted into the hospital. Of the 47 children with unknown HIV status, 89.4% (42/47) were enrolled in the surgical wards. The most frequent primary diagnoses were pneumonia, bone and soft tissue infection and acute diarrhoeal disease ([Table 1](#)).

Prevalence of ESBL-PE and CRE colonisation

Of 200 participants enrolled, 96 were colonized by at least one ESBL-PE, giving an ESBL-PE colonisation prevalence of 48% (95% CI 40.9–55.2%). Of these, 8.3% (8/96) participants were colonised by two different ESBL-PE, prevalence of 4% (95% CI 1.9–7.5%). One additional participant was colonised by a carbapenem-resistant *Enterobacter cloacae* (*E. cloacae*) giving a CRE colonisation prevalence of 0.5% (95% CI 0.02–2.4%). Of the total 104 ESBL-PE isolates collected from the 96 ESBL-PE colonized participants, *K. pneumoniae* accounted for 62.5% (65/104), *E. coli* 34.6% (36/104), *Klebsiella oxytoca* (*K. oxytoca*) 1.9% (2/104), and *E. cloacae* 1.0% (1/104). There was no significant difference in the proportions of colonised and non-colonised children with pre-existing co-morbid conditions ([Table 2](#)).

Nine percent (18/200) of the enrolled children were transferred-in from another healthcare facility, primarily to the medical wards at RCWMCH, 72.2% (13/18). The median duration of hospital stay at the time of enrolment was 4 days (range 1–64 days) and 30% of the participants had been hospitalised for more than 7 days prior to enrolment ([Table 2](#)). About a third of the participants, 32.5% (65/200), had been admitted into other wards in the course of the current admission, aside from the wards in which they were enrolled during the study, and the majority of these children were enrolled in the medical wards, 96.9% (63/65). Of the children admitted into other wards before enrolment, 52.3% (34/65) had been treated in the ICU. In all, 19% (38/200) had been treated in the ICU at RCWMCH either in the current admission or in the preceding 12 months ([Table 2](#)).

Overall, 25% (50/200) of the participants reported antibiotic usage in the 12-month period before current hospitalisation, 29.9% (29/97) of colonised children and 20.4% (20/103) of non-colonised children. The most common antibiotics administered during this period were penicillins in

