ANTIMICROBIAL SENSITIVITY OF GRAM-NEGATIVE BACTERIA WITH BETA LACTAM /BETA LACTAMASE INHIBITOR COMBINATION OF DRUGS IN COMPARISON TO OTHER ANTIBIOTICS IN TERTIARY CARE CENTER

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ABSTRACT

BACKGROUND

Objectives of the study were- 1. isolation and speciation of Gram-negative bacteria from urine, pus sample, wound swab, blood, tissue, BAL and other body fluids. 2. To identify bacteria most commonly isolated in ICU and Non-ICU Samples. 3. To compare sensitivity and resistant patterns of beta lactam and beta lactamase inhibitor combination of drugs, carbapenem, and aminoglycosides in various Gram-negative bacterial isolates.

Bacterial isolates are compared with MIC testing (E test) for Cefepime Tazobactam (CPT) and disc diffusion method.

MATERIALS AND METHODS

Gram negative bacterial pathogens from various clinical samples (urine, blood, broncho-alveolar lavage [BAL], other body fluids (bile, endometrial secretions), pus, wound swab, sputum and vaginal swab) were included in this study. The Isolates were identified with a battery of standard biochemical tests. Cefepime/tazobactam (CPT) (30/10 µg HiMedia, Mumbai) against various bacterial isolates and their susceptibility were compared with other β-lactam/β-lactamase inhibitor combinations like piperacillin/ tazobactam (PTZ), cefoperazone/sulbactam (CFS), carbapenems [imipenem (IMP), meropenem (MRP) and Amikacin]. MIC determination paper strip which is coated with Cefepime/Tazobactam on a single paper strip in a concentration gradient capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the test organism.

RESULTS

A total of 130 isolates from patients attending the tertiary care teaching hospital were included in the study. Most common age groups of isolation of Gram-negative bacilli was 40-60 yrs. and above 60 yrs. Out of 130 isolates isolated, 68 isolates were from male patients and 53 isolates were from female patients, 7 isolates were from male children and 2 isolates were from female children. Out of 130 isolates isolated, most common area of isolation of Gram-negative bacilli were from Inpatient (IP) from various wards, Out Patient (OP) and ICU. Out of 130 isolates isolated, most common samples were from urine, blood, pus and wound swab. Out of 130 samples isolated, 43% were from Escherichia coli, 24% were from Klebsiella pneumoniae, 18% were from Pseudomonas aeruginosa, 5% were from Enterobacter species and 3% were from Proteus species. Among 43% E. coli isolates, the sensitivity pattern towards, Cefepime Tazobactam was 90%, Cefepime - 32%, Piperacillin Tazobactam -75%, Cefoperazone sulbactam -71%, Carbapenem– 91%, and Amikacin- 92%. Klebsiella Pneumoniae (24%) showed susceptibility of 42% on Cefepime Tazobactam, Cefepime-16%, Piperacillin Tazobactam -48%, Cefoperazone sulbactam -46%, Carbapenem-55%, and Amikacin 55%. Pseudomonas aeruginosa (18%) showed highest susceptibility of 87% Sensitivity to Cefepime Tazobactam, Cefepime, Carbapenem and Amikacin followed by 70% Sensitivity to Piperacillin Tazobactam and Cefoperazone sulbactam. Proteus spp. showed 100 % susceptibility to Cefepime Tazobactam, Cefoperazone sulbactam, Carbapenem and Amikacin followed by 80% sensitivity to Cefepime, Piperacillin Tazobactam. Citrobacter spp. showed highest percentage of 100% susceptibility to Cefepime Tazobactam, Cefepime followed by 75% sensitivity to CFS, PIT, Carbapenem and Amikacin. Acinetobacter spp. Showed 50% sensitivity to Carbapenem and Amikacin respectively. E. Coli showed 68% resistant to Cefepime. Klebsiella showed resistant to 58%-Cefepime Tazobactam, 84%-, Cefepime, 52%-Piperacillin Tazobactam and 54% Cefoperazone sulbactam. Acinetobacter showed 100% resistant to Cefepime Tazobactam, Cefepime, Piperacillin Tazobactam and Cefoperazone sulbactam. Out of 130 Samples, Cefepime Tazobactam showed highest sensitivity to E. Coli, Enterobacter, Pseudomonas, Proteus, Serratia and Citrobacter. It showed resistant to Klebsiella and Acinetobacter.

