Section: Microbiology

Original Research Article

A STUDY ON SUSCEPTIBILITY PATTERN OF CEFTAZIDIME-AVIBACTAM COMBINATION ON CARBAPENEM RESISTANT ENTEROBACTERIACEAE ISOLATES AMONG VARIOUS SAMPLES IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Carbapenem Resistant Enterobacteriaceae (CRE) pose a significant threat to public health due to their limited treatment options and potential for causing severe, even life-threatening illnesses, particularly in healthcare settings where they can spread rapidly among vulnerable patients. The aim of this study is to determine the prevalence of Carbapenem Resistant Enterobacteriaceae among various clinical samples received in the Department of Microbiology of our hospital and to determine the susceptibility pattern of CRE to Ceftazidime-Avibactam combination. **Materials and Methods:** Bacterial colonies were identified and appropriate biochemical reactions and Antibiotic susceptibility testing were done to find out the Enterobacteriaceae group of organisms and CRE isolates by various methods according to CLSI 2021 guidelines. Commercially available ceftazidime-avibactam (30µg/20µg) E strip were performed for CRE isolates and Minimum Inhibitory Concentration (MIC) were interpreted as per CLSI guidelines. **Result:** A total of 4,162 clinical samples were collected out of which 697 were belonging to the Enterobacteriaceae family. Out of this, 81 samples were confirmed to be carbapenem resistant giving a prevalence of 11.62%. Pus was found to be highest contributor among the clinical samples to contain carbapenem resistant Enterobacteriaceae. Klebsiella pneumoniae was the predominant organism among the Carbapenem resistant Enterobacteriaceae. 94% of Klebsiella pneumoniae were sensitive to Ceftazidime/ Avibactam combination while 100% sensitivity was seen with Escherichia coli, Klebsiella oxytoca, Proteus mirabilis and Enterobacter species giving overall sensitivity of CRE to ceftazidime/ Avibactam combination of 96%.

INTRODUCTION

Antimicrobial resistance is one among the top ten global public health threats facing humanity.[1] Among them Carbapenem Resistant Enterobacteriaceae (CRE) is becoming an important threat affecting human health as Enterobacteriaceae are one of the most common causes of both community and healthcare associated infections. Carbapenem Resistant Enterobacteriaceae (CRE) can be defined as Enterobacteriaceae that are resistant to one or all of the following carbapenems: ertapenem, meropenem, imipenem or doripenem and resistant to all of the following third-generation cephalosporins: ceftriaxone, cefotaxime, and ceftazidime.[2] The Enterobacteriaceae family includes many bacteria out of which the most commonly isolated from clinical cultures include Escherichia coli, Klebsiella spp., and Enterobacter spp.[3] Carbapenem-resistant bacteria cause pneumonia, urinary tract infections, septicaemia, endocarditis, meningitis, and severe intra-abdominal infections which are only a few of the illnesses caused by Enterobacteriaceae.[4] Carbapenem-resistant Enterobacteriaceae (CRE) infections result in longer hospital admissions, higher healthcare costs, and increased mortality than carbapenem-susceptible bacterial infections.[5] According to the World Health Organization's

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antimicrobial resistance report, Enterobacteriaceae resistant to carbapenem are classified as a critical group and developing drug resistant infections.[6,7] According to Centers for Disease Control and Prevention (CDC) description of the antimicrobial-resistant pathogens, CRE such as Klebsiella species, Escherichia coli (E. coli) and Enterobacter species are the most crucial emerging resistance threats in the global.[8] Rise of carbapenem resistance and rapid dissemination of the Enterobacteriaceae family are referred to as "superbugs bacteria".[9,10]

Carbapenem resistance in Enterobacteriaceae is mostly expressed by the synthesis of carbapenemase enzymes, which are encoded by numerous genotypes and can be transferred among Enterobacteriaceae via transferable genetic elements. Commonly pronounced enzymes include Class A Klebsiella pneumoniae carbapenemase, Class B metallo-β-lactamases, and Class D OXA βlactamases.20 Resistance can also be developed through efflux pumps, permeability changes caused by the loss of outer membrane porin, or target mutations.[11]

Treatment options available for CRE are limited which include drugs like polymyxins, tigecycline and aminoglycosides.[12] There is an increasing resistance, limited efficacy and toxicity with these available drugs.