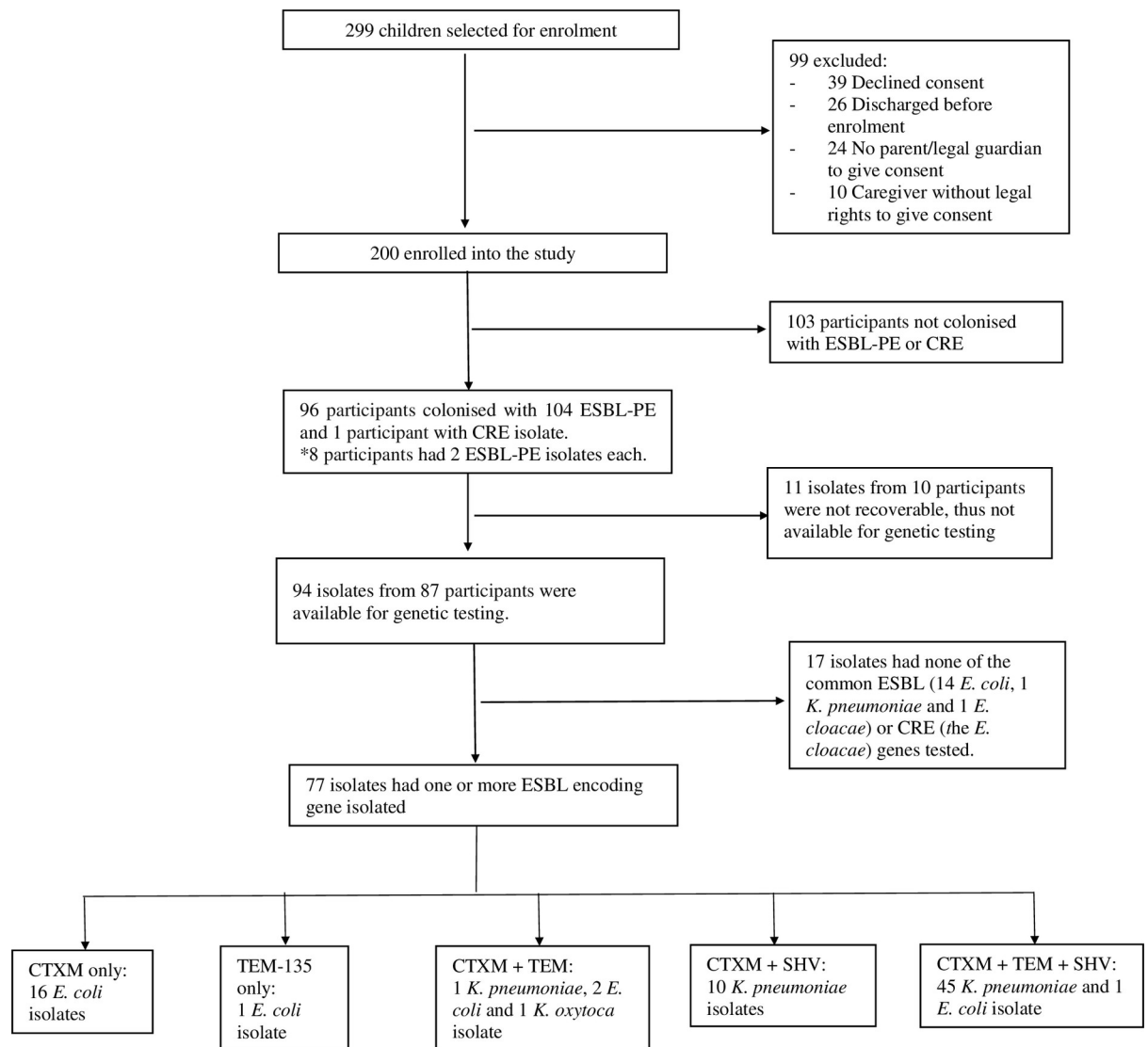


Fig 1. Flow chart depicting participant enrolment, phenotypic and genotypic testing results. # ESBL-PE, Extended spectrum beta-lactamase producing Enterobacterales, CRE, Carbapenem-resistant Enterobacterales, CTX-M, Cefotaxime-hydrolyzing beta-lactamase, TEM, Temoneira resistance encoding genes, SHV, Sulfhydryl variable, *K. pneumoniae*—*Klebsiella pneumoniae*, *E. coli*—*Escherichia coli*, *K oxytoca*—*Klebsiella oxytoca*, *E. cloacae*—*Enterobacter cloacae*.

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15.0% (30/200) of children, while 4.5% (9/200) received a carbapenem. During the current admission, 80.5% (165/200) had received one or more antibiotics, 84.5% (82/97) of colonised children and 76.7% (79/103) of non-colonised children. The antibiotics that these children received during the current period of hospitalisation included penicillins in 55.0% (110/200), aminoglycosides in 32% (64/200), cephalosporins in 19.5% (39/200), beta-lactam/beta-lactam inhibitor combination in 16% (32/200), carbapenems in 9% (18/200), cotrimoxazole in 7.5% (15/200), macrolides in 5.5% (11/200), metronidazole in 4.0% (8/200) and fluoroquinolones in 3.0% (6/200).

Factors associated with ESBL-PE colonisation

Using univariate analysis, age less than 12 months, admission to a medical ward at the time of study enrolment, hospitalisation for more than 7 days before study enrolment, and nasogastric

Table 1. Characteristics of the study subjects at the time of enrolment.

	Total (N = 200) n (%)	Colonised (N = 97) n (%)	Not colonised (N = 103) n (%)
Gender			
Male	121 (60.5)	58 (59.8)	63 (61.2)
Female	79 (39.5)	39 (40.2)	40 (38.8)
Age category			
≤28 days	9 (4.5)	6 (6.2)	3 (2.9)
>28 days– 12 months	89 (44.5)	50 (51.5)	39 (37.9)
>12 months—<60 months	47 (23.5)	20 (20.6)	27 (26.2)
≥60 months	55 (27.5)	21 (21.6)	34 (33.0)
Median age in months (IQR)	12 (2–68)	7 (2–45)	18 (4–82)
Median (IQR) days in hospital before enrolment	4 (2–9)	5 (2–12)	3 (2–7)
HIV status			
Infected	7 (3.5)	4 (4.1)	3 (2.9)
Exposed, uninfected	39 (19.5)	16 (16.5)	23 (22.3)
Unexposed, uninfected	107 (53.5)	65 (67.0)	42 (40.8)
Unknown	47 (23.5)	12 (12.4)	35 (34.0)
Primary clinical diagnosis			
Pneumonia	64 (32.0)	33 (34.0)	31 (30.1)
Bone and soft tissue infection	20 (10.0)	7 (7.2)	13 (12.6)
Acute diarrhoeal disease	11 (5.5)	8 (8.2)	3 (2.9)
Central nervous system malformation	10 (5.0)	6 (6.2)	4 (3.9)
Appendicitis	8 (4.0)	1 (1.0)	7 (6.8)
Meningitis	8 (4.0)	2 (2.1)	6 (5.8)
Urogenital malformations	7 (3.5)	2 (2.1)	5 (4.9)
Hydrocephalus	7 (3.5)	0 (0.0)	7 (6.8)
Hirschsprung disease	5 (2.5)	2 (2.1)	3 (2.9)
Trauma	5 (2.5)	2 (2.1)	3 (2.9)
Neonatal sepsis	4 (2.0)	4 (4.1)	0 (0.0)
Inguinal hernia	4 (2.0)	2 (2.1)	2 (1.9)
Tuberculosis	3 (1.5)	1 (1.0)	2 (1.9)
Solid tumour	3 (1.5)	2 (2.1)	1 (1.0)
Bloodstream infection	2 (1.0)	0 (0.0)	2 (1.9)

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intubation at the time of enrolment were significantly associated with ESBL-PE colonisation. However, on binomial logistic regression analysis, only hospitalisation for more than 7 days before study enrolment remained significantly associated with ESBL-PE colonisation ($p = 0.013$) (Table 2).

Antibiotic susceptibility profile of the ESBL-PE and CRE isolates

Table 3 summarises the antibiotic susceptibility profiles of the 104 ESBL-PE isolates. All ESBL-PE isolates were resistant to the cephalosporins except for one *E. coli* isolate and one *E. cloacae* isolate which retained susceptibility to cefepime, using Vitek 2. All the ESBL-PE isolates were however susceptible to the carbapenems and colistin. Most of the *K. pneumoniae* (72.3%) were susceptible to piperacillin/tazobactam and to amikacin (86.2%). Similarly, 94.4% and 97.2% of the *E. coli* isolates were susceptible to piperacillin/tazobactam and amikacin, respectively (Table 3). Susceptibility to both piperacillin/tazobactam and amikacin was observed in 73.1% of all isolates, 64.6% and 91.7% of all ESBL-PE *K. pneumoniae* and *E. coli*

Table 2. Factors associated with extended spectrum beta-lactamase-producing Enterobacterales colonisation determined by univariate analyses and binomial logistic regression.

