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ORIGINAL ARTICLE

ANTIBACTERIAL ACTIVITY OF CEFTAZIDIME-AVIBACTAM AGAINST CLINICAL ISOLATES OF XDR *SALMONELLA ENTERICA SEROVAR TYPHI*

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ABSTRACT

Typhoid fever continues to be a major healthcare problem in low and middle-income countries. The emergence of extensively drug-resistant (XDR) typhoid has further narrowed down the way to already limited therapeutic options. WHO has listed *S.typhi* amongst the pathogens for which new treatment options should be explored. To determine In-vitro activity of Ceftazidime-avibactam against clinical isolates of XDR *S.typhi.* This is a cross-sectional study. The Department of Microbiology, University of health sciences, Lahore from January to June 2021. Antimicrobial susceptibility was performed initially by Kirby Bauer disc diffusion method for 150 of XDR *Salmonella enterica Serovar typhi* and MICs of all the recommended antibiotics was determined by VITEK 2 (bioMérieux) fully automated system using Clinical Laboratory Standard Institute (CLSI) 2021 guidelines. MICs by the E-test method were determined for Ceftazidime-avibactam and Azithromycin only. All 150 (100%) isolates were sensitive to Ceftazidime-avibactam by disc diffusion and E-test methods. Out of 150 isolates 8(5.3%) were having high MICs against Azithromycin. Ceftazidime-avibactam can be used wisely to treat ESBL producing XDR typhoid fever cases especially in countries like Pakistan where Typhoid fever is endemic and majority of isolates are extensively drug resistant.

INTRODUCTION

Typhoid fever remains a significant public health threat in low and middle-income countries. According to the most recent estimates, between 11 and 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually worldwide (WHO, 2018). Antimicrobial resistance in Salmonella can be associated with horizontal transference of antibiotic resistant genes characteristically found on mobile genetic elements among Salmonella strains and other Enterobacteria or by clonal spread of antimicrobial drug resistant serovars that are particularly nominated in worldwide dissemination. Since November 2016, a large proportion of ceftriaxone-resistant cases have been identified in the province of Sindh. Similar case were also identified in the United Kingdom from a traveler returning from Pakistan. These *S.typhi* strains are resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, and third-generation cephalosporins. This new strain has been designated as XDR (Extensively drug resistant) leaving behind azithromycin and carbapenems, the only treatment options (Klemm *et al*., 2018).This Pakistan outbreak caused by an XDR H58 strain of *S.typhi* should be regarded as a clarion call that notifies public health authorities globally that we are rapidly approaching a scenario where the acquisition of one more resistance might result in *S. typhi* pathogen that is, in practical terms, virtually untreatable in most developing countries.

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Now is the time for global action to prevent a gathering storm from becoming a perfect storm and an enormous public health crisis. Ceftazidime is a third generation cephalosporin antibiotic useful for the treatment of a number of bacterial infections. Specifically it is used for joint infections, meningitis, pneumonia, sepsis, urinary tract infections, malignant otitis externa, *Pseudomonas aeruginosa* infection, and vibrio infection (Lagacé *et al*., 2014). Like all other third generation cephalosporins, it is active against *Salmonella enterica serovars* including *S.typhi.* But recent emergence of XDR *S.typhi* has knocked this drug out of the arsenal because of ESBL production. Avibactam is a non-β-lactam β-lactamase inhibitor developed by Actavis.

A new drug application for avibactam in combination with ceftazidime (branded as Avycaz) was approved by the FDA on February 25, 2015, for treating complicated urinary tract (cUTI) and complicated intra-abdominal infections (cIAI) caused by antibiotic resistant-pathogens, including those caused by multi-drug resistant Gram-negative bacterial pathogens (Sader *et al*., 2015). Recently approval has been given for bacterial pneumonia and ventilator associated pneumonia caused by multi drug resistant pathogens.

