

Molecular characterization of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* among the pediatric population in Qatar

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Abstract

Background: Although extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* are a public health problem in the Arabian Peninsula, data on the molecular characteristic of their antimicrobial resistance determinants in children is limited.

Methods: Whole-genome sequencing (WGS) was performed on ESBL-producing *E. coli* and *K. pneumoniae* isolates recovered from screening and clinical specimens from pediatric patients at Sidra Medicine in Doha from January to December 2018.

Results: WGS was performed on 327 ESBL producers: 255 *E. coli* and 72 *K. pneumoniae*. The most common sequence types (ST) were ST131 (16.5%), ST38 and ST10 (8.2 each%) in *E. coli* and ST307 (9.7%), ST45 and ST268 (6.9% each) in *K. pneumoniae*. CTX-M type ESBL were found in all but one isolate, with CTX-M-15 accounting for 87.8%. Co-carriage of OXA-1 alone or in combination with TEM-1B was associated with reduced susceptibility to

amoxicillin/clavulanate ($P=0.002$ and $P<0.0001$) and piperacillin/tazobactam ($P=0.02$ and $P=0.004$). The most common plasmid-mediated quinolone resistance genes co-carried were *qnr A/B/E/S* (45.3%). Ninety percent of gentamicin non-susceptible isolates harboured genes encoding AAC(3) enzymes, mainly *aac(3)-IIa*. Only 2 of 57 isolates harbouring *aac(6')-Ib-cr* were non-susceptible to amikacin. Eighty-five percent of isolates carried IncF plasmids.

Conclusions: Our data show that CTX-M is largely the most prevalent ESBL type in the pediatric population in Qatar with a predominance of CTX-M-15. Carbapenem-sparing options to treat ESBL infections are limited given the frequent co-production of OXA-1 and TEM-1B enzymes and co-resistance to antibiotic classes other than β -lactams.

Background

Over the last four decades, extended-spectrum β -lactamases (ESBLs) have presented a major challenge to treating infections caused by *Enterobacteriaceae* in healthcare and community settings [1–3]. Resistance to third- and fourth-generation cephalosporins mediated by ESBLs and the often associated co-resistance to other antibiotic classes such as fluoroquinolones, aminoglycosides and sulphonamides, has led to the increased use of carbapenems worldwide, which in turn is linked to increasing resistance to these agents [3–4]. Currently, the global epidemiology of ESBLs is dominated by CTX-M type enzymes, mainly due to the explosive dissemination of CTX-M-15 among *Escherichia coli* and *Klebsiella pneumoniae*. Nevertheless, regional variations in the prevalence, circulating enzyme types and sequence types (STs) among ESBL producers have been reported among different patient groups in different parts of the world [1–2].

Although the Arabian Peninsula is a region with one of the highest prevalence rates of ESBL-producing *Enterobacteriaceae* [5], the molecular epidemiology in children has never been studied using high-

resolution genomics. This study used whole-genome sequencing (WGS) to comprehensively characterize ESBL in *E. coli* and *K. pneumoniae* isolates recovered from screening and clinical specimens from pediatric patients at Sidra Medicine in Doha.

Methods

Setting, isolates and screening protocol

The study was conducted at Sidra Medicine, a new 400-bed tertiary care women's and children's hospital in Doha that has been functioning at full capacity since January 2018. The hospital serves as a referral centre for the pediatric population of Qatar for all medical subspecialties, including oncology, neonatal intensive care and pediatric intensive care, and surgical specialties, including cardiac surgery and neurosurgery.

All non-duplicate ESBL-producing *E. coli* and *K. pneumoniae* isolates from clinical specimens as well as rectal swabs submitted for carbapenemase-producing organism (CPO) screening from children younger than 18 years of age with medical or surgical conditions that require an emergency or elective hospital admission were retrospectively studied for a one-year period, between January and December 2018.

As a part of the infection control surveillance program in our institution, a CPO questionnaire-based risk assessment is performed on emergency and elective admissions is performed. The questionnaire consists of three questions to identify previous colonization with multidrug-resistant organisms, and admission and history of invasive procedures in the last 12 months in other health care facilities within or outside of Qatar. Rectal swabs are collected from patients who answered affirmatively to any of the questions or from those who are unsure about the answer to any of the questions. In addition, patients admitted to the Neonatal Intensive Care Unit (NICU) and Pediatric Intensive Care Unit (PICU) are routinely screened for CPO carriage on admission and then once a week.

