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Evaluation of *in Vitro* Activity of Ceftazidime/Avibactam and Ceftolozane/Tazobactam against ESBL-producing Enterobacterales Isolated from Intensive Care Units from Qatar

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ABSTRACT

Objectives: Extended-spectrum β-lactamases (ESBLs) mechanism of resistance in Enterobacterales leads to poor clinical outcomes. Ceftazidime/avibactam and ceftolozane/tazobactam are two broad-spectrum antimicrobial combinations that are effective against multidrug-resistant organisms with regional variations. This study aims to evaluate the antimicrobial susceptibility test (AST) for both combinations against ESBL-producing Enterobacterales isolated from intensive care units (ICUs) in tertiary hospitals from November 2012 to October 2013 in Qatar. ***Methods***: A total of 629 Enterobacterales isolates from ICUs were screened for ESBL production using BD-Phoenix™ confirmed by double-disk potentiation, while ESBL-genes were detected by polymerase chain reaction. The ASTs for ceftazidime/avibactam and ceftolozane/tazobactam were assessed by minimum inhibitory concentration (MIC) test strips. A single isolate that was resistant to both combinations was subjected to whole-genome sequencing. ***Results:*** The prevalence of ESBL-producing Enterobacterales isolated from ICUs was 17.3% (109/629) with predominance of *Klebsiella pneumoniae* (56/109; 51.4%) and *Escherichia coli* (38/109; 34.9%). The susceptibility of ceftazidime/avibactam and ceftolozane/tazobactam against ESBL-producers was 99.1% (108/109) and most (81/109; 74.3%) had MICs < 0.5 for both combinations. The predominant ESBL-gene was $bla_{\text{CTX-M}}$ (72/109; 66.1%). A single isolate that was resistant to both combinations harbored multiple ESBL resistant-genes including $bla_{\text{VEB-5}}$ and $bla_{\text{VIM-2}}$. ***Conclusions:*** ESBL-producing Enterobacterales isolated from ICUs were predominantly *K. pneumoniae* and *E. coli,* mainly harboring $bla_{\text{CTX-M}}$ gene. They were highly susceptible to ceftazidime/avibactam and ceftolozane/tazobactam suggesting potential alternatives to currently available therapeutic options.

The management of infections secondary to multidrug-resistant organisms (MDROs) that encompasses gram-negative bacteria (GNB) is a global healthcare challenge not only because of the limited available treatment options but also for their associated significant morbidity and mortality as well as the substantial cost of management.[1,2]

In secondary and tertiary hospitals, the ultimate antimicrobial resistance (AMR) is encountered at intensive care units (ICUs) where the critical nature of patients' cohort, concurrent comorbidities, invasive procedures, prior colonization as well as environmental exposure to MDROs that is accelerated by high antibiotics consumption are inevitable acquisition hazards.[3,4] Over the past decade in Qatar, internal microbiological surveillance and monitoring of GNB, particularly the Enterobacterales, has revealed alarmingly rising trends of AMR, particularly for extended-spectrum β-lactamases (ESBLs) in line with shifting regional epidemiology.[5,6] In critical care settings typical

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recommended approach for the management of ESBL-producing Enterobacterales is treatment with carbapenems, particularly if there is an associated serious invasive or high inoculum disease.[7] The concern of diminishing efficacy of the limited treatment options against the ever-rising resistant bacterial strains, led infection specialists to seek alternatives to carbapenem therapy.

Ceftazidime/avibactam and ceftolozane/tazobactam are β-lactam/β-lactamase inhibitors (BLBLIs) combinations that are approved by both the United States Food and Drug Administration and the European Medicines Agency, demonstrating comparable or superior activity against MDROs particularly in GNB for the treatment of complicated urinary tract and intra-abdominal infections as well as infections secondary to hospital or ventilation associated pneumonia.[8] Avibactam is a non-BLBLI that potently inhibits most (but not all) class A ESBLs, class C (including AmpC enzymes), and some class D β-lactamases.[9] Furthermore, due to its different mode of action, avibactam is considered as one of the most effective BLBLIs displaying a broader inhibitory range and spectrum.[10] On the other hand, ceftolozane is a novel cephalosporin that is not affected by outer membrane protein loss which is a weak substrate for drug efflux pump mechanism, rendering the drug exhibiting less affinity for hydrolysis by AmpC, and hence better efficacy.[11] Pairing ceftolozane with the classic β-lactamase inhibitor tazobactam has broadened their capacity to act on most ESBL-producing GNB.[12]

The presented study aims mainly to evaluate the antimicrobial activity of ceftazidime/avibactam and ceftolozane/tazobactam against 109 ESBL-producing Enterobacterales isolates from ICUs in Qatar,[13] describe its microbiological characteristics, as well as the underlying genomic resistance profiles.