Cephalosporins are the drug of choice for many bacterial infections due to their broad- spectrum activity. Five generations of cephalosporins are known so far and ceftazidime is a third- generation cephalosporin having a broad- spectrum activity against Gram positive cocci and Gram -negative bacilli. Avibactam is a novel non-βlactam βlactamase inhibitor (BLBLI). CZA combination is known to be effective against Ambler Class A, Class C and some Class D βlactamase producing organisms in contrary to ceftazidime alone which is hydrolysed by these enzymes. It is not effective against Class B β-lactamase producers, Gram positive organisms and Gram-negative anaerobes.[13] Antibiotic coverage of CAZ-AVI is more than 99% for Enterobacterales. CAZ-AVI was approved in 2015 by FDA for treating infections like complicated Urinary Tract Infections, Pneumonia etc. Treatment of CRE infections with CAZ-AVI showed significantly lower mortality and higher clinical cure.[14] Susceptibility pattern of CRE isolates to CAZ-AVI must evaluated to know the response of the drug against these resistant isolates and helping in administration of this combination drug to the patients to know the efficacy and clinical improvement.

Aim & objective

- To know the prevalence of Carbapenem Resistant Enterobacteriaceae (CRE) among various samples.
- To know the susceptibility pattern of Ceftazidime-Avibactam (30µg/20µg) among CRE isolates by Kirby-Bauer disc diffusion method.
- To determine the Minimum Inhibitory Concentration (MIC) of Ceftazidime- Avibactam (30µg/20µg) among CRE isolates by Epsilometer test (E-Strip).

MATERIALS AND METHODS

Inclusion Criteria

All the properly labelled samples sent in sterile container collected from patients with intra-abdominal, urinary tract, skin and soft-tissue, lower respiratory tract and bloodstream infections caused by Enterobacteriaceae group of organisms.

Exclusion Criteria

Samples with the improperly documented request, leaky containers, samples sent in unsterile containers. Paediatric population.

The study was conducted in the Department of Microbiology, Stanley Medical College, Chennai for a period of two months from August 2022 to September 2022. After obtaining Institutional Ethical clearance, the Clinical samples including pus, exudates, body fluids, respiratory specimens, urine and blood received in the Department of Microbiology were processed by standard laboratory techniques. After performing Gram stain, the specimens were inoculated on to MacConkey agar plates, Blood agar plates and Nutrient agar plates and the plates were incubated at 37°C for 18 to 24 hours. The colonies were processed as per standard Microbiological procedures and Antibiotic susceptibility testing was done based on CLSI 2021 guidelines. CRE isolates were confirmed by Modified Carbapenem Inactivation method (mCIM), EDTA Carbapenem Inactivation method (eCIM). E strips and media used were checked for quality control by using ATCC 25922 Escherichia coli. Commercial ceftazidime-avibactam (30µg/20µg) E strips were kept using sterile tooth picks provided in the kit in a 100mm MHA plate. The plates were incubated at 37°C for 18 to 24 hours. The zone of inhibition and Minimum Inhibitory Concentration (MIC) were interpreted as per CLSI guidelines.

RESULTS

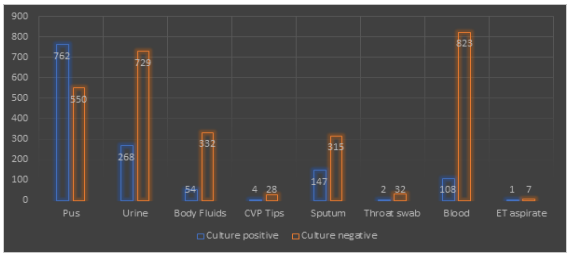

Figure 1: culture outcome of various clinical samples

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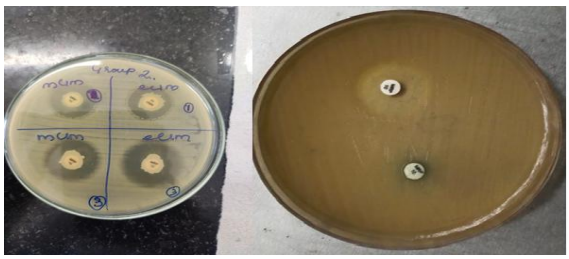

Figure 2: confirmation of CRE by MCIM and ECIM methods

Among the total 697 isolates of Enterobacteriales, 81 samples were confirmed to be carbapenem resistant showing a prevalence of 11.6%

Table 1: culture outcome of various clinical samples (N=4162).