CONCLUSION

This study showed a high level of antibiotic resistance among Gram-negative bacilli, particularly E. coli, K. pneumoniae and Acinetobacter spp. to the third generation cephalosporins. Cefepime Tazobactam is highly sensitive than Cefoperazone Sulbactam, Ceftazidime Sulbactam and Piperacillin Tazobactam. Cefepime, Tazobactam can be used to limit the Carbapenem usage in hospitals. E test and Antibiotic disc diffusion test showed equally sensitivity and resistant patterns.

KEY WORDS

Cefepime Tazobactam, Gram Negative Bacteria, E Test.

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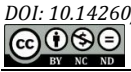

BACKGROUND

Gram negative bacilli (GNB) continue to be an important cause of health care associated infections. Antimicrobial resistance among these bacilli is increasing on a worldwide basis, especially resistance against β lactam antibiotics due to the development of β lactamase enzymes. As a result, it creates therapeutic failure or increases the morbidity among the patients.[1] Cefepime is a fourth-generation cephalosporin antibiotic. Cefepime has an extended spectrum of activity against Gram-positive and Gram-negative bacteria, with greater activity against both types of organism than third-generation agents. It is stable against AmpC & OXA. Cefepime has good activity against important pathogens including Pseudomonas aeruginosa, Staphylococcus aureus, and multiple drug-resistant Streptococcus pneumoniae. Cefepime is usually reserved to treat moderate to severe nosocomial pneumonia, infections caused by multiple drug-resistant microorganisms.[2] Tazobactam inhibits the action of bacterial β-lactamases, especially those belonging to the SHV-1 and TEM groups. It can be added to certain antibiotics to make them less vulnerable to bacteria's antimicrobial resistance. Tazobactam is combined with the fourth-generation cephalosporin Cefepime.[3] Cefepime and Tazobactam are prescribed for the treatment of uncomplicated skin and skin structure infections, uncomplicated and complicated urinary tract infections (UTI) and complicated intra-abdominal infections in adults and children, and it is also approved for use for empirical therapy for febrile neutropenic patients.[4] Cefepime and Tazobactam are indicated as a parenteral therapy for the treatment of various moderate to severe forms of infections due to susceptible beta-lactamase producing microbial organisms.[5,6] Cefepime and Tazobactam combination are particularly indicated if the Cefepime monotherapy is ineffective. Considering these therapeutic challenges, this study was aimed to compare the in-vitro antimicrobial effect of carbapenems, piperacillin/Tazobactam and cefoperazone/sulbactam with Cefepime/ tazobactam – a new β-lactam/β-lactamase inhibitor combination. This study was done to evaluate the current status of the drugs which will help the clinicians to prescribe the appropriate drugs to the patients to reduce their hospital stay and cost of treatment.

MATERIALS AND METHODS

Type of Study

Descriptive study.

Settings

The study was conducted in a Department of Microbiology, Government Sivagangai Medical College, Sivagangai.

Sample Size

130 isolates received from various Patients Samples with different Clinical condition.

Study Period

From July to December 2018.

Inclusion Criteria

Newly admitted patients without any antibiotic treatment.

Exclusion Criteria

Patients already with antibiotic treatments. Newborn child. Repeated isolates from the same patients.