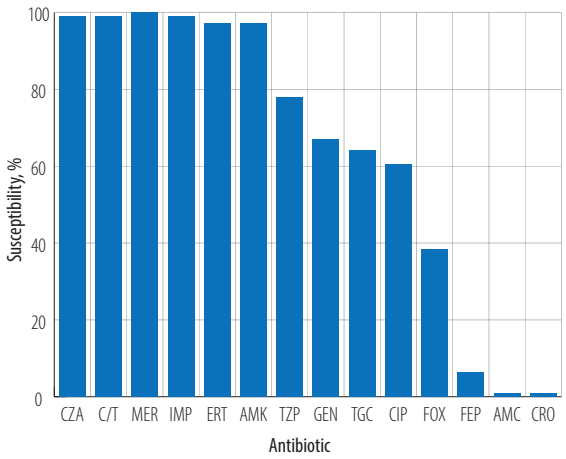
METHODS

This research project was approved by the Institutional Review Board at Hamad Medical Corporation (HMC), which complies with international ethical standards and regulations (Protocol no. RC/75813/2013). The study was conducted on routine specimens processed by the Microbiology Division, Department of Laboratory Medicine and Pathology, HMC, Qatar. All samples were collected prospectively over one year (1 November 2012 to 31 October 2013) from patients admitted to all ICUs (medical 29%, surgical 29%, trauma 16%, pediatric 16%, and neonatal 10%) at HMC. These were then analyzed for the presence of resistant pathogens.

The study definitions recognized duplicates of the same species of bacteria as isolates from the same patient displaying identical antimicrobial susceptibility patterns when isolated within 30 days regardless of sample sites which were considered repetitive and excluded. Isolates with major differences in antimicrobial susceptibilities were counted as new even within the defined 30 days time frame. The single isolate that was resistant to ceftazidime/avibactam and ceftolozane/tazobactam underwent standard diagnostic work-up, and then was stored at -80 °C pending further genomic analysis.

Microbiological identification and antimicrobial susceptibility tests (AST) were performed using BD Phoenix™ automated system according to manufacturer recommendations. Samples that tested positive for ESBL by Phoenix, or showed a minimum inhibitory concentration (MIC) of > 8 µg/mL for 3rd generation cephalosporins or aztreonam, were subsequently confirmed by a double-disk potentiation test with ceftazidime, amoxicillin/clavulanic acid, ceftriaxone, and cefoxitin antibiotics, interpreted as recommended by Clinical Laboratory Standards Institute standards for ESBL identification.[14] AST and MIC for ceftazidime/avibactam and ceftolozane/tazobactam were performed using MIC Test Strips (Liofilchem®, Diagnostics, Italy). *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Susceptibility reporting was based on the Clinical Laboratory Standards Institute recommendations.[14] Since there were no recommended intermediate susceptibility categories available for ceftazidime/avibactam against Enterobacterales, isolates were described as 'susceptible' if the MIC was ≤ 8 mg/L and 'non-susceptible' if the MIC was > 8 mg/L as outlined in the supplementary Table A1 in Appendix.[14] To achieve consistency, intermediate and resistant categories were grouped as non-susceptible for all reported antimicrobial agents.

Bacterial DNA extraction and detection of ESBL resistance genes were performed through an in-house polymerase chain reaction (PCR) technique, using the boiling lysis methods.[15] PCR reactions for the



AMC: amoxicillin/clavulanic acid; AMK: amikacin; CIP: ciprofloxacin; CRO: ceftriaxone; C/T: ceftolozane/tazobactam; CZA: ceftazidime/avibactam; ERT: ertapenem; FEP: cefepime; FOX: cefoxitin; IMP: imipenem; GEN: gentamicin; MEM: meropenem; TGC: tigecycline; TZP: piperacillin/tazobactam; TOB: tobramycin.

Figure 1: Antimicrobial susceptibility results for ceftazidime/avibactam, ceftolozane/tazobactam, and comparator agents against clinical extended-spectrum β-lactamase-producing Enterobacterales isolates from Qatar.

ESBL genes (TEM, SHV, and CTX-M-1) were conducted using previously described protocols.[16]

Whole-genome sequencing (WGS) was performed to study isolated genomic relationships for annotating antibiotic resistance genes (ARGs). Extracted DNA was sent to GATC Service (Eurofins Genomics, Germany) for sequencing using Illumina HiSeq 2000 system (Illumina, San Diego, California). The genes were assembled using SPAdes, Version 3.13.0 (https://cab.spbu.ru/software/spades/) while Multi-locus sequence typing (MLST) of the described resistant isolate of *E. coli* was performed on MLST server 1.8 provided (https://cge.cbs.dtu.dk/services/MLST/). ARGs were annotated using Comprehensive Antibiotic Resistance Database (CARD), Version 1.2.0 (https://card.mcmaster.ca/).

Demographics of patients, characteristics of isolates, as well as the patterns of antimicrobial susceptibility of ESBL-producing Enterobacterales including resistant genes were presented as numbers and percentages using Stata statistical software (Stata Corp LLC, College Station, Texas version 16.1).

RESULTS

Out of 629 Enterobacterales isolates investigated, 109 (17.3%) isolates from 87 patients were found to be ESBL positive. The source samples of these were: respiratory 35.8% (39/109), blood 27.5% (30/109), urine 24.8% (27/109), fluids 6.4% (7/109), and others 5.5% (6/109). The ESBL-positive isolates were predominantly *Klebsiella pneumoniae* (50.5%) and *E. coli* (34.9%) while other species comprised 13.7%. The majority of isolates were from male patients 65 (59.6%) and those aged between one month and 86 years. The patients were categorized into three age groups labeled adult, pediatric, and geriatrics. 'Adult' group (14–65 years) contributed to more than half (57/109; 52.3%) of the resistant isolates, followed by 'pediatric' (9/109; 8.3%) < 14 years, and 'geriatric' (26/109; 23.9%) > 65 years.

