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Research Article

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Breaking Barriers: Investigating the Potential of Ceftazidime-Avibactam in the Treatment of Salmonella Typhi Bacteremia through In-vitro Susceptibility Testing

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Abstract

Introduction

The prevalence of typhoid fever has been rising steadily in Pakistan with extensively drug-resistant (XDR) cases posing a major challenge to the national health care system. Physicians are hence, pushed towards prescribing last resort antibiotics which have concerns of cost-effectiveness, frequent in-patient monitoring and growing drug resistance. Hence, we evaluated susceptibility of Ceftazidime-avibactam (CAZ-AVI) against Salmonella typhi isolates from cases of bacteremia.

Methods

This was a cross-sectional study conducted at the Clinical Microbiology Laboratory of The Indus Hospital Karachi, Pakistan from 1st May to 31st October 2023. Two hundred and eighty-nine blood culture isolates identified as S. typhi were included in the study and were tested for CAZ-AVI susceptibility by Disc diffusion method. Interpretation of zone diameters was done using antimicrobial susceptibility breakpoints mentioned in Clinical & Laboratory Standards Institute (CLSI) M100. A zone diameter of ≥ 21 mm was considered as sensitive and ≤ 20 mm was considered resistant.

Results

Of the 289 isolates, 59.9% (n=173) were from male patients and 40.1% (n=116) from females. Patients mostly belonged to ≤ 18 years age group (n=249; 86.2%). Majority of the S. typhi isolates were characterized as XDR (n=171; 59.2%) followed by non-drug-resistant isolates (n=90; 31.1%) whereas, least were characterized as multi drug-resistant (MDR) strains (n=28; 9.7%). All the isolates tested for CAZ-AVI susceptibility were found to be sensitive.

Conclusion

We report 100% susceptibility of CAZ-AVI in our set of S. typhi bacteremia isolates. The findings of this study provide valuable data to physicians for exploring other treatment options for typhoid as well as guiding further multi-center in-vitro studies and clinical trials.

Keywords: Ceftazidime-avibactam, CAZ-AVI, Salmonella typhi, XDR typhoid

1. Introduction

Typhoid fever, mainly caused by Salmonella typhi, has become a global menace with an estimated burden of 9 million cases and 110,000 deaths annually [1]. Of particular concern are the South Asian countries where limited access to clean water and adequate sanitation significantly contributes to its spread. The highest prevalence among South Asian countries has been estimated to be in Pakistan where the cases dramatically increased after a new extensively drug-resistant (XDR) strain was first isolated in 2016 [2]. Since then, thousands of XDR cases are reported each year to the National Institute of Health (NIH) in Islamabad, raising grave concerns for the national health care systems [3]. The physicians often resort to Azithromycin in out-patient settings as a last available oral option in such cases. However, the recent emergence of Azithromycin-resistant XDR strains reported from India, Singapore and Pakistan among other countries have further complicated the issue [4,5,6]. Such situations have been forcing physicians to prescribe carbapenems as a last option. Although carbapenems are seen as an effective antibiotic in XDR cases, concerns of cost-effectiveness and frequent in-patient monitoring

limits its availability for the underprivileged population of Pakistan. Moreover, frequent and injudicious use of carbapenems has been associated with the rising trend of Carbapenem-resistant Enterobacterales (CRE) in the country over the past decade [7,8]. Therefore, there is an urgent need to explore other options for the treatment of typhoid fever, which are readily available in the country and associated with lesser treatment related morbidity. Ceftazidime-avibactam (CAZ-AVI), a combination of a third-generation cephalosporin and a beta-lactamase inhibitor, exhibits bactericidal activity against a wide number of gram-negative bacteria including Enterobacterales and Pseudomonas aeruginosa [9]. Several laboratory-based and clinical observational studies have demonstrated that CAZ-AVI is non-inferior to meropenem, in-vitro as well as in treating certain clinical infections [10,11,12]. Hence, we set out to evaluate the susceptibility of CAZ-AVI in S. typhi isolates of bacteremia.