Sample	Number of samples	Culture positive	Culture negative
Pus	1312	762	550
Urine	997	268	729
Body fluids	386	54	332
CVP tips	32	4	28
Sputum	462	147	315
Throat swab	34	2	32
Blood	931	108	823
ET aspirate	8	1	7
Total	4162	1346	2816

Table 2: distribution of enterobacteriales in various clinical samples (N=697)

Species	Samples					
	Urine	PUS	Blood	Fluids	Sputum	Total
Escherichia coli	108	157	22	6	7	300
Klebsiella pneumoniae	63	172	28	5	52	320
Klebsiella oxytoca	8	19	-	-	-	27
Enterobacter species	-	11	-	-	-	11
Proteus mirabilis	25	6	-	-	-	31
Citrobacter koseri	-	2	-	-	-	2
Providencia species	3	-	-	-	-	3
Salmonella typhi	-	-	3	-	-	3
Total	207	367	53	11	59	697

Table 3: Antibiogram of Enterobacteriales Across Various Clinical Samples

	AMP	SAM	CTX	AMC	TZP	GEN	CIP	TMP-SMX	FEP	IMP	MEM	AMK	TIG	COL
Escherichia coli	12.6 %	44%	44%	18.6 %	69%	56.3 %	50.3 %	18.6 %	50.3 %	94%	94%	62.6 %	100 %	100 %
Klebsiella oxytoca	IR	11.1 %	33.3 %	22.2 %	44.4 %	66.6 %	44.4 %	11.1 %	44.4 %	55.5 %	55.5 %	33.3 %	100 %	100 %
Klebsiella pneumonia	IR	28.4 %	47.5 %	14.3 %	90.6 %	71.5 %	57.1 %	33.4 %	66.5 %	80.9 %	85.6 %	61.8 %	100 %	100 %
Enterobacter	18.1 %	63.6 %	81.8 %	63.6 %	81.8 %	IR	72.7 %	IR	IR	81.8 %	81.8 %	IR	100 %	100 %
Proteus mirabilis	29%	54.8 %	67.7 %	45.1 %	87%	67.7 %	93.5 %	16.1 %	71%	87%	87%	71%	100 %	100 %

AMP-Ampicillin, SAM-Ampicillin-sulbactam, CTX-Cefotaxime, AMC-Amoxicillin-clavulanic , TZP-Piperacillin/Tazobactam, GEN-Gentamicin, CIP-Ciprofloxacin, TMP-SMX-Cotrimoxazole, FEP-Cefepime, IMP-Imipenem, MEM-Meropenem, AMK-Amikacin, TIG- Tigecycline, COL- Colistin

Table 4: distribution of CRE in various clinical samples (n=81)

Sample	Number	Percentage
Pus	66	81.48%
Urine	9	11.11%
Sputum	5	6.17%
Ascitic fluid	1	1.23%

Table 5: distribution of CRE among various species of enterobacteriales (n=81)

Species	Number	Percentage
Klebsiella pneumoniae	45	56%
Escherichia coli	18	22%
Enterobacter species	2	2%
Klebsiella oxytoca	12	15%
Proteus mirabilis	4	5%

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Table 6: antibiogram of CRE

	AMP	SAM	CTX	AMC	TZP	GEN	CIP	TMP-SMX	FEP	IMP	MEM	AMK	CAZ/AVI	ATM	TIG	COL
Escherichia coli	R	R	R	R	89 %	56 %	61 %	R	61 %	R	R	83%	100 %	72%	100 %	100 %
Klebsiella oxytoca	IR	R	R	R	52 %	33 %	42 %	R	42 %	R	R	33%	100 %	58%	100 %	100 %
Klebsiella pneumoniae	IR	R	R	R	84 %	78 %	62 %	R	67 %	R	R	82%	94%	84%	100 %	100 %
Enterobacter	R	R	R	R	100 %	100 %	50 %	R	100 %	R	R	100 %	100 %	100 %	100 %	100 %
Proteus mirabilis	R	R	R	R	50 %	50 %	50 %	R	75 %	R	R	25%	100 %	100 %	100 %	100 %