	Colonised N = 96 n (%)	Not colonised N = 104 n (%)	OR (95% CI)	aOR (95% CI)
Male gender	38 (39.6)	41 (39.4)	1.01 (0.57–1.78)	
Age < 12 months	55 (57.3)	43 (41.3)	1.90 (1.09–3.34) *	1.36 (0.70–2.65)
Transferred to RCWMCH [®]	10 (10.4)	9 (8.7)	1.23 (0.48–3.16)	
Previous RCWMCH admission	23 (24.0)	15 (14.4)	1.87 (0.91–3.84)	1.23 (0.52–2.91)
Current admission to medical ward (vs. surgical ward)	63 (65.6)	49 (47.1)	2.14 (1.21–3.79) *	0.78 (0.35–1.77)
Hospitalisation for >7 days before enrolment	40 (41.7)	20 (19.2)	3.0 (1.59–5.66) *	2.83 (1.40–5.72) *
Cardiac co-morbidity	8 (8.3)	4 (3.8)	2.27 (0.66–7.81)	
Gastrointestinal tract co-morbidity	20 (20.9)	22 (21.2)	0.98 (0.50–1.94)	
Neurological co-morbidity	17 (17.7)	16 (15.4)	1.18 (0.56–2.50)	
Concomitant chronic lung disease	7 (7.3)	3 (2.9)	2.65 (0.67–10.55)	2.67 (0.57–12.59)
Concomitant congenital anomaly	25 (26.0)	25 (24.0)	1.11 (0.59–2.11)	
Major surgery in current admission	12 (12.5)	23 (22.1)	0.50 (0.24–1.08)	0.40 (0.15–1.04)
Peripheral venous line	88 (91.7.7)	87 (83.7)	2.15 (0.88–5.24)	1.81 (0.65–5.02)
Nasotracheal intubation ± ventilation	8 (8.3)	4 (3.8)	2.27 (0.66–7.81)	
PEG feeding	6 (6.2)	1 (1.0)	6.87 (0.81–58.1)	
Nasogastric intubation	38 (39.6)	24 (23.1)	2.18 (1.18–4.03) *	1.60 (0.76–3.39)
Decreased level of consciousness	5 (5.2)	1 (1.0)	5.66 (0.65–49.3)	
Receiving gastric acid inhibitor therapy	12 (12.5)	6 (5.8)	2.33 (0.84–6.49)	
Receiving immunosuppressive therapy	11 (11.2)	11 (10.6)	1.09 (0.45–2.65)	
Admission to ICU in 12 months preceding current admission	6 (6.2)	2 (1.9)	3.40 (0.68–17.27)	
Admission to ICU during current admission	19 (19.8)	15 (14.4)	1.46 (0.70–3.08)	
Antibiotic administration in the 12-month period preceding admission	29 (30.2)	21 (20.2)	1.71 (0.90–3.27)	1.35 (0.63–2.90)

*p<0.05, OR Odds ratio, 95% CI 95% confidence interval, aOR adjusted Odds ratio, RCWMCH Red Cross War Memorial Children's Hospital, PEG percutaneous endoscopic gastrostomy, ICU intensive care unit. [®]Tranferred to RCWMCH refers to admitted from another hospital or a convalescent home.

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isolates respectively. The CRE *E. cloacae* isolate exhibited intermediate susceptibility to ertapenem on E-test, but was susceptible to imipenem and meropenem.

Table 3. Antibiotic susceptibility profile of the extended spectrum beta-lactamase-producing Enterobacterales isolates showing proportion susceptible.

Antibiotic	Total N = 104 (%)	<i>Klebsiella pneumoniae</i> N = 65 n (%)	<i>Escherichia coli</i> N = 36 n (%)	<i>Klebsiella oxytoca</i> N = 2 n (%)	<i>Enterobacter cloacae</i> N = 1 n (%)
Cotrimoxazole	10 (9.6)	5 (7.7)	4 (11.1)	0 (0.0)	1 (100.0)
Ampicillin/amoxicillin	1 (1.0)	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)
Amoxicillin plus clavulanic acid	19 (18.3)	1 (1.5)	16 (44.4)	2 (100.0)	0 (0.0)
Piperacillin plus tazobactam	83 (79.8)	47 (72.3)	34 (94.4)	2 (100.0)	0 (0.0)
Cefepime	2 (1.9)	0 (0.0)	1 (2.8)	0 (0.0)	1 (100.0)
Ciprofloxacin	687 (64.4)	41 (63.1)	23 (63.9)	2 (100.0)	1 (100.0)
Gentamicin	33 (31.7)	8 (12.3)	22 (61.1)	2 (100.0)	1 (100.0)
Amikacin	93 (89.4)	56 (86.2)	35 (97.2)	1 (50.0)	1 (100.0)
Tigecycline	104 (100.0)	65 (100.0)	36 (100.0)	2 (100.0)	0 (0.0)

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Genetic testing of ESBL-PE and CRE isolates