Bacterial identification and antimicrobial susceptibility testing

Screening rectal swabs were directly inoculated onto two selective chromogenic media (CHROMagar™ ESBL and CHROMagar™ mSuperCARBA, CHROMagar, France) to detect ESBL and carbapenemase producers, respectively. Colonies isolated from any of the screening media and *Enterobacteriaceae* isolated from clinical specimens, were identified using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF) (Bruker, Germany) and tested for antimicrobial susceptibility using the BD Phoenix™ automated identification and susceptibility testing system (Becton Dickinson, USA). Minimum inhibitory concentrations (MIC) and breakpoints were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [6]. Isolates with intermediate susceptibility and resistant to each antibiotic tested were grouped in a single non-susceptible category. The CLSI susceptible-dose-

dependent category for cefepime was considered as intermediate for the purpose of this study. Non-susceptible isolates to any of the third-generation cephalosporins or cefepime were considered as potential ESBL producers.

Whole-genome sequencing and bioinformatic analysis

WGS was performed on all organisms with a susceptibility pattern consistent with ESBL production on the Illumina Miseq platform. Paired-end (PE) DNA libraries were constructed using Nextera XT (Illumina, San Diego, CA). The DNA was tagmented, amplified by index primers and purified with AMPure XP beads (Beckman Coulter, Brea, CA) to remove the smaller fragments. DNA libraries were then normalized, pooled and sequenced using the MiSeq platform generation 300 bp PE reads (MiSeq Reagent Kit V3). Raw reads were assessed by Fastqc (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and quality trimmed by Trim Galore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) to eliminate adaptors and low quality sequences. Trimmed reads were assembled de novo using SPAdes v.3.9.0 [7], and smaller contigs (< 500 bp) were excluded. The assessment of all genome assemblies was performed using QUAST [8], contaminant reads were excluded after analysis by Kraken [9]. Sequence types (ST) and the genes encoding antimicrobial resistance for β -lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulphamethoxazole (co-trimoxazole) were predicted based on the PubMLST typing schemes implemented in *mlst* (<https://github.com/tseemann/mlst>) and the ResFinder database [10] implemented in *abricate* (<https://github.com/tseemann/abricate>), respectively. The plasmids and incompatibility scheme were defined based on the PlasmidFinder database [11] and Kleborate [12]. The phylogroup and serotypes of *E. coli* were predicted using ClermonTyping [13] and ECTyper <https://github.com/phac-nml/ecoli_serotyping>. The relatedness of strains was inferred using Parsnp [14].

Statistical analysis

Associations were assessed by the chi-squared test with Yates' correction for continuity, or Fisher's exact test when an expected value was less than 5. In addition, the analysis of some variables was broken down into three different nursing units based on the CPO screening policy: PICU and NICU, where active surveillance of ESBL carriage was performed on a weekly basis; and General Pediatrics (GPED) included the remaining units in which ESBL colonisation were studied depending on the result of the risk assessment survey.

Results

A total of 327 ESBL producers were sequenced, 255 *E. coli* and 72 *K. pneumoniae* (Additional file 1). Of them, 203 *E. coli* (79.6%) and 55 *K. pneumoniae* (76.4%) were recovered from screening specimens. Sixty-nine strains were isolated from clinical specimens (*E. coli* 52 isolates; 25.5% and *K. pneumoniae* 17 isolates; 31.5%), including urine (36 isolates; 52.2%), peritoneal fluid (8 isolates; 11.6%), respiratory tract (7 isolates; 10.1%), blood stream (6 isolates; 8.7%), pus and wound (5 isolates; 7.2% each) and

cerebrospinal fluid (2 isolates; 2.9%). The proportion of clinical isolates in GPED, PICU and NICU were 27.6% (55 isolates), 8.9% (8 isolates) and 15.8% (6 isolates), respectively.