The combination contains the well-established third-generation cephalosporin ceftazidime paired with the novel non-β-lactam β-lactamase inhibitor avibactam (Karlowsky *et al*., 2016). Avibactam lacks clinically significant antibacterial activity; however, it inhibits a broad spectrum of β-lactamases, with high affinity for class A, C, and some D enzymes, restoring the *in vitro* activity of ceftazidime.

Ceftazidime-avibactam demonstrated potent activity against molecularly confirmed ESBL-producing (*n* = 5,354; MIC_{90}, 0.5 μg/ml; 99.9% susceptible), plasmid-mediated AmpC-producing (*n* = 246; MIC_{90}, 0.5 μg/ml; 100% susceptible), and ESBL and AmpC-producing (*n* = 152; MIC_{90}, 1 μg/ml; 100% susceptible) isolates of *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* (Karlowsky *et al*., 2016).

Ceftazidime/avibactam has been shown to be highly active against *Enterobacteriaceae* in in-vitro studies, inhibiting a broad spectrum of β-lactamases. In a study, the activities of ceftazidime-avibactam and comparator antimicrobial agents were tested against 20,709 clinical *Enterobacteriaceae* isolates collected in U.S. hospitals during the period from 2011 to 2013. Overall, 99.9% (20,698 of 20,709) of *Enterobacteriaceae* strains were inhibited at a ceftazidime-avibactam MIC of 8 μg/ml or less, which is the ceftazidime-avibactam-susceptible breakpoint. This study included 284 CTX_{M-15} producing isolates and 107 CTX_{M-14} producers. The potent Gram-negative spectrum of activity of ceftazidime-avibactam, including activity against resistant organisms, demonstrates that it warrants further study in difficult to treat serious infections where resistant gram negative bacteria may occur. (Castanheira *et al*., 2015). Ceftazidime-avibactam activity has been reported in *Salmonella enterica* having $MIC_{50/90}$ equal to 0.25/0.5 with range of MIC $\leq$ 0.03-0.5 and 100% isolates were sensitive to this combination (Lagacé *et al*., 2014.

Study design

This was a Cross-sectional study.

Setting

This study was conducted at The Department of Microbiology, University of Health Sciences, Lahore.

Duration

6 months (January 2021 to June 2021).

Sample collection

A total of 150 positive blood cultures meeting the inclusion criteria were collected from blood different tertiary care hospitals of Lahore.

Bacterial identification

The isolates were cultured and purified on Tryptic Soya agar. The blood culture bottle detected as positive was sub-cultured onto Blood agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) incubated at 35-37 °C. The isolate identification was initially performed by Gram-staining. Biochemical identification was done by using Analytical Profile Index-20 Enterobacteriacae system (BioMerieux, France). France) and VITEK2 (bioMérieux) consistent with the manufacturer instructions. *S.typhi* was confirmed by agglutination with genus- and serotype-specific antisera (Salmonella poly antiserum A-I (Difco), Salmonella O antiserum (Difco) and Salmonella Vi antiserum (Difco).

Antimicrobial Susceptibility

Antimicrobial susceptibility testing was performed by Standard Kirby-Bauer Disk Diffusion method using cation adjusted Mueller-Hinton agar (MHA) (Oxoid UK), according to Clinical Laboratory Standards Institute (CLSI) guidelines 2021 and zones of inhibition were interpreted according to the breakpoints. The reference strains of *E. coli* ATCC® 25922 and *P. aeruginosa* ATCC® 2785 were used for consistency.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration of all the recommended antibiotics was determined by Vitek 2 (bioMérieux) fully automated system. *S.typhi* that demonstrated high MICs against ceftriaxone and cefotaxime (>4ug/mL) was considered as XDR. For Ceftazidime-avibactam MIC was determined by E-Strip (liofilchem, Italy) method. A value of ≤ 8/4 mg/L was taken as sensitive according to CLSI 2021 criteria for *Enterobacterales.*

This study was conducted using the bacterial strains which were isolated for diagnostic and treatment purpose. Moreover, the study was completely anonymous, any demographic data or identifiable information was not obtained therefore informed consent was not required for such type of study according to the local legislation.