Of the 104 ESBL-PE and 1 CRE isolated from 97 colonised children, 94 isolates from 87 participants were available for genetic testing. Eighty ESBL-PE isolates (85.1%) from 73 participants had one or more resistance-conferring genes (Table 4). Of these, 25% (20/80) had only one resistant-conferring gene, 14 (17.5%) had 2 genes, and 46 (57.5%) had 3 genes identified. The most common resistance conferring gene was *bla*_{CTX-M} found in 95% (76/80) of ESBL-PE i.e. in 98.2% (56/57) of *K. pneumoniae* isolates, 55.9% (19/34) of *E. coli* isolates, and the only *K. oxytoca* (1.3%) isolate tested. The *bla*_{TEM} and *bla*_{SHV} genes were found in 66.3% (53/80) and 13.8% (11/80) of ESBL-PE isolates respectively, often in combination with *bla*_{CTX-M} gene. Three *E. coli* isolates had *bla*_{TEM} as the only resistance gene, and one *K. pneumoniae* isolate had *bla*_{SHV} as the only resistance gene. The 3 *E. coli* TEM-only positive amplicons were sequenced, and two were identified as TEM-1 genotypes i.e. narrow-spectrum beta-lactamase, and the remaining one as a TEM-135 genotype i.e. ESBL. The DNA from the SHV-only positive *K. pneumoniae* isolate was not sequenced as *bla*_{SHV} is intrinsic to *K. pneumoniae* and would not be differentiated using conventional sequencing. For the two ESBL-PE isolates which retained susceptibility to cefepime, no gene was found in the *E. cloacae* isolate, while the *E. coli* isolate had both CTX-M and TEM genes.

The only CRE isolate, an *E. cloacae*, was negative for common CRE conferring genes, namely *bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48} and variants, *bla*_{IMP}, *bla*_{VIM} and *bla*_{GES}.

Discussion

In this prospective cross-sectional study, we showed that approximately 50% of children hospitalised in RCWMCH are colonised with ESBL-PE, predominately with *K. pneumoniae* and *E. coli*, findings which are consistent with previous paediatric colonisation studies [11, 12, 13]. The CRE colonisation prevalence was extremely low in our participants, but comparable to previous studies which also reported lower CRE colonisation [30, 34, 50]. Similarly, blood-stream infection (BSI) studies from our hospital have shown that while *K. pneumoniae* and *E. coli* are the predominant Gram-negative aetiological agents, CRE only sporadically caused

Table 4. Frequency of genes conferring extended spectrum beta-lactam resistance to the colonising extended spectrum beta-lactamase-producing Enterobacterales isolates.

ESBL Gene	Extended spectrum beta-lactamase producing Enterobacterales N = 104				Total
	<i>Klebsiella pneumoniae</i> n = 65	<i>Escherichia coli</i> n = 36	<i>Klebsiella oxytoca</i> n = 2	<i>Enterobacter cloacae</i> n = 1	
Not available	8	2	1	0	11
No gene found	0	12	0	1	13
No EBSL gene confirmed					
TEM 1 only	0	2	0	0	2
SHV 1 only	1	0	0	0	1
EBSL gene confirmed					
TEM-135 only	0	1	0	0	1
CTX-M only	0	16	0	0	16
CTX-M + TEM	1	2	1	0	4
CTX-M + SHV	10	0	0	0	10
TEM + SHV	0	0	0	0	0
CTXM + TEM + SHV	45	1	0	0	46

ESBL-PE, extended spectrum beta-lactamase-producing Enterobacteriaceae, ESBL, extended spectrum beta-lactamase, CTX-M, cefotaxime-hydrolyzing beta-lactamase, TEM, Temoneira resistance encoding genes, SHV, sulphhydryl variable.

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invasive infection [44, 51]. Furthermore, of *K. pneumoniae* and *E. coli* isolates causing BSI at RCWMCH, 83% and 30% are ESBL-PE, respectively [41, 42].

This study was not designed to determine the timing of colonisation of ESBL-PE and CRE and hence we were unable to establish the extent of community versus hospital acquisition. Colonisation was documented in some participants who had only been hospitalised for one day, suggesting that some of the participants may have been colonised outside the hospital environment. However, the risk factor analysis showed that hospitalisation for more than 7 days before enrolment was an independent risk factor for ESBL-PE colonisation. This finding strongly suggests that the healthcare environment is an important site of colonisation and is consistent with previous BSI studies that showed that 95% and 55% of *K. pneumoniae* and *E. coli* BSIs respectively are hospital-acquired or healthcare-associated [41, 42].

The predominant ESBL gene identified was bla_{CTX-M} (95%), often occurring in combination with other ESBL-conferring genes (78.9%). This was notable for the *K. pneumoniae* isolates, most of which carried more than one ESBL-conferring gene. In contrast, most *E. coli* isolates harboured only the bla_{CTX-M} gene. This is in keeping with the global pattern where CTX-M has become the predominant ESBL-conferring gene in resistant *K. pneumoniae* and *E. coli* isolates [4, 21, 26]. It has been suggested that the CTX-M plasmid has adapted to *K. pneumoniae* resulting in better transmission efficiency [4, 26]. Three *E. coli* isolates each harboured a TEM gene only. One isolate had a TEM-135 gene which encodes an enzyme that hydrolyses 3rd generation cephalosporins and thus confers ESBL-PE properties on the isolate [52]. This is unlike the TEM-1 gene found in the other 2 isolates, the first TEM gene which encodes an enzyme that hydrolyses earlier penicillins like ampicillin but not 3rd generation cephalosporins, and therefore does not confer ESBL-PE characteristics on the isolates [53]. Most *K. pneumoniae* isolates have chromosomal SHV genes which encodes enzymes that do not hydrolyse 3rd generation cephalosporins [53]. One of our *K. pneumoniae* isolates was shown to only carry an SHV gene. The ESBL-PE phenotype of the three isolates with TEM-1 only or SHV only genes might be due to hyperproduction of the encoded enzymes along with porin changes, or the presence of other ESBL's or enzymes not detected by this assay.