The predominantly identified ESBL-producing genes were $bla_{\text{CTX-M-1}}$ (72/109; 66.1%) followed by bla_{SHV} (58/109; 53.2 %) and bla_{TEM} (44/109; 40.4%). All three β-lactamase genes (TEM, SHV, and CTX-M-1) were detected in 26/56 (46.4%) of *K. pneumoniae* isolates, while two genes (SHV/CTX-M-1) were present in 10/56 (17.8%) of *K. pneumoniae* and only 1/38 (2.6%) of *E. coli* isolates, with TEM/CTX-M-1 being present in 7/38 (18.4%) of *E. coli* and 4/56 (7.1%) of *K. pneumoniae,* while TEM/SHV was detected in only 2/38 (5.3%) of *E. coli* isolates.

The activity of ceftazidime/avibactam and ceftolozane/tazobactam against 109 ESBL-producing Enterobacterales isolates demonstrated 99.1% (108/109) susceptibility for both combinations. Only meropenem showed 100% (109/109) susceptibility followed by imipenem at 99.1% while ertapenem and amikacin susceptibility was 97.2%. Other antimicrobials demonstrated moderate-to-low susceptibility rates with 78.0% for piperacillin/tazobactam, 64.2% for tigecycline, 60.6% for ciprofloxacin, and 38.5% for co-trimoxazole while as predicted cephalosporin had high-level resistance (99.1% for ceftriaxone and 93.6% for cefepime) [Figure 1]. Furthermore, most of the ESBL-producing Enterobacterales were highly susceptible to ceftazidime/avibactam at low MICs ($MIC_{50/90}$ 0.19/0.38 µg/mL) and ceftolozane/tazobactam ($MIC_{50/90}$ 0.38/1.00 µg/mL) [Table 1], with the majority of isolates demonstrating MICs < 0.5 (n = 81; 74.3%) [Table 2]. The additional microbiological and molecular characterization including susceptibility testing results are shown in Appendix [Table A1].

Our findings are distinctively different from other regional studies where ceftazidime/avibactam

Table 1: Minimum inhibitory concentration (MIC) for ceftazidime/avibactam and ceftolozane/tazobactam against 109 clinical extended-spectrum β-lactamase-producing Enterobacterales isolates collected from intensive care units, Hamad Medical Corporation, Qatar

Organism	Number of isolates	Antibiotic	Range	Susceptible isolates, n (%)	MIC50	MIC90
Klebsiella pneumoniae ssp pneumoniae	55	CZA	0.09–0.75	55 (100)	0.25	0.38
		C/T	0.25–1.50	55 (100)	0.38	1.00
Escherichia coli	38	CZA	0.06–256.00	37 (97.4)	0.12	0.38
		C/T	0.19–256.00	37 (97.4)	0.38	0.75
Enterobacter aerogenes	4	CZA	0.09–0.25	4 (100)	0.19	0.25
		C/T	0.38–0.50	4 (100)	0.38	0.50
Enterobacter cloacae	4	CZA	0.09–0.19	4 (100)	0.09	0.19
		C/T	0.19–0.25	4 (100)	0.25	0.25
Serratia marcescens	2	CZA	0.02–0.12	2 (100)	0.02	0.12
		C/T	0.19	2 (100)	0.19	0.19
Citrobacter braakii	1	CZA	0.25	1 (100)	0.25	0.25
		C/T	0.75	1 (100)	0.75	0.75
Citrobacter freundii	1	CZA	0.06	1 (100)	0.06	0.06
		C/T	0.38	1 (100)	0.38	0.38
Citrobacter amalonaticus	1	CZA	0.12	1 (100)	0.12	0.12
		C/T	0.19	1 (100)	0.19	0.19
Klebsiella oxytoca	1	CZA	0.12	1 (100)	0.12	0.12
		C/T	0.38	1 (100)	0.38	0.38
Klebsiella pneumoniae ssp ozaenae	1	CZA	0.09	1 (100)	0.09	0.09
		C/T	0.25	1 (100)	0.25	0.25
Proteus penneri	1	CZA	0.04	1 (100)	0.04	0.04
		C/T	1.00	1 (100)	1.00	1.00
Total	109	CZA	0.02–256.00	108 (99.1)	0.19	0.38
		C/T	0.19–256.00	108 (99.1)	0.38	1.00

CZA: ceftazidime-avibactam; C/T: ceftolozane-tazobactam.

Table 2: Comparison of minimum inhibitory concentration for ceftazidime/avibactam vs ceftolozane/tazobactam against 109 clinical extended-spectrum β-lactamase-producing Enterobacterales isolates from samples from intensive care unit patients at Hamad Medical Corporation, Qatar.