2. Materials and Methods:

2.1 Study Design and Setting: This was a cross-sectional study conducted at the Clinical Microbiology Laboratory of The Indus Hospital Karachi, Pakistan from 1st May to 31st October 2023. Two hundred and eighty-nine blood culture isolates identified as S. typhi were included in the study and were tested for CAZ-AVI susceptibility by Disc diffusion method. S. typhi isolates from specimens other than blood and which were sent outside the timeframe of sample collection were excluded.

2.2 Sample Size

The sample size was calculated from the findings of a previous in-vitro study, evaluating the efficacy of CAZ-AVI against clinical isolates of Enterobacter ales from 2017 to 2019, using the WHO sample size software [13]. We estimated that a minimum sample size of 279 isolates would be needed to detect an expected CAZ-AVI efficacy of 98.1%, keeping a 95% confidence interval and a 1.6% margin of error.

2.3 CAZ-AVI Susceptibility Testing

S. typhi isolates were sub-cultured on sheep blood agar (SBA) and incubated at 37oC for 24 hours. After which isolated colonies from SBA were inoculated in phosphate-buffered saline and a 0.5 MacFarland suspension was prepared. A sterile swab was used to streak the entire surface of a Mueller-Hinton Agar (MHA) plate and a 30/20 µg concentration CAZ-AVI antibiotic disc was placed on the agar surface. The agar plate was incubated at 37oC for 24 hours. After incubation the MHA plates were examined for measurement of CAZ-AVI zone diameter. Interpretation of zone diameters was done using antimicrobial susceptibility breakpoints mentioned in Clinical & Laboratory Standards Institute (CLSI) M100. A zone diameter of $\geq$ 21 mm was considered as sensitive and $\leq$ 20 mm was considered resistant.

2.4 Statistical Analysis

Information regarding patient's age, gender, drug-resistance pattern of isolates and susceptibility for CAZ-AVI was recorded on a standardized study proforma. Baseline data collected on hard copies of the study proformas was entered in the Microsoft Excel software (Microsoft Excel 2013 {15.0.5553.1000} 32-bit). Statistical significance was computed using MedCalc Statistical Software version 20.027 (MedCalc Software bv, Ostend, Belgium) to determine any association of patient age groups with the frequency of drug-resistant isolates, using the Chi-square test. A p value of $\leq$0.05 was considered as significant.

4. Results

Of the 289 isolates, 59.9% were from male patients and 40.1% from females. Patients mostly belonged to $\leq$ 18 years age group (86.2%). Majority of the S. typhi isolates were characterized as XDR (59.2%) followed by non-drug-resistant isolates (31.1%) whereas, least were characterized as MDR strains (9.7%). All the isolates tested for CAZ-AVI susceptibility were found to be sensitive. Characteristics, drug resistance pattern and CAZ-AVI susceptibility of the isolates is tabulated in Table 1.

Characteristic	n (%)
Age	
≤18 years	249 (86.2%)
>18 – 45 years	38 (13.1%)
>45 years	2 (0.7%)
Gender	
Male	173 (59.9%)
Female	116 (40.1%)
Drug Resistance	
No drug resistance	90 (31.1%)
Multi drug resistance (MDR)	28 (9.7%)
Extensive drug resistance (XDR)	171 (59.2%)
Ceftazidime-avibactam (CAZ-AVI) susceptibility	
Sensitive isolates	289 (100%)

Table 1: Characteristics, Drug Resistance Pattern and Ceftazidime-Avibactam (CAZ-AVI) Susceptibility of Salmonella Typhi Isolates (n=289).

The association of age groups of ≤18 years and >18 – 45 years with XDR strains of S. typhi was found to be statistically significant (p=0.02). Furthermore, statistical significance was also noted for association of both these age groups with non-drug resistant strains of S. typhi (p=0.04 & p=0.02). Association of different age groups with frequency of drug-resistant isolates is shown in Table 2.