Methodology

A total of 130 non-repetitive, consecutive aerobic Gram-negative bacterial pathogens from various clinical samples (Urine, Blood, broncho-alveolar lavage [BAL], other body fluids (Bile, endometrial secretions), Pus, Wound Swab, Sputum and Vaginal Swab) were included in this study. The samples were transported to the lab and streaked on MacConkey Agar and Blood Agar medium. After the incubation period at 37^{o}C, the culture plates were examined, and bacterial isolates were observed by Grams Staining and motility test. Isolates were identified with a battery of standard biochemical tests[7] Cefepime/tazobactam (CPT) (30/10 µg Hi Media, Mumbai) against various bacterial isolates and to compare its susceptibility with other β-lactam/β-lactamase inhibitor combination like Piperacillin/ tazobactam (PTZ), Cefoperazone/sulbactam (CFS), Carbapenems [imipenem (IMP), meropenem (MRP) and Amikacin]. The antibiotic sensitivity was done with Mueller Hinton agar according to Kirby- Bauer technique.[8] Cefepime/tazobactam interpretative criteria was not available, Cefoperazone and Cefepime zone size as per CLSI 2018 was used to interpret these two drug combinations (CLSI 2018). MIC determination paper strip which is coated with Cefepime/Tazobactam on a Single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, on testing against the test organism. Enterobacteriaceae group of organisms for which the Cefepime Tazobactam zone diameters below 2 mm are considered susceptible, 4-8 mm are Intermediate sensitivity, >16 mm are Resistant. Non-fermenters group of organisms for which the Cefepime Tazobactam zone diameters below 8 mm are considered susceptible, 16 mm are Intermediate sensitivity, >32 mm are Resistant.[9] ATCC control strains, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as controls.

The study was approved by the Institutional ethical committee.

RESULTS

Age in Years	Numbers (n=130)
1-12	9
13-40	23
40-60	42
61 and Above	56
Total	**130**

Table 1. Age Wise Distribution of Samples

A total of 130 isolates from patients attending the tertiary care teaching hospital. Most common age group of isolation of Gram-negative bacilli were from 40-60 yrs. and above 60 yrs. [Table. 1]

Sex	Numbers (n=130)
Male	68
Female	53
Male Child	7
Female Child	2
Total	**130**
Table 2. Sex Wise Distribution of Samples	

Out of 130 isolates isolated, 68 isolates were from male patients and 53 isolates were from female patients. 7 isolates were from male children and 2 isolates were from female children. [Table. 2]

Places	Numbers(n=130)
OP	43
IP	69
ICU	16
PICU	1
CTICU	1
Total	**130**
Table 3. Places of Isolation of Organisms	

Out of 130 isolates isolated, most common area of isolation of Gram-Negative Bacilli were from Inpatient (IP) from various wards, Out Patient (OP) and ICU. [Table. 3]

Samples	Numbers (n=130)
Urine	39 (30%)
Blood	16 (12%)
PUS	31 (24%)
Sputum	3
BAL	6
Other Body fluids	2
Tissue	5
Vaginal Swab	2
Wound Swab	26 (20%)
Total	**130**
Table 4. Samples Isolation	

Out of 130 isolates isolated, most common Samples were from Urine, Blood, Pus and Wound Swab. [Table. 4]

Organism in Percentage	Numbers (n=130)
E. Coli (43%)	56: - Urine-30, Blood-6, Wound swab-7, Pus-9, BAL-1, Tissue-1, Other fluids-1, Vaginal Swab-1
Klebsiella pneumonia (24%)	31: - Urine-5, Blood-3, Bal-4, Pus-7, Wound Swab-6, Sputum-1, Tissue-3, Other Fluids-1, Vaginal Swab-1
Enterobacter Species (5%)	7: - Urine-1, Blood-2, Wound Swab-1, Pus-3
Proteus species (3%)	5: - Pus-2, Blood-1, Tissue-1, Wound Swab-1
Pseudomonas aeruginosa (18%)	23: - Urine-2, Wound Swab-8, Pus-8, Sputum-2, Blood-2, Bal-1
Serratia marcescens	2: -Blood-2
Acinetobacter	2: - Wound Swab-2
Citrobacter koseri (2%)	4: - Pus-2, Wound Swab-1, Urine-1
Total	**130**
Table 5. Gram Negative Bacteria's Isolated from Various Samples	

Table 5, Out of 130 samples isolated, 43% were from Escherichia Coli, 24% were from Klebsiella Pneumoniae, 18% were from Pseudomonas aeruginosa, 5% were from Enterobacter Species and 3% were from Proteus Species.