Eighty-eight different sequence types (STs) were detected in *E. coli*, whereas 36 STs were found in *K. pneumoniae*. The 5 most prevalent STs in *E. coli* were ST131 (42 isolates; 16.5%) followed by ST38 and ST10 (21 isolates; 8.2% each), ST1193 (10 isolates; 3.9%) and ST73 (8 isolates; 3.1%). All ST131 isolates belonged to phylogroup B2. O25:H4 accounted for 23 ST131 clones (54.8%). The 3 most common STs among *K. pneumoniae* isolates were ST307 (7 isolates; 9.7%) followed by ST45 and ST268 (5 isolates; 6.9% each) (Additional file 1 and Additional file 2).

Eighty percent of NICU isolates, including all *K. pneumoniae* isolates, were detected during hospital stay. Carriage acquisition rate and infection acquisition rate in NICU during hospitalisation for the study period were 3.28/1000 patient-days and 0.82/1000 patient-days. Twenty-nine percent of PICU isolates were detected during hospitalization. Carriage acquisition rate and infection acquisition rate in PICU were 5.82/1000 patient-days and 0.76/1000 patient-days. There were no outbreaks detected in these units during the study.

Overall, non-susceptibility rates among ESBL-producing *E. coli* and *K. pneumoniae* isolates were 99.2% and 98.6% for ceftriaxone, 60.2% and 87.3% for ceftazidime, 68.8% and 73.2% for cefepime, 46.5% and 70.8% for amoxicillin/clavulanate, 12.5% and 31% for piperacillin/tazobactam, 5.1% and 9.9% for ertapenem, 3.1% and 8.5% for meropenem, 18.4% and 29.6% for gentamicin, 0.8% and 4.2% for amikacin, 41.4% and 40.8% for ciprofloxacin, 66% and 76.1% for co-trimoxazole, and 7.8% and 60.6% for nitrofurantoin. There were no significant differences in non-susceptibility rates between clinical and screening isolates by nursing unit. Of the total 69 bacterial isolates recovered from clinical specimens, 42% (29 isolates) and 20.3% (14 isolates) were non-susceptible to amoxicillin/clavulanate and piperacillin/tazobactam. By contrast, non-susceptibility among clinical isolates to ertapenem and amikacin was 3% (2 isolates) and 1.5% (1 isolate). All 29 urinary isolates of *E. coli* were susceptible to nitrofurantoin.

WGS revealed that all 327 isolates except one carried CTX-M genes. The predominant gene detected was *bla*_{CTX-M-15} in 287 isolates (87.8%), followed by *bla*_{CTX-M-14} and *bla*_{SHV-106} in 9 isolates each (2.8%). ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* were isolated simultaneously from 17 samples with *bla*_{CTX-M-15} detected in both species in 16 of those samples.

Sixty-seven percent of isolates co-produced one or more β -lactamases. The most common genes were *bla*_{TEM-1B} and *bla*_{OXA-1} in 134 (41%) and 64 (19.6%) isolates, respectively. CTX-M-15 was present in all isolates carrying *bla*_{OXA-1} (Table 1).

Table 1. Distribution of β -lactamase, quinolone, aminoglycoside and co-trimoxazole resistance genes among ESBL-producing *E. coli* and *K. pneumoniae*