Antibiotic		Ceftolozane/tazobactam, n (%)					
	MIC	< 0.25	< 0.5	< 0.75	< 4	> 256	Total
Ceftazidime/avibactam	< 0.1	13 (11.9)	6 (5.5)	1 (0.9)	1 (0.9)	0.0	21 (19.3)
	< 0.25	7 (6.4)	13 (11.9)	0.0	0.0	0.0	20 (18.3)
	< 0.5	5 (4.6)	37 (33.9)	14 (12.8)	7 (6.4)	0.0	63 (57.8)
	< 0.75	0.0	0.0	0.0	3 (2.8)	0.0	3 (2.8)
	< 1	0.0	1 (0.9)	0.0	0.0	0.0	1 (0.9)
	> 256	0.0	0.0	0.0	0.0	1 (0.9)	1 (0.9)
	Total	25 (22.9)	57 (52.3)	15 (13.8)	11 (10.1)	1 (0.9)	109 (100)

demonstrated superior activity when compared to ceftolozane/tazobactam against ESBL-producer [Table 3], which suggests a potential correlation of embedded ESBL resistance genes not demonstrated in our study because of paucity of resistant isolates [Table 4].[22]

Among the 109 identified ESBL-producing Enterobacteralesw only one (0.9%) *E. coli* isolate was completely resistant to both ceftolozane/tazobactam and ceftolozane/tazobactam, with MIC > 256 [Table 1]. The resistant isolate was collected from peritoneal fluid of a fatal case of complicated

Table 3: Summary of studies comparing *in vitro* activity of ceftazidime/avibactam and ceftolozane/tazobactam against extended-spectrum β-lactamase-producing Enterobacterales from different geographical regions worldwide.

Study	Geographic location	Susceptibility testing method	Inclusion criteria	Collection years	Number included	Susceptible to MEM, n (%)	Susceptible to CZAn, (%)	Susceptible to C/T, n (%)
Alatoom et al,[17] 2017	Abu Dhabi, UAE	Etest	Resistant to ≥ 1 agent from ≥ 3 antimicrobial classes	2015–2016	31	NA	29 (93.5)	30 (96.8)
Sader et al,[18] 2020	70 medical centers, USA	Broth microdilution	ESBL-producing Enterobacterales from patients hospitalized with pneumonia	2017–2018	285	283 (99.3)	285 (100)	219 (76.8)
Viala et al,[19] 2019	Montpellier, France	Etest	3rd G cephalosporin resistant Enterobacteriaceae	2017	62	NA	60 (97)	34 (65)
Araj et al,[20] 2020	Beirut, Lebanon	MIC gradient Strip Test	MDR and ESBLs *E. coli and K. pneumoniae*	2017–2018	199	NA	NA	159 (79.9)
Hirsch et al,[21] 2020	Boston, MA; and, Philadelphia, PA	Broth microdilution	carbapenem-susceptible (meropenem MIC ≤ 1 mg/L)	2013-2016	119	119 (100)	119 (100)	109 (91.6)

CZA: ceftazidime/avibactam; C/T: ceftolozane/tazobactam; MDR: multi-drug resistant; MEM: meropenem; NS: non-susceptible.
**All studies reported the isolates as susceptible if the MIC was ≤ 8 mg/L for ceftazidime/avibactam and ≤ 4 mg/L for ceftolozane/tazobactam.*

Table 4: Genotypic profiles of different β-lactamase enzymes detected among extended-spectrum β-lactamase-producing *E. coli* isolated from samples from intensive care unit patients at Hamad Medical Corporation, Qatar.

Resistance gene	Gene family	Gene identity, %
CTX-M-15	Class A β-lactamase	100
VEB-5	Class A β-lactamase	100
VIM-2	Class B β-lactamase	100
E. coli ampC	Class C β-lactamase	97.9
E. coli ampC1	Class C β-lactamase	99.3
E. coli ampH	Class C β-lactamase	99.2
CMY-42	Class C β-lactamase	100
OXA-10	Class D β-lactamase	100
OXA-4	Class D β-lactamase	100
OXA-486	Class D β-lactamase	100

intra-abdominal infection, and was subsequently identified as sequence type ST38. Genomic data analysis revealed that the resistant isolate possessed different ARGs including 11 different β-lactamase genes from all classes; Class A ESBL (CTX-M-1 and VEB-5), Class B metallo-β-lactamase (MBL) including $bla_{\text{VIM-2}}$, class C β-lactamase including $bla_{PCMY-42}$. Class D β-lactamase such as $bla_{\text{OXA-4}}$, $bla_{\text{OXA-10,}}$ and $bla_{\text{OXA-486}}$ [Table 4].

DISCUSSION

AMR is a major global healthcare challenge with ominous outcomes. Its ultimate manifestation occurs at critical care units where potent risk factors converge—such as a hazardous environment, vulnerable host, and highly resistant pathogens.[23] Thus, one of the foremost challenges in critical care is prevention and management of infections caused by MDR gram-negative organisms, particularly ESBL-producing Enterobacterales resistant to most antimicrobial classes including most β-lactam penicillins, BLBLIs, and cephalosporins.[23,24] To combat the growing problem of ESBL-producing Enterobacterales, recent decades have witnessed exponentially rising reliance on carbapenems, to the point of their becoming the *sine qua non* for its management, especially in the context of invasive or high-burden disease.[23,25] In complicated ESBL infections, randomized control trials have demonstrated the superiority of carbapenems over comparators including BLBIs.[25] However, the near-

universal use of carbapenems has not prevented the problem from increasing; hence, the relentless search for ever-more powerful antibiotic regimens.[8] Among the most promising new combinations are ceftazidime/avibactam and ceftolozane/tazobactam. Several global studies have evaluated the spectrum of their efficacy *in vitro* and *in vivo* against highly resistant gram-negative strains including ESBL producers and their regional variations.[17–21,26]