The antibiotic susceptibility profiles of the ESBL-PE isolates as summarised in Table 4 correlate with published data. High proportions of the *K. pneumoniae* and *E. coli* isolates were susceptible to piperacillin-tazobactam and to amikacin, similar to findings among ESBL-producing bloodstream isolates of *K. pneumoniae* and *E. coli* reported in previous studies from RCWMCH [41]. Despite these findings, a randomised control trial among adults that determined whether piperacillin-tazobactam was as effective as meropenem for treating BSI caused by *K. pneumoniae* or *E. coli* with non-susceptibility to third generation cephalosporins showed that the 30-day mortality was significantly higher in the piperacillin-tazobactam group, suggesting that piperacillin-tazobactam alone may no longer be the recommended definitive therapy in this context [54]. The high proportion of *E. coli* (91.6%) susceptible to both piperacillin-tazobactam and amikacin suggest this may be a consideration in BSI caused by ESBL-PE *E. coli*. This is unlikely to be an option for ESBL-PE *K. pneumoniae* where only 64.6% were susceptible to both piperacillin-tazobactam and amikacin.

Study strengths and limitations

A strength of this study is that it is one of the first prospective studies to estimate the prevalence of ESBL-PE and CRE carriage in children hospitalised in sub-Saharan Africa. Limited funds for completing the microbiology investigation of the enrolled participants restricted our surveillance to one specimen per participant and prevented us from sampling the participants at multiple time points during hospitalisation, and prevented differentiation between

community and hospital acquisition of ESBL-PE. Our sampling approach may have underestimated the colonisation rate as multiple rectal swabs may have increased the yield of colonised children or serial sampling may have identified additional children who became colonised later on in the course of their hospital admission. The sample size of 200 children was not determined scientifically but influenced by the available funding. Thus, the study may have been underpowered to explore risk factors associated with colonisation definitively. Screening for genes conferring extended spectrum beta-lactam resistance was limited to the three commonly occurring resistance genes in our setting. Furthermore, we did not screen the isolates for the presence of Ambler group C beta-lactamases which produce similar antibiograms to the extended spectrum beta-lactamases. Lastly, relatedness of the ESBL-PE isolates was not evaluated. This may have assisted us to understand the extent of hospital transmission.

Notwithstanding these limitations the study showed that ESBL-PE colonisation prevalence is high in this setting, and prolonged hospitalisation is a risk factor for colonisation, suggesting the need for improved infection control practices.

Conclusion

The current study extends our understanding of ESBL-PE and CRE colonisation at our institution. Major findings were high ESBL-PE colonisation prevalence, very low CRE colonisation prevalence, prolonged hospitalisation as an independent risk factor for ESBL-PE colonisation, and common resistance genes responsible for conferring extended spectrum beta-lactam resistance. Further research, adequately funded to include more participants, is required to provide robust colonization estimates, explore colonisation prevalence changes over time, quantify the extent of community versus hospital acquisition, determine the pattern of acquisition of colonisation in the course of admission and relatedness of the isolates, and whether improved infection control practice can moderate colonisation rates. Whole genome sequencing will additionally help interrogate other resistance conferring genes not identified by PCR technology among the ESBL-PE and CRE isolates, especially among those where no resistance conferring gene was found.

Supporting information

S1 File. References for ESBL and carbapenemase PCR.
(DOCX)

S2 File. Data set of study participants and isolates.
(SAV)

S3 File. ESBL and CRE PCR results.
(XLSX)

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References

1. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! *Trends Mol Med*. 2012; 18(5):263–72. <https://doi.org/10.1016/j.molmed.2012.03.003> PubMed PMID: WOS:000304232000004. PMID: 22480775
2. Pitout JD, Laupland KB. Extended-spectrum β -lactamase-producing Enterobacteriaceae: an emerging public-health concern. *The Lancet infectious diseases*. 2008; 8(3):159–66. [https://doi.org/10.1016/S1473-3099\(08\)70041-0](https://doi.org/10.1016/S1473-3099(08)70041-0) PMID: 18291338
3. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*. 2018; 18(3):318–27. [https://doi.org/10.1016/S1473-3099\(17\)30753-3](https://doi.org/10.1016/S1473-3099(17)30753-3) PMID: 29276051
4. Woerther PL, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev*. 2013; 26(4):744–58. <https://doi.org/10.1128/CMR.00023-13> PMID: 24092853; PubMed Central PMCID: PMC3811232.
5. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. *Infect Control Hosp Epidemiol*. 2008; 29(12):1099–106. <https://doi.org/10.1086/592412> PMID: 18973455.
6. Souverein D, Euser SM, Herpers BL, Kluytmans J, Rossen JW, Den Boer JW. Association between rectal colonization with Highly Resistant Gram-negative Rods (HR-GNRs) and subsequent infection with HR-GNRs in clinical patients: A one year historical cohort study. *PloS one*. 2019; 14(1):e0211016. <https://doi.org/10.1371/journal.pone.0211016> PMID: 30682095
7. Denkel LA, Maechler F, Schwab F, Kola A, Weber A, Gastmeier P, et al. Infections caused by extended-spectrum β -lactamase-producing Enterobacteriales after rectal colonization with ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae*. *Clinical Microbiology and Infection*. 2019. <https://doi.org/10.1016/j.cmi.2019.11.025> PMID: 31809805
8. Shakil S, Ali SZ, Akram M, Ali SM, Khan AU. Risk factors for extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. *J Trop Pediatr*. 2010; 56(2):90–6. <https://doi.org/10.1093/tropej/fmp060> PMID: 19608665.
9. Pessoa-Silva CL, Meurer Moreira B, Camara Almeida V, Flannery B, Almeida Lins MC, Mello Sampaio JL, et al. Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive

- care unit: risk factors for infection and colonization. *J Hosp Infect.* 2003; 53(3):198–206. <https://doi.org/10.1053/jhin.2002.1373> PMID: 12623321.
10. Cassettari V, Da Silveira I, Dropa M, Lincopan N, Mamizuka E, Matté M, et al. Risk factors for colonisation of newborn infants during an outbreak of extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* in an intermediate-risk neonatal unit. *Journal of Hospital Infection.* 2009; 71(4):340–7. <https://doi.org/10.1016/j.jhin.2008.11.019> PMID: 19147256.
 11. Demir S, Soysal A, Bakir M, Kaufmann ME, Yagci A. Extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in paediatric wards: a nested case-control study. *J Paediatr Child Health.* 2008; 44(10):548–53. <https://doi.org/10.1111/j.1440-1754.2008.01326.x> PMID: 18557808.
 12. Andriatahina T, Randrianirina F, Hariniana ER, Talarmin A, Raobijaona H, Buisson Y, et al. High prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a pediatric unit in Madagascar. *BMC Infect Dis.* 2010; 10:204. <https://doi.org/10.1186/1471-2334-10-204> PMID: 20624313; PubMed Central PMCID: PMC2912907.
 13. Woerther PL, Angebault C, Jacquier H, Hugede HC, Janssens AC, Sayadi S, et al. Massive increase, spread, and exchange of extended spectrum beta-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. *Clin Infect Dis.* 2011; 53(7):677–85. <https://doi.org/10.1093/cid/cir522> PMID: 21890771.
 14. Pallecchi L, Malossi M, Mantella A, Gotuzzo E, Trigoso C, Bartoloni A, et al. Detection of CTX-M-type beta-lactamase genes in fecal *Escherichia coli* isolates from healthy children in Bolivia and Peru. *Antimicrob Agents Chemother.* 2004; 48(12):4556–61. <https://doi.org/10.1128/AAC.48.12.4556-4561.2004> PMID: 15561825; PubMed Central PMCID: PMC529195.
 15. Ruppe E, Woerther PL, Diop A, Sene AM, Da Costa A, Arlet G, et al. Carriage of CTX-M-15-producing *Escherichia coli* isolates among children living in a remote village in Senegal. *Antimicrob Agents Chemother.* 2009; 53(7):3135–7. <https://doi.org/10.1128/AAC.00139-09> PMID: 19364858; PubMed Central PMCID: PMC2704652.
 16. Birgy A, Cohen R, Levy C, Bidet P, Courroux C, Benani M, et al. Community faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in French children. *BMC Infect Dis.* 2012; 12:315. <https://doi.org/10.1186/1471-2334-12-315> PMID: 23171127; PubMed Central PMCID: PMC3579697.
 17. Ahmed SF, Ali MM, Mohamed ZK, Moussa TA, Klena JD. Fecal carriage of extended-spectrum beta-lactamases and AmpC-producing *Escherichia coli* in a Libyan community. *Ann Clin Microbiol Antimicrob.* 2014; 13:22. <https://doi.org/10.1186/1476-0711-13-22> PMID: 24934873; PubMed Central PMCID: PMC4107601.
 18. Bartoloni A, Pallecchi L, Riccobono E, Mantella A, Magnelli D, Di Maggio T, et al. Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in *Escherichia coli*: 20 years of surveillance in resource-limited settings from Latin America. *Clin Microbiol Infect.* 2013; 19(4):356–61. <https://doi.org/10.1111/j.1469-0691.2012.03807.x> PMID: 22414066.
 19. Pallecchi L, Bartoloni A, Fiorelli C, Mantella A, Di Maggio T, Gamboa H, et al. Rapid dissemination and diversity of CTX-M extended-spectrum beta-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother.* 2007; 51(8):2720–5. <https://doi.org/10.1128/AAC.00026-07> PMID: 17548490; PubMed Central PMCID: PMC1932529.
 20. Boo NY, Ng SF, Lim VK. A case-control study of risk factors associated with rectal colonization of extended-spectrum beta-lactamase producing *Klebsiella* sp. in newborn infants. *J Hosp Infect.* 2005; 61(1):68–74. <https://doi.org/10.1016/j.jhin.2005.01.025> PMID: 15953660.
 21. Nordberg V, Quizhpe Peralta A, Galindo T, Turlej-Rogacka A, Iversen A, Giske CG, et al. High proportion of intestinal colonization with successful epidemic clones of ESBL-producing Enterobacteriaceae in a neonatal intensive care unit in Ecuador. *PloS one.* 2013; 8(10):e76597. <https://doi.org/10.1371/journal.pone.0076597> PMID: 24146896; PubMed Central PMCID: PMC3795716.
 22. Crivaro V, Bagattini M, Salza MF, Raimondi F, Rossano F, Triassi M, et al. Risk factors for extended-spectrum beta-lactamase-producing *Serratia marcescens* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. *J Hosp Infect.* 2007; 67(2):135–41. <https://doi.org/10.1016/j.jhin.2007.07.026> PMID: 17884248.
 23. Denkel LA, Schwab F, Kola A, Leistner R, Garten L, von Weizsacker K, et al. The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E). *J Antimicrob Chemother.* 2014; 69(8):2230–7. <https://doi.org/10.1093/jac/dku097> PMID: 24729603.
 24. Bulabula A, Dramowski A, Mehtar S. Transmission of multidrug-resistant Gram-negative bacteria from colonized mothers to their infants: a systematic review and meta-analysis. *Journal of Hospital Infection.* 2020; 104(1):57–67. <https://doi.org/10.1016/j.jhin.2019.10.001> PMID: 31604126