Organism in Percentage	Cefepime Tazobactam (CPT)	Cefepime	PIT	CFS	Carbapenem	Amikacin
	Sen %	Sen %	Sen %	Sen %	Sen %	Sen %
E. Coli (43)	90	32	75	71	91	92
Klebsiella Pneumoniae (24)	42	16	48	46	55	55
Pseudomonas aeruginosa (18)	87	86	70	70	87	87
Enterobacter Species (5)	100	100	100	100	100	100
Proteus Species (3)	100	80	80	100	100	100
Citrobacter Species (2)	100	100	75	75	75	75
Serratia	100	100	100	100	100	100
Acinetobacter Species	0	0	0	0	50	50
Table 6. List of Antibiotics and their Sensitivity Pattern: - (N=130)						

Table 6, among 43% E. coli isolates, the sensitivity pattern towards, Cefepime Tazobactam was 90%, Cefepime - 32%, Piperacillin Tazobactam -75%, Cefoperazone sulbactam - 71%, Carbapenem– 91%, and Amikacin- 92%. Klebsiella Pneumoniae (24%) showed susceptibility of 42% on Cefepime Tazobactam, Cefepime-16%, Piperacillin Tazobactam -48%, Cefoperazone sulbactam -46%, Carbapenem-55%, and Amikacin 55%. Pseudomonas aeruginosa (18%) showed highest susceptibility to 87% Sensitivity to Cefepime Tazobactam, Cefepime, Carbapenem and Amikacin followed by 70% Sensitivity to Piperacillin Tazobactam and Cefoperazone sulbactam. Proteus spp. showed 100 % susceptibility to Cefepime Tazobactam, Cefoperazone sulbactam, Carbapenem and Amikacin followed by 80% sensitivity to Cefepime, Piperacillin Tazobactam. Citrobacter spp. showed highest percentage of 100% susceptibility to Cefepime Tazobactam, Cefepime followed by 75% sensitivity to CFS, PIT, Carbapenem and Amikacin. Acinetobacter spp. Showed 50% sensitivity to Carbapenem and Amikacin respectively.

Organism	Cefepime Tazobactam	Cefepime	PIT	CFS	Carba	Amikacin
	Res %	Res %	Res %	Res %	Res %	Res %
E. coli	10	68	25	29	9	8
Klebsiella pneumoniae	58	84	52	54	45	45
Pseudomonas aeruginosa	13	14	30	30	13	13
Proteus Species	0	20	20	0	0	0
Citrobacter Species	0	0	25	25	25	25
Acinetobacter Species	100	100	100	100	50	50
Table 7. Distribution of Resistant Pattern						

E. Coli showed 68% resistant to Cefepime. Klebsiella showed resistant to 58%-Cefepime Tazobactam, 84%-, Cefepime, 52%-Piperacillin Tazobactam and 54% Cefoperazone sulbactam. Acinetobacter showed 100% resistant to Cefepime Tazobactam, Cefepime, Piperacillin Tazobactam and Cefoperazone sulbactam. (Table. 7).

Cefepime Interpretation	S	SDD	R	ATCC - QC
Enterobacteriaceae	<2	4_8	>=16	Escherichia coli

Table 8. MIC of Cefepime Tazobactam with E Strips Method (HiMedia) – CLSI 2018.

Organism	Cefepime Tazobactam MIC (E Test %)	
	Sen %	Res %
E. Coli	90	10
Klebsiella	45	55
Pseudomonas	87	13
Enterobacter	100	0
Proteus Spp.	100	0
Citrobacter	100	0
Acinetobacter	0	100
Serratia	100	0

Table 9

Out of 130 Samples, Cefepime Tazobactam showed highest sensitivity to E. Coli, Enterobacter, Pseudomonas, Proteus, Serratia and Citrobacter. It showed resistant to Klebsiella and Acinetobacter. (Table 8).