A 2010 study in Qatar that investigated 450 episodes of invasive bacteremia from a single institution found 61% prevalence of GNB, the most prevalent of which were *E. coli (*27.8%) *and K. pneumoniae* (17.9%), most of them ESBL producers.[27] The scale of the growing problem at the same institution became clearer in a 2020 study which analyzed the results of culture-positive complicated urinary tract infections among adults admitted to surgical ICUs over a 10-year period (2008–2018). The study found that 36% of the isolated pathogens were ESBLs.[28] Healthcare leaders in the Gulf Cooperation Council countries have recognized the problem of resistant pathogens as a health priority and initiated regional collaboration against this common threat.[29]

In our study, 99.1% of ESBL isolates were highly susceptible and most isolates (74.3%) exhibited MIC < 0.5 for both ceftazidime/avibactam and ceftolozane/tazobactam [Table 2]. Notably, the observed high-level susceptibility for ceftazidime/avibactam and ceftolozane/tazobactam against ESBL-producing Enterobacterales isolates collected from prospective critical care clinical cases, predates the introduction of these agents into clinical practice in Qatar. Our microbiological evaluation suggests these novel agents might be rational empirical treatment options sparing carbapenems.

In the Arabian Gulf region, the high volume of international travel coupled with population diversity and high antibiotic consumption are contributing factors towards the rising and diversifying trends of ESBLs in GNB. The problem has reached an endemic state requiring alternative management options.[6,30]

In line with regional and global ESBL genomic studies, the observation that $bla_{\text{CTX-M}}$ in conjunction with bla_{SHV} and bla_{TEM} are the main ARGs for ESBL-producing Enterobacterales, points towards the role of cephalosporins as the potential driving precipitant.[5,21,31] In Qatar, the molecular epidemiology of Enterobacterales from the pediatric population follows the same trends in the region when a large study of 327 sequenced ESBL producers from clinical samples at the largest children's hospital in the region demonstrated dominance of *E. coli and K. pneumoniae* as main pathogens with predominance of $bal_{\text{CTX-M-1}}$ and coproduction of $bla_{\text{OXA-1}}$ and $bla_{\text{TEM-1B}}$ as ARGs.[32] In contrast, in the adults population, there are no detailed recent studies to evaluate the wider molecular epidemiology of ESBL in the country but the study of 149 non-repetitive carbapenem-resistant Enterobacterales confirmed regional preponderance of bla_{NDM} and bla_{OXA48}.[33]

Not surprisingly, following undergoing WGS, the only concomitant isolate resistant to both ceftazidime/avibactam and ceftolozane/tazobactam harbored multitude of different ARGs.

The ESBL-producing *E. coli* which belonged to ST38 possessed β-lactamase genes from all classes as shown in Table 3. Intriguingly, the detailed study demonstrated the presence of $bla_{\text{VIM-2}}$ MBL which is known to play a fundamental role in ceftazidime/avibactam and ceftolozane/tazobactam resistance.[34] In addition to the endemic class A $bla_{\text{CTX-M}}$ the resistant isolate also harbored $bla_{\text{VEB-5}}$, which was initially detected in *E. coli* in the USA (GenBank accession number EF420108). The ARG, bla_{VEB} confers high-level resistance to cephalosporins as well as monobactams and has been shown to inactivate ceftolozane/tazobactam.[35] However, $bla_{\text{VEB-5}}$ is known to be inhibited by avibactam which restored the MIC of ceftazidime from 256 μg/mL to 2 μg/mL for ceftazidime/avibactam combination.[36] In addition to that, the resistant isolate has multiple underlying ARGs including $bla_{PCMY\text{-}42}$ (AmpC), which drives ceftolozane/tazobactam resistance[37] as well as class D β-lactamases $bla_{\text{OXA-10,}}$ which has been recently reported to enhance ceftolozane/tazobactam and ceftazidime/avibactam resistance.[38]

Despite its wide mechanism of action against MDROs including class A, C, and D β-lactamases, both ceftazidime/avibactam and ceftolozane/tazobactam remain vulnerable when encountering embedded class B β-lactamases such as the potent carbapenemase $bla_{\text{VIM-2}}$ MBL, as described in the isolate of the study.[21,39] Although some molecular tests have been developed to screen for ceftazidime/avibactam and ceftolozane/tazobactam resistance, the current recommendations to interpret activity

through the golden routes of ASTs hold true.[40]

As a consequence, from our study, the prime recommendation is to conduct an urgent clinical evaluation of the novel antibiotics as alternative therapeutic options for MDROs including ESBLs, particularly in critical care settings. This should be supported by data from surveillance and monitoring mechanisms to evaluate the prevalence and characteristics of AMR in the region.