	GPED (No=199)		NICU (No=38)		PICU (No=90)	
	<i>E. coli</i> (No=169)	<i>K. pneumoniae</i> (No=30)	<i>E. coli</i> (No=19)	<i>K. pneumoniae</i> (No=19)	<i>E. coli</i> (No=67)	<i>K. pneumoniae</i> (No=23)
No (%)						
ESBL genes						
<i>bla</i> _{CTX-M-15}	143 (84.1)	30 (100)	19 (100)	18 (94.7)	56 (83.5)	21 (91.3)
<i>bla</i> _{CTX-M-14}	8 (4.7)	-	-	-	-	1 (4.3)
<i>bla</i> _{SHV-106}	0	6 (20)	-	2 (10.5)	-	1 (4.3)
<i>bla</i> _{TEM-104}	5 (3)	-	-	-	3 (4.5)	-
<i>bla</i> _{CTX-M-102}	5 (3)	-	-	-	3 (4.5)	-
<i>bla</i> _{CTX-M-159}	5 (3)	-	-	-	2 (3)	-
<i>bla</i> _{CTX-M-3}	2 (1.2)	-	-	-	1 (1.5)	-
<i>bla</i> _{CTX-M-27}	1 (0.6)	-	-	-	2 (3)	-
<i>bla</i> _{SHV-27}	-	4 (13.3)	-	2 (10.5)	-	2 (8.6)
<i>bla</i> _{CTX-M-1}	1 (0.6)	-	-	-	2 (3)	1 (4.3)
Other βL genes						
<i>bla</i> _{TEM-1B}	61 (36.1)	16 (53.3)	14 (73.7)	12 (63.2)	24 (35.8)	7 (34.8)
<i>bla</i> _{OXA-1}	23 (13.6)	17 (56.7)	5 (26.3)	8 (42.1)	7 (10.4)	4 (17.4)
<i>bla</i> _{NDM-1/5}	5 (3)	4 (13.3)	-	-	2 (3)	1 (4.3)
<i>bla</i> _{DHA}	6 (3.6)	2 (6.7)	-	-	-	1 (4)
<i>bla</i> _{TEM-33/35}	6 (3.6)	-	-	-	-	1 (4.3)
<i>bla</i> _{OXA-48}	4 (2.4)	1 (3.3)	-	-	1 (1.5)	-
<i>bla</i> _{CMV}	2 (1.2)	-	-	-	3 (4.5)	-
PMQR-G						
<i>aac</i> (6)-Ib-cr	21 (12.4)	14 (46.7)	3 (15.8)	7 (36.7)	8 (11.9)	4 (17.4)
<i>oqxAB</i>	-	26 (86.7)	-	18 (94.7)	1 (1.5)	23 (100)
<i>qepA1</i>	-	-	2 (10.5)	-	-	-
<i>qnr A/B/E/S</i>	64 (37.9)	21 (70)	7 (36.8)	11 (57.9)	28 (41.8)	17 (73.9)
AME-G						
<i>aac</i> (3)-II*	28 (16.6)	11 (36.7)	5 (26.3)	8 (42.1)	11 (16.4)	1 (4.3)
<i>aadA1</i>	18 (10.7)	4 (13.3)	-	2 (10.5)	10 (14.9)	1 (4.3)
<i>aadA2</i>	6 (3.6)	6 (20)	6 (31.6)	2 (10.5)	5 (7.7)	5 (21.7)
<i>aadA5</i>	51 (30)	2 (6.7)	1 (5.3)	-	22 (32.3)	1 (4.3)
<i>aph</i> (3'')-Ia	13 (7.7)	4 (13.3)	-	2 (10.5)	2 (3)	4 (17.4)
<i>aph</i> (3'')-Ib	72 (42.6)	21 (70)	6 (31.6)	14 (73.7)	32 (47.8)	15 (65.2)
<i>aph</i> (6)-Id	69 (40.8)	21 (70)	6 (31.6)	14 (73.7)	33 (49.3)	16 (65.2)
SXT-RG						
<i>dfrA/B</i>	114 (67.4)	25 (83.3)	13 (68.4)	12 (63.2)	46 (68.7)	14 (60.9)
<i>sul1-3</i>	112 (66.3)	24 (80)	14 (73.7)	15 (78.9)	46 (68.7)	14 (60.9)

PMQR-G: Plasmid-mediated quinolone resistance genes; AME-G: Aminoglycoside modifying enzyme genes; SXT-RG: co-trimoxazole resistance genes.

*Comprised *aac*(3)-IIa and *aac*(3)-IIc, *aac*(3)-IVa and *aac*(3)-Via.

We further assessed the association between co-production of TEM-1B and OXA-1 enzymes and non-susceptibility to amoxicillin/clavulanate and piperacillin/tazobactam, after excluding the confounding influence of other β -lactamases active against β -lactamase inhibitors, namely plasmid-mediated AmpC β -lactamases, (DHA-1, CMY 1/2/135), inhibitor-resistant TEM mutants (TEM 33/35) and carbapenemases (OXA-48-type and NDM-type). Non-susceptibility to amoxicillin/clavulanate was observed in 53.9% of isolates (48/89) carrying *bla*_{TEM-1B} alone ($P < 0.001$) and 75% (15/20) carrying *bla*_{OXA-1} alone ($P = 0.002$), whereas non-susceptibility to piperacillin/tazobactam was observed in 5.6% of isolates (5/89) carrying

*bla*_{TEM-1B} alone ($P = 0.5$) and 25% (5/20) carrying *bla*_{OXA-1} alone ($P = 0.02$). Thirty-one of the 32 isolates (96.8%) carrying both enzymes simultaneously were non-susceptible to amoxicillin/clavulanate ($P < 0.0001$), whereas 15 (46.9%) of them were non-susceptible to piperacillin/tazobactam ($P = 0.004$).