RESULTS

In this study, a total of 150 XDR *S. typhi* isolates were included which were collected over a period of 6 months from different tertiary care hospitals of Lahore.

The antimicrobial susceptibility testing showed that all the *Salmonella* typhi isolates were susceptible to Carbapenems (Imipenem & Meropenem) but 7 isolates had higher Azithromycin MIC and 1 isolate was resistant to Azithromycin (table 1). The MIC values for Imipenem & Meropenem were between ≤ 0.06 to 1 μg/mL. Out of 150 isolates 7(4.6%) were resistant to Piperacillin-tazobactam and rest had MIC between 1 to ≥ 128.Out of 150 isolates 7(4.6%) had MIC of 16 μg/mL and only 1(0.6%) had MIC of 32μg/mL. All the isolates were resistant to Ampicillin, Chloramphenicol, Co-trimoxazole, Ciprofloxacin and Third generation Cephalosporins. For Ceftazidime-avibactam MIC range was between 0.016 to 0.064 mg/ mL. Overall 150(100%) isolates were sensitive to Ceftazidime-avibactam (table 2).

DISCUSSION

Typhoid fever is a significant health problem for countries like Pakistan. Emergence and spread of XDR *S.typhi* across Pakistan has generated a huge problem for the control and prevention of Typhoid fever. These *S.typhi* strains are resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, and third-generation cephalosporins. With very limited treatment options already available emergence of Azithromycin resistance would create further problem. There is dire need to look for new therapeutic options as urged by WHO (World health organization).

In this study we explored In-vitro activity of Ceftazidime-avibactam against clinical isolates of XDR *S. typhi* collected from different tertiary care hospitals of Lahore.

Table 1: MIC distribution of *S. typhi* isolated from blood cultures

No. of isolates having MICs in µg/mL														
Antibacterial Agent	Breakpoint	≤ 0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128	Total
Ampicillin	≥ 32	0	0	0	0	0	0	0	0	0	0	95	55	150
Piperacillin-tazobactam*	≥ 128/4	0	0	0	8	12	10	45	33	2	13	20	7	150
Ceftriaxone	≥ 4	0	0	0	0	0	0	0	30	42	35	15	28	150
Cefixime	≥ 4	0	0	0	0	0	0	0	38	15	48	29	20	150
Cefotaxime	≥ 4	0	0	0	0	0	0	0	20	12	48	33	37	150
Cefepime	≥ 16	0	0	0	0	0	0	0	0	0	31	73	46	150
Imipenem	≥ 4	0	30	28	80	12	0	0	0	0	0	0	0	150
Meropenem	≥ 4	5	13	32	55	45	0	0	0	0	0	0	0	150
Azithromycin	≥ 32	0	0	0	0	0	92	50	0	7	1	0	0	150

*The MIC value of Piperacillin-tazobactam are expressed as MIC values of Piperacillin with equal (4µg/mL) concentration of tazobactam

Table 2: MIC of CTZ-AVI* for XDR *S. typhi*

MIC (mg/mL)	n (% isolates)	MIC (mg/mL)	n (% isolates)
0.016	07 (4.6%)	3	00 (0%)
0.023	40 (26.6%)	4	00 (0%)
0.032	36 (24%)	6	00 (0%)
0.047	37 (24.6%)	8	00 (0%)
0.064	22 (14.6%)	12	00 (0%)
0.094	08 (5.3%)	16	00 (0%)
0.125	00 (0%)	24	00 (0%)
0.19	00 (0%)	32	00 (0%)
0.25	00 (0%)	48	00 (0%)
0.38	00 (0%)	64	00 (0%)
0.50	00 (0%)	96	00 (0%)
0.75	00 (0%)	128	00 (0%)
1.0	00 (0%)	192	00 (0%)
1.5	00 (0%)	256	00 (0%)
2	00 (0%)		