DISCUSSION

In Our Study, Infections due to Gram negative organisms are commonly isolated in the clinical Sample. Similar observations have been made by others; Bhat et al and Shrestha et al.[10,11]

In this study, E. coli, Klebsiella spp, and P. aeruginosa are the most commonly isolated organism. The bacterial isolates were collected mainly from patients with urinary tract infections (30%), Pus and wound swab (44%) and bloodstream infections (12%) correlates with Veeraraghavan B et al.[12]

In this study, among 43% E. coli isolates, the sensitivity pattern towards, Cefepime Tazobactam was 90%, Cefepime - 32%, Piperacillin Tazobactam -75%, Cefoperazone sulbactam -71%, Carbapenem– 91%, and Amikacin- 92%. Klebsiella Pneumoniae (24%) showed susceptibility to 42% on Cefepime Tazobactam, Cefepime-16%, Piperacillin Tazobactam -48%, Cefoperazone sulbactam -46%, Carbapenem-55%, and Amikacin 55%. Pseudomonas aeruginosa (18%) showed highest susceptibility of 87% Sensitivity to Cefepime Tazobactam, Cefepime, Carbapenem and Amikacin followed by 70% Sensitivity to Piperacillin Tazobactam and Cefoperazone sulbactam. Proteus spp. showed 100 % susceptibility to Cefepime Tazobactam, Cefoperazone sulbactam, Carbapenem and Amikacin followed by 80% sensitivity to Cefepime, Piperacillin Tazobactam. Citrobacter spp. showed highest percentage of 100% susceptibility towards Cefepime Tazobactam, Cefepime followed by 75% sensitivity to CFS, PIT, Carbapenem and Amikacin. Acinetobacter spp. Showed 50% sensitivity to Carbapenem and Amikacin respectively.

Addition of tazobactam increased the susceptibility of from 32% to 90% in E. coli, from 16 to 41.0% in Klebsiella, from 86.0 to 87% in Pseudomonas and Proteus from 80% to 100%. This study highly correlates with Abdul Kafur et al.[13]

Cefepime/tazobactam having better coverage than Cefepime, Cefoperazone-sulbactam, Piperacillin Tazobactam and Ceftazidime Tazobactam correlates with my previous sudy.[14]

Cefepime tazobactam (30/10 µg) combination was found to be very effective against many ESBL producing Gram negative organism. As susceptibility rates to Cefepime are severely decreasing, Cephalosporins should not be a useful for treatment.

In this study, E. Coli showed 68% resistant to Cefepime. Klebsiella showed resistant to 58%-Cefepime Tazobactam, 84%-, Cefepime, 52%-Piperacillin Tazobactam and 54% Cefoperazone sulbactam. Acinetobacter showed 100% resistant to Cefepime Tazobactam, Cefepime, Piperacillin Tazobactam and Cefoperazone sulbactam correlates with Bhat V et al.[15]

Gram negative isolates were most frequently sensitive to Carbapenems and Cefepime Tazobactam followed by aminoglycosides in this study.

The high rate of Enterobacteriaceae resistance to Carbapenem is a serious concern. At the same time reducing Carbapenem resistance in Pseudomonas is encouraging correlates with Dalal p et al.[16]

Cefepime Tazobactam E test (Mic) showed highest sensitivity to E. Coli, Enterobacter, Pseudomonas, Proteus, Serratia and Citrobacter. It showed resistant to Klebsiella and Acinetobacter. Same findings are confirmed with disc diffusion method also.

CONCLUSION

This study showed a high level of antibiotic resistance among Gram-negative bacilli, particularly E. coli, K. pneumoniae and Acinetobacter spp. to the third generation cephalosporins. Cefepime Tazobactam is more effective than Cefoperazone Sulbactam, Ceftazidime Sulbactam and Piperacillin Tazobactam. Cefepime Tazobactam can be used us to limit the Carbapenem usage in hospitals. E test and Antibiotic disc diffusion test showed equally sensitivity and resistant patterns.

Limitations

This is a single center study.

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