AMP-Ampicillin, SAM-Ampicillin-sulbactam, CTX-Cefotaxime, AMC-Amoxicillin-clavulanic , TZP-Piperacillin/Tazobactam, GEN-Gentamicin, CIP-Ciprofloxacin, TMP-SMX-Cotrimoxazole, FEP-Cefepime, IMP-Imipenem, MEM-Meropenem, AMK-Amikacin, CAZ/AVI- Ceftazidime-Avibactam, ATM- Aztreonam, TIG- Tigecycline, COL- Colistin

Table 7: distribution of ceftazidime-avibactam sensitivity among CRE by MIC

Organism	MIC RANGE(≤8/4 µg/mL)	
	SENSITIVE(%)	RESISTANT(%)
Klebsiella pneumoniae (45)	42(94%)	3(6%)
Escherichia coli (18)	18(100%)	-
Klebsiella oxytoca (12)	12(100%)	-
Proteus mirabilis (4)	4(100%)	-
Enterobacter species (2)	2(100%)	-

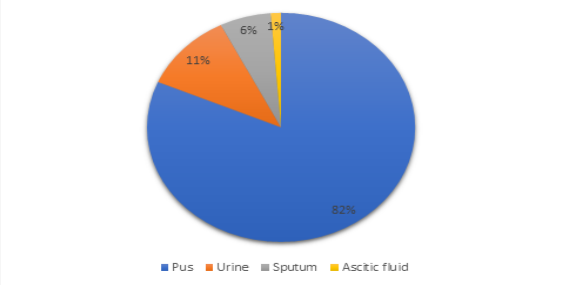

Figure 3: distribution of CRE in various clinical samples

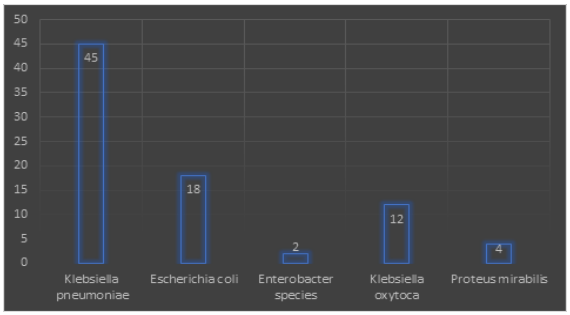

Figure 4: distribution of CRE among various species of enterobacteriales

Figure 5: CEFTAZIDIME – AVIBACTUM SUSCEPTIBILITY OF CRE BY E-TEST- (A) Shows as Minimal Inhibitory Concentraion (MIC) of 48µg/ml, hence resistance. (B) Shows a Minimal Inhibitory Concentration (MIC) of 0.47 µg/ml, hence sensitive.

DISCUSSION

A total of 4,162 clinical samples (table 1) were received at the Department of Microbiology during the study period from July 2022 to August 2022 out of which 697 isolates were identified as members of the Enterobacteriales family (table 2). In our study it was evident that the majority of Enterobacteriales was of Klebsiella pneumoniae (45.91%) followed by Escheria coli (43.04%) (table 2). Prabala et al had a majority of Klebsiella penumoniae (42.79%) and followed by Escheria coli (36.49%).[15]

Antimicrobial susceptibility testing showed that out of the 320 Klebsiella pneumoniae only 85.6% were sensitive to meropenem and 80.9% were sensitive to imipenem. Klebsiella pneumoniae showed 28.4% sensitivity to ampicillin-sulbactam, 47.5% sensitivity to cefotaxime, 14.3% sensitivity to amoxicillin-clavulanic acid, 90.6% sensitivity to

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piperacillin/tazobactam, 71.5% sensitivity to gentamicin, 57.1% sensitivity to ciprofloxacin, 33.4% sensitivity to cotrimoxazole, 66.5% sensitivity to cefepime and 61.8% sensitivity to amikacin.