25. Mammina C, Di Carlo P, Cipolla D, Giuffre M, Casuccio A, Di Gaetano V, et al. Surveillance of multi-drug-resistant gram-negative bacilli in a neonatal intensive care unit: prominent role of cross transmission. *Am J Infect Control*. 2007; 35(4):222–30. <https://doi.org/10.1016/j.ajic.2006.04.210> PMID: 17482993.
26. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, et al. Extended-spectrum beta-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and wide-spread prevalence of SHV- and CTX-M-type beta-lactamases. *Antimicrob Agents Chemother*. 2003; 47(11):3554–60. <https://doi.org/10.1128/aac.47.11.3554-3560.2003> PMID: 14576117; PubMed Central PMCID: PMC253771.
27. Wiener-Well Y, Rudensky B, Yinnon AM, Kopuit P, Schlesinger Y, Broide E, et al. Carriage rate of carbapenem-resistant *Klebsiella pneumoniae* in hospitalised patients during a national outbreak. *J Hosp Infect*. 2010; 74(4):344–9. <https://doi.org/10.1016/j.jhin.2009.07.022> PMID: 19783067.
28. Birgy A, Doit C, Mariani-Kurkdjian P, Genel N, Faye A, Arlet G, et al. Early detection of colonization by VIM-1-producing *Klebsiella pneumoniae* and NDM-1-producing *Escherichia coli* in two children returning to France. *Journal of clinical microbiology*. 2011; 49(8):3085–7. PubMed PMID: Medline:21653781. <https://doi.org/10.1128/JCM.00540-11> PMID: 21653781
29. Viau RA, Hujer AM, Marshall SH, Perez F, Hujer KM, Briceno DF, et al. "Silent" dissemination of *Klebsiella pneumoniae* isolates bearing K. pneumoniae carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 2012; 54(9):1314–21. PubMed PMID: Medline:22492318.
30. Drew RJ, Turton JF, Hill RL, Livermore DM, Woodford N, Paulus S, et al. Emergence of carbapenem-resistant Enterobacteriaceae in a UK paediatric hospital. *J Hosp Infect*. 2013; 84(4):300–4. <https://doi.org/10.1016/j.jhin.2013.05.003> PMID: 23831281.
31. Giuffre M, Bonura C, Geraci DM, Saporito L, Catalano R, Di Noto S, et al. Successful control of an outbreak of colonization by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* sequence type 258 in a neonatal intensive care unit, Italy. *The Journal of hospital infection*. 2013; 85(3):233–6. PubMed PMID: Medline:24074641. <https://doi.org/10.1016/j.jhin.2013.08.004> PMID: 24074641
32. Mougkou K, Michos A, Spyridopoulou K, Daikos GL, Spyridis N, Syriopoulou V, et al. Colonization of high-risk children with carbapenemase-producing Enterobacteriaceae in Greece. *Infection control and hospital epidemiology*. 2013; 34(7):757–9. PubMed PMID: Medline:23739084. <https://doi.org/10.1086/670997> PMID: 23739084
33. Dirajlal-Fargo S, DeBiasi R, Campos J, Song X. Carbapenem-resistant Enterobacteriaceae in pediatric patients: epidemiology and risk factors. *Infect Control Hosp Epidemiol*. 2014; 35(4):447–9. <https://doi.org/10.1086/675593> PMID: 24602957.
34. Ulu-Kilic A, Alp E, Percin D, Cevahir F, Altay-Kurkcu C, Ozturk A, et al. Risk factors for carbapenem resistant *Klebsiella pneumoniae* rectal colonization in pediatric units. *J Infect Dev Ctries*. 2014; 8(10):1361–4. <https://doi.org/10.3855/jidc.4593> PMID: 25313618.
35. Abdalhamid B, Elhadi N, Alabdulqader N, Alsamman K, Aljindan R. Rates of gastrointestinal tract colonization of carbapenem-resistant Enterobacteriaceae and *Pseudomonas aeruginosa* in hospitals in Saudi Arabia. *New Microbes New Infect*. 2016; 10:77–83. <https://doi.org/10.1016/j.nmni.2016.01.014> PMID: 26933499; PubMed Central PMCID: PMC4765740.
36. Akturk H, Sutcu M, Somer A, Aydin D, Cihan R, Ozdemir A, et al. Carbapenem-resistant *Klebsiella pneumoniae* colonization in pediatric and neonatal intensive care units: risk factors for progression to infection. *Braz J Infect Dis*. 2016; 20(2):134–40. <https://doi.org/10.1016/j.bjid.2015.12.004> PMID: 26867474.
37. Caselli D, Cesaro S, Fagioli F, Carraro F, Ziino O, Zanazzo G, et al. Incidence of colonization and bloodstream infection with carbapenem-resistant Enterobacteriaceae in children receiving antineoplastic chemotherapy in Italy. *Infect Dis (Lond)*. 2016; 48(2):152–5. <https://doi.org/10.3109/23744235.2015.1087647> PMID: 26393496.
38. Diaz A, Ortiz DC, Trujillo M, Garces C, Jaimes F, Restrepo AV. Clinical Characteristics of Carbapenem-resistant *Klebsiella pneumoniae* Infections in Ill and Colonized Children in Colombia. *Pediatr Infect Dis J*. 2016; 35(3):237–41. <https://doi.org/10.1097/INF.0000000000000987> PMID: 26569194.
39. Karaaslan A, Soysal A, Altinkanat Gelmez G, Kepenekli Kadayifci E, Soyletir G, Bakir M. Molecular characterization and risk factors for carbapenem-resistant Gram-negative bacilli colonization in children: emergence of NDM-producing *Acinetobacter baumannii* in a newborn intensive care unit in Turkey. *J Hosp Infect*. 2016; 92(1):67–72. <https://doi.org/10.1016/j.jhin.2015.09.011> PMID: 26601601.
40. Essack SY, Hall LM, Pillay DG, McFadyen ML, Livermore DM. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum beta-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. *Antimicrob Agents Chemother*. 2001; 45(1):88–95. <https://doi.org/10.1128/AAC.45.1.88-95.2001> PMID: 11120950; PubMed Central PMCID: PMC90245.