At least one plasmid-mediated quinolone resistance (PMQR) gene was detected in 199 isolates (60.9%). PMQR genes found were *qnr A/B/E/S* in 148 isolates (45.3%), *oqxAB* in 68 isolates (20.8%), *aac(6')-Ib-cr* in 57 isolates (17.4%) and *qepA* in 2 isolates (0.6%). *oqxAB* were mostly detected in *K. pneumoniae* (67/68 isolates; 98.5%). Fifteen different aminoglycoside modifying enzymes genes were detected. Ninety percent of isolates non-susceptible to gentamicin carried genes encoding AAC(3) enzymes (61/68 isolates). Conversely, only 2 (3.5%) of the 57 isolates harbouring *aac(6')-Ib-cr* were non-susceptible to amikacin. All 4 isolates with high-level resistance to amikacin carried 16S rRNA methylase genes (2 *rmtB* and 2 *armA*). The simultaneous carriage of *bla*_{OXA-1}, *aac(6')-Ib-cr* and *aac(3)-II* was strongly associated ($P < 0.0001$). Eighty-nine percent of the isolates harbouring the *dfrA17* gene also carried the *aadA5* gene while all isolates harbouring *dfrA12* carried *aadA2* genes (Table 1).

Narrow host-range IncF plasmids were present in 278 isolates (85%). FIB was the most common F replicon found in 243 isolates (74.3%) followed by FII in 162 isolates (49.5%) and FIA 99 isolates (30.3%). In addition, 10 more incompatibility group plasmids were detected, mostly as a part of multiple combinations with F replicons. The 3 most prevalent were: IncI1 (62 isolates; 19%), IncX (27 isolates; 8.3%) and IncB/O/K/Z (25 isolates; 7.6%). On the other hand, 189 isolates (57.8%) contained col-type plasmids. RNAI (119 isolates, 36.4%), col156 (88 isolates; 26.9%) and MG828 (46 isolates; 14%) were the most common representatives within this group. Plasmid location of *bla*_{CTX-M} genes could be determined in 21 isolates (Table 2). Twenty-one isolates (6.4%) did not have any plasmid detected.

Table 2. Plasmid location of *bla*_{CTX-M} genes in 22 ESBL-producing *E. coli* and *K. pneumoniae* isolates

Sample ID*	Species	ST	Unit	Specimen	<i>bla</i> _{CTX-M}	Plasmid
E6	<i>E. coli</i>	421	GPED	Rectal swab	CTX-M-15	IncFIB
E19	<i>E. coli</i>	73	GPED	Rectal swab	CTX-M-15	IncFII
CP28	<i>K. pneumoniae</i>	337	GPED	Rectal swab	CTX-M-15	IncFII
E35	<i>E. coli</i>	1193	PICU	Urine	CTX-M-15	IncFIA
E55	<i>E. coli</i>	131	PICU	Rectal swab	CTX-M-15	IncFIB
E58	<i>K. pneumoniae</i>	101	PICU	Rectal swab	CTX-M-15	IncFII
E60	<i>E. coli</i>	28	NICU	Rectal swab	CTX-M-15	IncFII
E102	<i>E. coli</i>	ND	GPED	Wound	CTX-M-15	IncI1
E128	<i>E. coli</i>	131	GPED	Rectal swab	CTX-M-15	IncB/O/K/Z
E157	<i>K. pneumoniae</i>	37	PICU	Rectal swab	CTX-M-15	IncI1
E172	<i>E. coli</i>	998	GPED	Rectal Swab	CTX-M-15	IncX
E183	<i>E. coli</i>	69	GPED	Urine	CTX-M-15	IncFIA
E206	<i>E. coli</i>	ND	GPED	Rectal swab	CTX-M-15	IncFII
E215	<i>E. coli</i>	1611	GPED	Rectal swab	CTX-M-15	IncB/O/K/Z
E320	<i>K. pneumoniae</i>	133	PICU	Rectal swab	CTX-M-1	Col156
E360	<i>E. coli</i>	73	GPED	Urine	CTX-M-15	IncI1
E379	<i>K. pneumoniae</i>	37	PICU	Rectal swab	CTX-M-15	IncI1
E403	<i>K. pneumoniae</i>	37	NICU	Rectal swab	CTX-M-15	IncI1
E443	<i>E. coli</i>	773	GPED	Rectal swab	CTX-M-15	IncFIB
E486	<i>K. pneumoniae</i>	133	PICU	Rectal swab	CTX-M-15	Col156
E496	<i>E. coli</i>	517	GPED	Rectal swab	CTX-M-15	IncFIB
E497	<i>E. coli</i>	131	GPED	Rectal swab	CTX-M-15	IncFIB