* The MIC value of Ceftazidime-avibactam are expressed as MIC values based on dosage regimen of 2.5g (2g Ceftazidime and 0.5g of Avibactam) 8 hourly

Ceftazidime-avibactam MIc was tested using E-test method against 150 XDR *S.typhi* isolates. MIC range was between 0.016 to 0.064 mg/ mL. Out of 150 isolates 7(4.6%) had MIC of 0.016 mg/ mL, 40(26.6%) had MIC of 0.023 mg/ mL, 36(24%) had MIC of 0.032 mg/ mL, 37(24.6%) had MIC of 0.047 mg/ mL, 22(14.6%) had MIC of 0.064 mg/ mL and 8(5.3%) had MIC of 0.094 mg/ mL.In our study 150(100%) isolates were sensitive to Ceftazidime-avibactam.

Ceftazidime-avibactam has shown promising results against MDR and XDR pathogens producing ESBLs in previous studies. In a recent study, a total of 7051 Enterobacterale isolates and 2032 *Pseudomonas aeruginosa* isolates from hospitalized patients in Australia, Japan, South Korea, Malaysia, the Philippines, Taiwan and Thailand were studied. More than 90% of all Enterobacterales isolates, including the ESBL-positive, carbapenemase-negative and the

carbapenemase-positive, MBL-negative were susceptible to amikacin and ceftazidime–avibactam (Wen-Chien *et al*., 2020). Ceftazidime-avibactam and comparators were tested by reference broth microdilution against 372 Gram-negative bacilli collected from 11 teaching hospitals in China in 2011 and 2012. Avibactam potentiated the activity of ceftazidime against organisms with combinations of ESBLs, AmpCs, and KPC-2 (Xiaojuan Wang *et al*., 2014).

In another study Ceftazidime-avibactam was tested against 57 well-characterized Gram-negative strains producing beta lactamases from all molecular classes. Avibactam lowered ceftazidime MICs up to 2,048-fold against AmpC extended-spectrum beta lactamase (ESBL), and KPC-producing *Enterobacteriaceae* or *Pseudomonas aeruginosa* (Henry *et al*., 2015).

In another study conducted in Spain, Ceftazidime-avibactam was very active against ESBL producing Enterobactarales. *Escherichia coli* had MIC_{90} of 0.25 mg/liter and ESBL producing *Klebsiella pneumonia* had MIC_{90} of 0.5 mg/liter, Ceftazidime-resistant AmpC-producing species had MIC_{90} of 1 mg/liter (Pitart *et al.,* 2015).

Furthermore, in a large US focused surveillance program conducted in 2012, ceftazidime-avibactam had antimicrobial activity against 99.8% of *Enterobacteriaceae* including those with an ESBL phenotype.

Ceftazidime-avibactam can be used as a treatment option in ESBL producing XDR *S.typhi* isolates sparing carbapenems and azithromycin.This will reserve the use of these drugs in non-responding or difficult to treat cases without significantly creating resistance by overuse. Azithromycin resistance has been reported from several parts of the world especially typhoid endemic countries like India, Nepal, and Bangladesh. Several cases have been reported from Pakistan too (Muhammad *et al*., 2021; Iqbal J., 2020)

CONCLUSION

Azithromycin resistance in XDR *S. typhi* is on the rise and it may pose a significant threat to the clinicians especially where XDR *S. typhi* is endemic now. Antimicrobial stewardship especially in the post-COVID-19 era is crucial to prevent the development of resistance to this limited oral choice against XDR typhoid. There is vital need to look for new treatment options. Ceftazidime-avibactam has shown promising results in this in-vitro study and it can be used wisely to treat XDR typhoid cases.

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