CONCLUSION

ESBL-producing Enterobacterales represent a significant and growing threat to healthcare in Qatar and the Arabian Gulf region in general, particularly in critical care settings. MDROs such as *K. pneumoniae* and *E. coli* harboring multiple ARGs continue to predominate. Promising high *in vitro* antimicrobial susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam against ESBLs-producing Enterobacterales have added to the arsenal of alternative management options to overcome the growing resistance problem.

Disclosure

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Table A1: Microbiological characteristics, molecular characterization, and susceptibility testing results for 109 ESBL-producing Enterobacteriaceae isolates.

Isolate number	Collection mmm-yy	Organism	Location	Specimen type	Disk confirmation test	Molecular results			Antimicrobial susceptibility test (MIC)	
						SHV	TEM	CTXM1	CZA	C/T
1	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Sputum	Positive	Negative	Negative	Positive	0.25	0.75
2	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Urine	Positive	Positive	Positive	Positive	0.25	0.50
3	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Sputum	Positive	Positive	Positive	Positive	0.75	1.00
4	Nov-12	*Escherichia coli*	SICU	Wound Swab	Positive	Negative	Negative	Positive	0.12	0.38
5	Nov-12	*Serratia marcescens*	MICU	Blood	Positive	Negative	Negative	Negative	0.12	0.19
6	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Endotracheal Tube Secretion	Positive	Positive	Negative	Negative	0.19	0.38
7	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	NICU	Peritoneal fluid	Negative +AmpC	Negative	Positive	Positive	0.19	0.25
8	Nov-12	*Escherichia coli*	MICU	Urine	Negative +AmpC	Negative	Positive	Negative	0.50	2.00
9	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Negative	Negative	0.75	1.50
10	Nov-12	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Endotracheal Tube Secretion	Positive	Positive	Positive	Positive	0.19	0.38
11	Dec-12	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Endotracheal Tube Secretion	Positive	Positive	Positive	Positive	0.19	0.38
12	Dec-12	*Klebsiella oxytoca*	NICU	Tracheostomy Site Swab	Positive	Negative	Negative	Negative	0.12	0.38
13	Dec-12	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Urine	Positive	Positive	Positive	Positive	0.25	0.50
14	Dec-12	*Escherichia coli*	NICU	Conjunctival Swab	Positive	Negative	Negative	Positive	0.09	0.38
15	Dec-12	*Enterobacter aerogenes*	MICU	Blood	Positive	Positive	Negative	Negative	0.19	0.50
16	Dec-12	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Endotracheal Tube Secretion	Positive	Positive	Positive	Positive	0.19	0.38
17	Jan-13	*Escherichia coli*	MICU	Urine	Negative	Negative	Negative	Negative	0.38	0.50
18	Jan-13	*Escherichia coli*	SICU	Blood	Positive	Negative	Positive	Negative	0.25	0.38
19	Jan-13	*Escherichia coli*	TICU	Blood	Positive	Negative	Positive	Positive	0.125	0.25

Table A1: Microbiological characteristics, molecular characterization, and susceptibility testing results for 109 ESBL-producing Enterobacteriaceae isolates.

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Isolate number	Collection mmm-yy	Organism	Location	Specimen type	Disk confirmation test	Molecular results			Antimicrobial susceptibility test (MIC)	
						SHV	TEM	CTXM1	CZA	C/T
20	Jan-13	*Enterobacter cloacae*	TICU	Blood	Negative	Negative	Negative	Negative	0.12	0.25
21	Jan-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Sputum	Positive	Positive	Positive	Positive	0.25	0.38
22	Jan-13	*Escherichia coli*	MICU	Blood	Negative +AmpC	Negative	Positive	Negative	0.38	1.50
23	Jan-13	*Proteus penneri*	MICU	Blood	Positive	Negative	Negative	Negative	0.047	1.00
24	Jan-13	*Escherichia coli*	PICU	Urine	Positive	Negative	Negative	Positive	0.12	0.38
25	Jan-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Endotracheal Tube Secretion	Positive	Positive	Negative	Negative	0.25	0.38
26	Jan-13	*Klebsiella pneumoniae ssp pneumoniae*	TICU	Urine	Positive	Positive	Positive	Positive	0.25	0.75
27	Jan-13	*Citrobacter braakii*	TICU	Blood	Negative	Negative	Negative	Negative	0.25	0.75
28	Jan-13	*Escherichia coli*	TICU	Sputum	Positive	Negative	Negative	Positive	0.94	0.38
29	Jan-13	*Escherichia coli*	PICU	Urine	Positive	Negative	Positive	Positive	0.12	0.38
30	Feb-13	*Serratia marcescens*	MICU	Sputum	Negative	Negative	Negative	Negative	0.02	0.19
31	Feb-13	*Escherichia coli*	MICU	Urine	Positive	Negative	Positive	Positive	0.06	0.25
32	Feb-13	*Escherichia coli*	MICU	Blood	Positive	Negative	Negative	Positive	0.12	0.50
33	Feb-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Tracheal Aspirate	Positive	Positive	Positive	Positive	0.19	0.38
34	Feb-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Urine	Positive	Positive	Negative	Positive	0.09	0.38
35	Feb-13	*Escherichia coli*	MICU	Blood	Positive	Negative	Negative	Positive	0.19	0.38
36	Mar-13	*Escherichia coli*	TICU	Ascitic Fluid	Positive	Negative	Negative	Positive	0.12	0.38
37	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	TICU	Sputum	Positive	Positive	Negative	Negative	0.25	0.38
38	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Urine	Positive	Negative	Negative	Positive	0.38	0.38
39	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	NICU	Blood	Positive	Positive	Negative	Positive	0.25	0.38
40	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Negative	Positive	0.25	0.38
41	Mar-13	*Escherichia coli*	SICU	Peritoneal fluid	Positive + AmpC	Negative	Negative	Positive	256.00	256.00

Table A1: Microbiological characteristics, molecular characterization, and susceptibility testing results for 109 ESBL-producing Enterobacteriaceae isolates.