*Full characterization of these isolates can be found in Table 1, Supplemental; ST: Sequencing type

Discussion

Our data show that CTX-M are largely the most common ESBL type in the pediatric population in Qatar with CTX-M-15 as the main driving force. Our study also points out that narrow host range plasmids, mainly those belonging to the IncF family, are likely the main vehicle for the dissemination of *bla*_{CTX-M-15} along with other resistance genes such as *bla*_{OXA-1}, *aac(6)-Ib-cr* and *aac(3)-II* among a great diversity of genotypes within *E. coli* and *K. pneumoniae* [15–16]. These findings are not surprising taking into account that expatriates comprise around 87% of the Qatar's population with citizens from the Indian subcontinent representing almost 50% [17]. Therefore, it is reasonable to hypothesize that our data mainly reflect an epidemiological trend in the general population, which is likely shaped by a high rate of community carriage among residents from countries such as India, Nepal and Bangladesh, where rates of CTX-M-15 among faecal isolates from healthy individuals and clinical isolates have been reported above 90% [18–22]. In contrast, our NICU had a variety genotypes of both species without subsequent significant cross-transmission, suggesting a sporadic colonisation/infection within a group that is at particular high risk for ESBL acquisition [23].

Sequence analysis revealed that the management of ESBL infections in our paediatric population is challenging, particularly concerning carbapenem-sparing options. The effect of clavulanic acid and

tazobactam in inhibiting CTX-M enzymes is often counteracted in our isolates by the co-production of TEM-1B and OXA-1 [24], and to a lesser extent by other enzymes such as plasmid-mediated AmpC and carbapenemases. For example, amoxicillin/clavulanate does not seem to be a reliable agent for the treatment of uncomplicated ESBL lower urinary tract infections (UTI). Fortunately, nitrofurantoin remains a good option in this scenario, especially in cases caused by *E. coli*. On the other hand, although TEM-1B seems to have a poor inhibitory effect against tazobactam, the common co-production of OXA-1 alone or accompanied by TEM-1B, makes piperacillin/tazobactam an unreliable agent for the empirical or definitive treatment of systemic ESBL infections in our context, irrespective of the unresolved controversy concerning the usefulness of this agent in high-inoculum infections [4, 25–26]. Although ertapenem seems to be an appealing option, the non-susceptible rate in the whole collection of isolates was not negligible. It should be noted that 3 out of 20 non-susceptible isolates to ertapenem carrying *bla*_{CTX-M-15} lacked genes encoding carbapenemases or AmpC β -lactamases, suggesting that ertapenem can also be a substrate for CTX-M-15 when its penetration through the outer membrane is reduced due to porin deficiencies [27]. Particularly noteworthy was high proportion of isolates susceptible to amikacin despite the presence *aac(6′)-Ib-cr* genes in 17% of strains. Although monotherapy with amikacin has been proposed to treat febrile UTI in children in regions with low resistance rates among ESBL producers [23, 28–29], it should be kept in mind that it is not uncommon to find susceptible isolates according to the CLSI breakpoints (MIC \leq 16 mg/L) harbouring *aac(6′)-Ib-cr* with MICs between 4 and 8 mg/L [2430]. Therefore, it might be speculated that the ratio C_{\max}/MIC , the main pharmacodynamic parameter that predicts bacterial killing and prevent selection of resistance in aminoglycosides [31–32], could be often compromised when *aac(6′)-Ib-cr* is carried. The high ciprofloxacin non-susceptibility rate was also noticeable, given that quinolones are rarely prescribed in our pediatric institution. Although *oqxAB* genes are mainly chromosomally located in *K. pneumoniae* [33], the high proportion of PMQR determinants in our isolates suggests that transmission of these genes among commensal intestinal flora through the plasmid types reported here, may be facilitating the selection and spread of resistant mutants in the community [34–35]. Finally, it was not surprising to find a high co-resistance rate to co-trimoxazole, which renders this combination ineffective at least as empirical therapy. Interestingly, the strong association between *dfrA17* and *aadA5* genes and between *dfrA12* and *aadA2* genes suggests that class 1 integrons were not uncommon in our isolates. Class 1 integrons containing these gene cassette combinations are often located in IncF plasmids along with resistance genes such as *bla*_{CTX-M-15}, *bla*_{OXA-1} and *aac(6′)-Ib-cr* [36–37].