41. 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. <https://doi.org/10.1186/s12879-016-1919-y> PMID: 27751185; PubMed Central PMCID: PMC5067886.
42. 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).
43. Dramowski A, Cotton MF, Rabie H, Whitelaw A. Trends in paediatric bloodstream infections at a South African referral hospital. *BMC Pediatrics.* 2015; 15(1):33.
44. Malande OO, Du Plessis A, Rip D, Bamford C, Eley B. Invasive carbapenem-resistant Enterobacteriaceae infection at a paediatric hospital: A case series. *S Afr Med J.* 2016; 106(9):877–82. <https://doi.org/10.7196/SAMJ.2016.v106i9.11028> PMID: 27601110.
45. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100 (ISBN 1-56238-804-5) [Print]; ISBN 1-56238-805-3 [Electronic]. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2017.
46. Young AL, Nicol MP, Moodley C, Bamford CM. The accuracy of extended-spectrum beta-lactamase detection in *Escherichia coli* and *Klebsiella pneumoniae* in South African laboratories using the Vitek 2 Gram-negative susceptibility card AST-N255. *Southern African Journal of Infectious Diseases.* 2019; 34(1):1–6.
47. Revised surveillance case definition for HIV infection—United States, 2014. *MMWR Recommendations and reports: Morbidity and mortality weekly report Recommendations and reports.* 2014; 63(Rr-03):1–10. Epub 2014/04/11. PMID: 24717910.
48. Sanders CC, Peyret M, Moland ES, Shubert C, Thomson KS, Boeufgras J-M, et al. Ability of the VITEK 2 Advanced Expert System To Identify β -Lactam Phenotypes in Isolates of Enterobacteriaceae and *Pseudomonas aeruginosa*. *Journal of Clinical Microbiology.* 2000; 38(2):570–4. <https://doi.org/10.1128/JCM.38.2.570-574.2000> PMID: 10655347
49. Oren I, Sprecher H, Finkelstein R, Hadad S, Neuberger A, Hussein K, et al. Eradication of carbapenem-resistant Enterobacteriaceae gastrointestinal colonization with nonabsorbable oral antibiotic treatment: A prospective controlled trial. *Am J Infect Control.* 2013; 41(12):1167–72. <https://doi.org/10.1016/j.ajic.2013.04.018> PMID: 24274912.
50. Arhoune B, Oumokhtar B, Hmami F, Barguigua A, Timinouni M, El Fakir S, et al. Rectal carriage of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae among hospitalised neonates in a neonatal intensive care unit in Fez, Morocco. *J Glob Antimicrob Resist.* 2017; 8:90–6. <https://doi.org/10.1016/j.jgar.2016.11.004> PMID: 28039104.
51. Lochan H, Pillay V, Bamford C, Nuttall J, Eley B. Bloodstream infections at a tertiary level paediatric hospital in South Africa. *BMC infectious diseases.* 2017; 17(1):750.
52. Liu H, Wang Y, Wang G, Xing Q, Shao L, Dong X, et al. The prevalence of *Escherichia coli* strains with extended spectrum beta-lactamases isolated in China. *Frontiers in Microbiology.* 2015;6. <https://doi.org/10.3389/fmicb.2015.00006> PMID: 25713560
53. Paterson DL, Bonomo RA. Extended-spectrum β -lactamases: a clinical update. *Clinical microbiology reviews.* 2005; 18(4):657–86. <https://doi.org/10.1128/CMR.18.4.657-686.2005> PMID: 16223952
54. Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of Piperacillin-Tazobactam vs Meropenem on 30-Day Mortality for Patients With *E coli* or *Klebsiella pneumoniae* Bloodstream Infection and Ceftriaxone Resistance: A Randomized Clinical Trial. *Jama.* 2018; 320(10):984–94. Epub 2018/09/13. <https://doi.org/10.1001/jama.2018.12163> PMID: 30208454; PubMed Central PMCID: PMC6143100.