Antimicrobial susceptibility testing of Enterobacter showed that out of the 11, 81.8% were sensitive to both meropenem and imipenem. Enterobacter species showed 18.1% sensitivity to ampicillin, 63.6% sensitivity to ampicillin-sulbactam, 81.8% sensitivity to cefotaxime, 63.6% sensitivity to amoxicillin-clavulanic acid, 81.8% sensitivity to piperacillin/tazobactam, 72.7% sensitivity to ciprofloxacin and intrinsic resistance to cotrimoxazole, gentamicin, cefepime and amikacin. Hence among the total 697 isolates of Enterobacteriales, 81 samples were confirmed to be carbapenem resistant showing a prevalence of 11.6% [Table 3 and Figure 2] which was similar to the study in Greater Noida, India by Namitha Thomas et al16 with a prevalence of 18.54%. A study conducted by Shree et al17 in Karnataka, India showed a prevalence of 23.69%. Prabhala S et al,[15] showed a prevalence of 26.5% in their study.

In our study, the clinical sample which showed highest prevalence of CRE was pus (81.48%) followed by urine (11.11%) [Table 4]. In a study conducted by et al the predominant clinical sample was found to be urine.[15-17]

Out of the 81 carbapenem resistant Enterobacteriales, Klebsiella pneumonia holds the highest prevalence accounting 56% followed by Escherichia coli of 22% [Table 5]. Prabhala S et al,[15] shows similar results to our study with Klebsiella pneumoniae (59.9%) being the most common CRE organism followed by Escherichia coli (20.18%). In the study by Shree S R et al,[17] Escherichia coli (54%) was the predominant organism followed by Klebsiella pneumoniae (20%). The study conducted by Namitha Thomas et al,[16] shows Escherichia coli having the higher percentage of 63.75% followed by Klebsiella pneumoniae of 11.25%.

In this study, out of the total CRE, Klebsiella pneumoniae were 98% sensitive to ceftazidime-avibactam, 84% sensitive to Aztreonam, Piperacillin/tazobactam, 82% were sensitive to Amikacin, 78% sensitive to Gentamicin, 67% to Cefepime and 62% to Ciprofloxacin. Escherichia coli were 100% sensitive to ceftazidime-avibactam combination, 89% to piperacillin/tazobactam ,83% to amikacin, 72% sensitive to Aztreonam, 61% sensitive to ciprofloxacin, cefepime and 56% sensitive to Gentamicin. Klebsiella oxytoca were 83% sensitive to ceftazidime-avibactam and 33% sensitive to Amikacin, Gentamycin. [Table 6]

In our study, among the sensitivity pattern of the total 81 CRE to Ceftazidime-avibactum, 78 isolates had MIC values below 8/4 µg/mL and 3 isolates of Klebsiella pneumoniae had MIC values more than 16/4 µg/mL. Hence overall sensitivity of CRE to Ceftazidime –Avibactam in this study is 96% [Table 7]. A study by Ratish et al,[18] from Kochi, India showed a sensitivity of 79% while Bakthavatchalam et al,[19] from India showed that in their study CRE K. pneumoniae and E.coli had susceptibility of 72% and 87% respectively.

CONCLUSION

The prevalence of CRE in this study is 11.6% and the sensitivity to Ceftazidime –Avibactam is 96%.Since there are many factors contributing to antimicrobial resistance, there is an increasing prevalence of antimicrobial resistance and infections caused by MDR Gram-negative bacteria. According to the priority pathogen list 2024 released by WHO, CRE comes under critical group. These CREs if not identified and treated early may lead to increased mortality which makes a challenge for the treating physicians. Even though Tigecycline and Colistin are cost effective, the adverse effects produced by these drugs are more and serious when compared to the adverse effects of Ceftazidime –Avibactam. Hence, Ceftazidime –Avibactam is a safer drug to treat patients infected with CRE strains.A uniform susceptibility pattern of Ceftazidime-Avibactam makes it a right choice of drug for all CRE which shows a variable susceptibility to the other drugs. Early and appropriate use of ceftazidime-avibactam decreases the mortality caused by pathogens which are sensitive to ceftazidime-avibactam. Hence with strict and vigilant infection control practices along with the right antibiotic at right time, we can manage the infections caused by MDR organisms.

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