Our study has several limitations. Since MICs were determined by the Phoenix automated system, which does not always provide an exact value within the susceptibility range, low-level resistance conferred by certain genes could not be assessed when MICs did not exceed susceptible breakpoints, for instance, low-level amikacin resistance in isolates carrying *aac(6′)-Ib-cr* or low-level piperacillin/tazobactam resistance in the presence of OXA-1 enzyme [24, 30]. Moreover, the activity of other potential useful agents available in Qatar, particularly ceftazidime-avibactam, ceftolozane-tazobactam and fosfomicin, could not be assessed because susceptibility cards used in this study did not contain any of these antibiotics, although it could be assumed that ceftazidime-avibactam would have been effective against our isolates

in the absence of co-production of NDM-type carbapenemases [4]. It should be also taken into account that the population studied mostly comprised children at risk for acquisition of multidrug-resistant bacteria, which may have biased the actual burden of some resistance determinants in our entire pediatric population. Finally, plasmid replicons were analysed using genome assemblies derived from short-read sequence data, which hindered reliable plasmid sequence reconstruction and elucidation of plasmid location of CTX-M genes in the majority of isolates. In fact, plasmid sequences were not detected by PlasmidFinder database in 21 isolates, which indicated the challenge on the inference of plasmids from fragmented, small-sized contigs but also by a potential chromosomal integration of *bla*_{CTX-M} genes, a phenomenon increasingly reported in clinical isolates [38–39].

Conclusion

In summary, our study showed that CTX-M enzymes are overwhelmingly prevalent in the pediatric population of Qatar with CTX-M-15 as the major driving force. High community carriage rates among a multinational population appears to be the main source for *bla*_{CTX-M-15} and other resistance genes, which are likely vehiculized and spread by epidemic plasmids. Our study also highlighted the promising value of NGS-based technologies to improve antibiotic prescribing practices by uncovering resistance mechanisms not detected by conventional phenotypic antimicrobial susceptibility testing. To the best of our knowledge, this is the first study characterizing ESBL in children using WGS in the Arabian Peninsula. Since the labour force from the Indian subcontinent also represents the largest group of foreign citizens in other Gulf States, we believe that our results could be extrapolated at least to the middle and eastern part of the region.

Abbreviations

ESBL

extended-spectrum β -lactamase

WGS

whole-genome sequencing

CPO

carbapenemase-producing organism

CLSI

Clinical and Laboratory Standards Institute

MIC

minimum inhibitory concentration

NICU

Neonatal Intensive Care Unit

PICU

Pediatric Intensive Care Unit

GPED

General Pediatrics

ST

sequence type

PMQR

plasmid-mediated quinolone resistance

UTI

urinary tract infection

Declarations

Ethics approval and consent to participate

Ethics approval for the study was obtained from the Institutional Review Board of Sidra Medicine.

Consent for publication

Not applicable

Availability of data and materials

Comprehensive molecular data for all isolates can be found in Additional file 1.

Competing interests

The authors have no conflicts of interest.

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Authors' contributions

APL designed the study, analyzed data and drafted the manuscript; SS performed WGS, supported HAM in collecting isolates and performing antimicrobial susceptibility testing and revised the manuscript; HAM collected isolates, performed antimicrobial susceptibility testing on selected isolates, supported SS in performing WGS, carried out the statistical analysis and revised the manuscript; KMT performed the bioinformatic analysis and revised the manuscript; MH supervised WGS process and bioinformatic analysis and revised the manuscript; PT coordinated and supervised the project and substantially revised the manuscript. All authors read and approved the final version of the manuscript.

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