Age Group	Drug-resistant isolates	n (%)	P value
≤18 years	Non-drug resistant	72 (28.9%)	p=0.04*
	MDR	23 (9.2%)	p=0.5
	XDR	154 (61.9%)	p=0.02*
>18 – 45 years	Non-drug resistant	18 (47.4%)	p=0.02*
	MDR	4 (10.5%)	p=0.9
	XDR	16 (42.1%)	p=0.02*
>45 years	MDR	1 (50%)	p=0.1
	XDR	1 (0.8%)	p=0.8

Table 2: Association of patient age groups with frequency of drug-resistant isolates. (*p value ≤0.05)

5. Discussion

Our study results show 100% sensitivity of CAZ-AVI in all S. typhi isolates from bacteremic patients. Several antimicrobial surveillance studies conducted internationally and from South Asian region corroborate with our findings. A study conducted on patient isolates from 16 major teaching hospitals across Taiwan in 2017, evaluated the susceptibility of CAZ-AVI among several other antibiotics against Enterobacterales. Of the n=1359 isolates of Enterobacterales tested, more than 99% were sensitive to CAZ-AVI [14]. Similarly, another in-vitro study from USA, tested for CAZ-AVI susceptibility in pediatric patient isolates from 70 medical centers from 2016 to 2019. CAZ-AVI was found to be susceptible in more than 99.9% of the total n=37900 isolates of Enterobacterales tested [15].

Most of the isolates belonged to patients that were either 18 years of age or less (86.2%). Population-based data from Pakistan in this regard is scarce however, several epidemiological studies from different regions of the country have consistently shown higher incidence of typhoid fever in the younger age groups [16]. Also, prevalence data from India and Bangladesh is consistent with our findings that younger population is at higher risk for contracting typhoid [16, 17]. An analysis of risk factors for typhoid in the younger population of Karachi showed higher risk in children particularly living in high density areas of the city with lack of safe drinking water sources [18]. Moreover, school aged children and young adults belonging to underprivileged areas who often leave home are exposed to unhygienic and contaminated food on the streets [17].

More than half of the patient isolates in our study (59.2%) were XDR strains. This finding agrees with the existing and rising trend

of XDR cases throughout Pakistan [2,3]. Since, the isolation of the first case of XDR typhoid in Sindh province in 2016, there have been intermittent outbreaks of XDR cases in the country characterized by patients refractory to standard therapy [3]. Frequent disproportionate use of antibiotics constituting the first- and second-line therapy for typhoid is responsible for resistance to most of the drugs traditionally used for treating typhoid. Hence, there has been a push towards use of Azithromycin in out-patient settings, which has been unfortunately resulting in Azithromycin resistant cases [4,5,6]. Furthermore, prescription of carbapenems is not always feasible as an out-patient option and patients often require close in-patient monitoring. Injudicious use of carbapenems in the country is contributing to the rising pandemic of CRE infections, posing an imminent hazard for emergence of carbapenem-resistant XDR typhoid. In such scenario, other options such as CAZ-AVI may prove helpful for treating cases of XDR typhoid and curtailing the ever-increasing resistance to Azithromycin and Carbapenems.

There are a few limitations to our study. Firstly, this was a single center study and multi-center studies throughout the country are required to assess the efficacy of CAZ-AVI on a wider population. Nonetheless, Indus Hospital is one of the largest tertiary care hospitals of Karachi, situated in the Korangi district which caters to a population of more than 2.5 million people [19]. Secondly, other antibiotic combinations of beta-lactamase inhibitors viz. Ampicillin-sulbactam, Ceftolozane-tazobactam, Imipenem-relebactam and Meropenem-vaborbactam were not explored for in-vitro testing against S. typhi isolates in this study. Our findings regarding CAZ-AVI susceptibility against these isolates are robust and we intend to explore other beta-lactam/ beta-lactamase combinations for in-vitro testing in future. Lastly, minimum inhibitory concentration (MIC) of CAZ-AVI was not performed in the isolates to confirm susceptibility results obtained via Disk diffusion method. However, we employed the quality control measures recommended by CLSI for testing of CAZ-AVI by Disk diffusion method and reported our results only when all quality control criteria were adequately met.

6. Conclusion

We report 100% susceptibility of CAZ-AVI in our set of S. typhi bacteremia isolates. The findings of this study provide valuable data to physicians for exploring other treatment options for typhoid as well as guiding further multi-center in-vitro studies and clinical trials.

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