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Isolate number	Collection mmm-yy	Organism	Location	Specimen type	Disk confirmation test	Molecular results			Antimicrobial susceptibility test (MIC)	
						SHV	TEM	CTXM1	CZA	C/T
42	Mar-13	*Escherichia coli*	SICU	Peritoneal fluid	Negative + AmpC	Negative	Negative	Positive	0.19	0.75
43	Mar-13	*Escherichia coli*	SICU	Urine	Positive	Positive	Negative	Positive	0.12	0.38
44	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Urine	Positive	Positive	Positive	Positive	0.19	0.5
45	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Positive	Positive	0.38	1.00
46	Mar-13	*Klebsiella pneumoniae ssp ozaenae*	MICU	Sputum	Positive	Negative	Negative	Negative	0.09	0.25
47	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Tracheal Aspirate	Positive	Negative	Positive	Positive	0.12	0.25
48	Mar-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Urine	Positive	Positive	Positive	Positive	0.38	1.50
49	Mar-13	*Escherichia coli*	TICU	Urine	Positive	Negative	Positive	Positive	0.09	0.38
50	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	TICU	J VAC Fluid	Positive	Negative	Positive	Positive	0.19	0.5
51	Apr-13	*Escherichia coli*	TICU	J VAC Fluid	Positive	Negative	Negative	Positive	0.09	0.75
52	Apr-13	*Escherichia coli*	PICU	Urine	Positive	Negative	Negative	Positive	0.12	0.38
53	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Endotracheal Tube Secretion	Positive	Positive	Positive	Positive	0.19	0.75
54	Apr-13	*Enterobacter aerogenes*	MICU	Tracheal Aspirate	Positive	Positive	Positive	Positive	0.25	0.38
55	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Negative	Negative	0.75	1.00
56	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Endotracheal Tube Secretion	Positive	Positive	Negative	Negative	0.25	0.38
57	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Negative	Positive	0.19	0.38
58	Apr-13	*Escherichia coli*	SICU	Wound Swab	Positive	Negative	Negative	Positive	0.12	0.50
59	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Sputum	Positive	Positive	Negative	Negative	0.25	0.38
60	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Negative	Negative	0.19	0.25
61	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	NICU	Blood	Positive	Positive	Positive	Positive	0.19	0.38

Table A1: Microbiological characteristics, molecular characterization, and susceptibility testing results for 109 ESBL-producing Enterobacteriaceae isolates.

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Isolate number	Collection mmm-yy	Organism	Location	Specimen type	Disk confirmation test	Molecular results			Antimicrobial susceptibility test (MIC)	
						SHV	TEM	CTXM1	CZA	C/T
62	Apr-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Endotracheal Tube Secretion	Positive	Positive	Negative	Negative	0.25	0.25
63	May-13	*Citrobacter freundii*	TICU	Blood	Negative	Negative	Negative	Negative	0.06	0.38
64	May-13	*Escherichia coli*	MICU	Urine	Positive	Negative	Negative	Positive	0.06	0.25
65	May-13	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Tracheostomy Site Swab	Positive	Positive	Positive	Positive	0.25	0.75
66	May-13	*Klebsiella pneumoniae ssp pneumoniae*	NICU	Endotracheal Tube Secretion	Positive	Positive	Positive	Positive	0.25	0.38
67	May-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Sputum	Positive	Positive	Negative	Negative	0.19	0.38
68	May-13	*Klebsiella pneumoniae ssp pneumoniae*	TICU	Blood	Positive	Positive	Negative	Positive	0.19	0.75
69	May-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Negative	Negative	0.38	0.38
70	May-13	*Enterobacter cloacae*	SICU	Sputum	Positive	Positive	Negative	Negative	0.19	0.19
71	Jun-13	*Klebsiella pneumoniae ssp pneumoniae*	TICU	Wound Swab	Positive	Positive	Negative	Positive	0.38	0.38
72	Jun-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Negative	Negative	0.19	0.38
73	Jun-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Positive	Positive	0.19	1.00
74	Jun-13	*Escherichia coli*	PICU	Endotracheal Tube Secretion	Positive	Negative	Negative	Positive	0.12	0.38
75	Jun-13	*Escherichia coli*	PICU	Urine	Positive	Negative	Negative	Positive	0.12	0.38
76	Jun-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Positive	Positive	0.38	1.50
77	Jul-13	*Klebsiella pneumoniae ssp pneumoniae*	NICU	Blood	Positive	Positive	Negative	Negative	0.38	0.38
78	Jul-13	*Escherichia coli*	NICU	Blood	Positive	Negative	Negative	Negative	0.12	0.38
79	Jul-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Negative	Positive	0.25	0.75
80	Jul-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Endotracheal Tube Secretion	Positive	Positive	Positive	Positive	0.19	0.38
81	Jul-13	*Escherichia coli*	PICU	Urine	Positive	Negative	Negative	Positive	0.12	0.38

Table A1: Microbiological characteristics, molecular characterization, and susceptibility testing results for 109 ESBL-producing Enterobacteriaceae isolates.

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Isolate number	Collection mmm-yy	Organism	Location	Specimen type	Disk confirmation test	Molecular results			Antimicrobial susceptibility test (MIC)	
						SHV	TEM	CTXM1	CZA	C/T
82	Jul-13	*Escherichia coli*	SICU	Urine	Positive	Negative	Positive	Negative	0.25	0.50
83	Jul-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	BAL	Positive	Positive	Negative	Positive	0.25	0.75
84	Jul-13	*Escherichia coli*	MICU	Urine	Positive	Negative	Negative	Negative	0.09	0.25
85	Jul-13	*Citrobacter amalonaticus*	PICU	Urine	Positive	Positive	Negative	Negative	0.12	0.19
86	Jul-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Positive	Positive	0.25	0.50
87	Jul-13	*Enterobacter cloacae*	NICU	Eye Swab	Negative + AmpC	Negative	Negative	Negative	0.09	0.25
88	Jul-13	*Enterobacter cloacae*	TICU	Blood	Negative + AmpC	Negative	Negative	Negative	0.09	0.25
89	Jul-13	*Enterobacter aerogenes*	TICU	Blood	Positive	Positive	Negative	Negative	0.09	0.38
90	Aug-13	*Escherichia coli*	MICU	Ascitic fluid	Positive	Negative	Positive	Positive	0.12	0.25
91	Aug-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Urine	Positive	Positive	Negative	Positive	0.38	0.75
92	Aug-13	*Enterobacter aerogenes*	TICU	Sputum	Positive	Positive	Negative	Negative	0.25	0.38
93	Aug-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Positive	Positive	0.19	0.75
94	Sep-13	*Escherichia coli*	NICU	Blood	Positive	Negative	Negative	Positive	0.19	0.75
95	Sep-13	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Urine	Positive	Positive	Positive	Positive	0.25	0.5
96	Sep-13	*Escherichia coli*	MICU	Urine	Positive	Negative	Positive	Positive	0.09	0.38
97	Sep-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Urine	Positive	Positive	Positive	Positive	0.25	0.75
98	Sep-13	*Escherichia coli*	SICU	Sputum	Positive	Negative	Negative	Positive	0.12	0.50
99	Sep-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Positive	Positive	0.25	0.75
100	Sep-13	*Klebsiella pneumoniae ssp pneumoniae*	PICU	Blood	Positive	Negative	Positive	Positive	0.12	0.25
101	Sep-13	*Escherichia coli*	PICU	Urine	Positive	Negative	Negative	Positive	0.06	0.25
102	Sep-13	*Klebsiella pneumoniae ssp pneumoniae*	MICU	Sputum	Positive	Positive	Positive	Positive	0.19	0.38
103	Sep-13	*Klebsiella pneumoniae ssp pneumoniae*	NICU	Central line Tip	Positive	Positive	Negative	Positive	0.25	0.50
104	Sep-13	*Escherichia coli*	TICU	Sputum	Positive	Negative	Positive	Positive	0.19	0.25
105	Oct-13	*Escherichia coli*	SICU	Blood	Positive	Negative	Negative	Positive	0.06	0.19

Table A1: Microbiological characteristics, molecular characterization, and susceptibility testing results for 109 ESBL-producing Enterobacteriaceae isolates.

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Isolate number	Collection mmm-yy	Organism	Location	Specimen type	Disk confirmation test	Molecular results			Antimicrobial susceptibility test (MIC)	
						SHV	TEM	CTXM1	CZA	C/T
106	Oct-13	*Escherichia coli*	SICU	Sputum	Positive	Positive	Positive	Negative	0.06	0.19
107	Oct-13	*Escherichia coli*	SICU	Urine	Positive	Negative	Negative	Positive	0.09	0.25
108	Oct-13	*Klebsiella pneumoniae ssp pneumoniae*	SICU	Blood	Positive	Positive	Positive	Positive	0.38	1.00
109	Oct-13	*Escherichia coli*	TICU	Sputum	Positive	Positive	Positive	Negative	0.06	0.25

ESBL: extended-spectrum β-lactamase; MIC: minimum inhibitory concentration; White: susceptible; grey: non-susceptible, susceptibility was reported according to Clinical Laboratory Standards Institute (CLSI) breakpoints (Clinical Laboratory Standards Institute, 2020). m: month; y: year; MICU: Medical Intensive Care Unit; NICU: Neonatal Intensive Care Unit; PICU: Pediatric Intensive Care Unit; SICU: Surgical Intensive Care Unit; TICU: Trauma Intensive Care Unit; CZA: ceftazidime-avibactam; C/T: ceftolozane-tazobactam.