ORIGINAL ARTICLE

IN VITRO COMPARATIVE EFFICACY OF CARBAPENEMS AND β-LACTAM β-LACTAMASE INHIBITOR COMBINATIONS AGAINST MULTI DRUG RESISTANT GRAM NEGATIVE BACILLI

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ABSTRACT

Background: Gram-negative bacilli are commonly encountered in hospitalized and community acquired infections. Exposure to a wide range of antibiotics has led to multidrug resistant strains. Carbapenems and β-lactam β-lactamase inhibitor combinations are commonly used groups in such clinical settings. This study was conducted to find out susceptibility of multidrug resistant Gram-negative bacilli to these antimicrobials.

Material & Methods: This descriptive study was conducted at Microbiology Department, Fauji Foundation Hospital, Rawalpindi, from April 2004 to March 2006. Sensitivity pattern of 2413 Gram-negative rods was tested.

Results: From 4204 samples, 2413(57.39%) Gram-negative rods were isolated. These were Escherichia Coli 1039(43.0%), Pseudomonas aeruginosa 667(27.6%), Klebsiella pneumoniae 490(20.3%), Proteus 62(2.6%), Acinetobacter 40(1.7%), Providencia 35(1.5%) and Enterobacter 34(1.4%). To imipenem, 1.5% Klebsiella pneumoniae, 3.2% Escherichia coli, 11% Enterobacter 15%, Proteus mirabilis, 10.7% Pseudomonas aeruginosa and 20% Acinetobacter, while none of Providencia. were resistant. To piperacillin/tazobactam, 0.69% Klebsiella pneumoniae, 2.38% Proteus mirabilis, 7.5% Escherichhia coli & Pseudomonas aeruginosa, 20% Acinetobacter while none of Enterobacter & Providencia were resistant. To meropenem only 7.9% Pseudomonas aeruginosa were resistant. None of Klebsiella pneumoniae and Escherichia coli were resistant to it. To cefoperazone/sulbactam, 20% Klebsiella pneumoniae were resistant but none of Escherichia coli, Enterobacter, Proteus mirabillis, Pseudomonas aeroginosa, Providencia and Acinetobacter were resistant.

Conclusion: Resistance pattern of carbapenems and β-lactam, β-lactamase inhibitor combinations shows that both these groups of antibiotics are effective in treating serious life threatening infections caused by Gram-negative bacilli.

Key words: Gram negative bacilli, Carbapenem, β-lactam, β-lactamase inhibitor.

INTRODUCTION

The human life has always been in danger from diseases caused by microorganisms. The history still mourns the death toll of epidemics of influenza, plague and malaria, which occurred during the 20th century. Nosocomial or hospital acquired infections are the major causes of morbidity and mortality among hospitalized patients. There has been a major shift in the etiology of hospital-acquired infections during 1980s in contrast to 1970s i.e. an increase in the laboratory isolation of Coagulase negative Staphylococci, Candida, Staphylococcus aureus, Enterococci, Pseudomonas aeruginosa and Enterobacter.[1,2] Taken as a whole the shifts are away from more easily treated pathogens towards more resistant ones with fewer options for therapy.[2]

The indiscriminate use of antimicrobials over prolonged periods has led to the emergence of multidrug resistant (MDR) strains.[3] Whenever a new and effective antibiotic is introduced, bacteria after exposure to this antimicrobial, acquire resistance through different mechanisms, commonest being the production of β-lactamases.[4,5] The production of extended spectrum β-lactamases by these organisms have made even the 3rd generation cephalosporins ineffective. To combat these MDR strains new and more effective antibiotics are required.

Carbapenems (Imipenem, meropenem, ertapenem) and β-lactam β-lactamase inhibitor combinations (piperacillin / tazobactam, cefoperazone/sulbactam) are relatively new antibiotics, effective against a wide variety of bacteria. Carbapenems belong to β-lactam group of antibiotics, antimicrobial activity of which is the result of intrinsic molecular characteistics.[6,7]

β-lactamase inhibitors include clavulanic acid, sulbactam and tazobactam. These compounds have limited antimicrobial activity but their major value is an inherent ability to limit the destructive action of β-lactamases against more active β-lactam compounds such as penicillins and cephalosporins.[8] For example, co-amoxiclav is a combination of amoxicillin and a β-lactamase inhibitor clavulanic acid, where as tazobactam is combined with piperacillin and cefoperazone with sulbactam.[8] Piperacillin/tazobactam is a combination in which tazobactam is a penicillanic acid that inhibits a wide variety of β-lactamases. It has a spectrum of activity similar to that of the clavulanic acid and superior to that of sulbactam.[9] Piperacillin when combined with tazobactam has shown extended spectrum of activity against β-lactamase producing agents.[10] These combinations are indicated as an empirical therapy for infections caused by a wide range of potential pathogens in both immunocompromised and immunocompetent patients and for the treatment of mixed aerobic and anaerobic infections such as intra-abdominal infections.[11]

This study was conducted to compare the in vitro activity of carbapenems (imipenem and meropenem) and β-lactam β-lactamase inhibitor combinations (piperacillin / tazobactam and cefoperazone / sulbactam) against MDR Gram-negative bacilli in order to find out the effectiveness of these agents in our setup.

MATERIALS AND METHODS

This study was conducted at Microbiology Department of Fauji Foundation University Hospital, Rawalpindi, over a period of two years, from April 2004 to March 2006. Two thousand four hundred and thirteen Gram-negative rods were isolated from various samples (pus, sputum, blood, urine and high vaginal swabs).

Blood agar and MacConkey's agar were used for the primary isolation of organisms from the specimens. Primary identification of the isolates was done by the colonial morphology, lactose fermentation on MacConkey's agar, Gram staining, catalase and oxidase tests. Identification to the species level was confirmed by using API 20-E.

Sensitivity was performed on Mueller Hinton agar using the modified Kirby-Bauer method.[12] Only those Gram-negative bacteria were selected which showed resistance to three or more of the following four antibiotic groups; Penicillins, Aminoglycosides, Quinolones and Third generation cephalosporins.

RESULTS

From 4204 samples, 2413 (57.39%) Gram-negative rods were isolated. (Figure-1) The highest frequency of Gram-negative rods were isolated from urine specimens (87.6%), followed by sputum (50.3%), blood and pus samples (49.7%) each and high vaginal swabs (30.8%). (Table-1).

Out of 2413 Gram-negative rods, 1039 (43.0%) were Escherichia Coli, 667 (27.6%) Pseudomonas aeruginosa, 490 (20.3%) Klebsiella pneumoniae, 62 (2.6%) Proteus spp, 40 (1.7%) Acinetobacter spp, 35 (1.5%), Providencia spp, and 34 (1.4%) were Enterobacter spp. While Citobacter spp, Salmonella spp, Morganella spp, Hafnia, Serratia, Aeromonas, Xanthomonas were

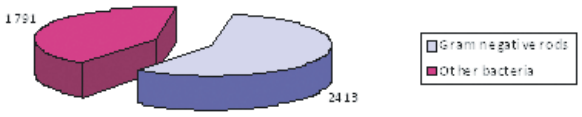

Fig-1: Frequency of Gram negative rods in 4204 samples.

Table 1: Distribution of Gram-negative rods in various samples

Samples	Total Number	Gram-Negative Rods	
		Number	Percentage
Urine	1303	1141	87.6
Sputum	262	132	50.3
Pus	1562	777	49.7
Blood	165	82	49.7
HVS	912	281	30.8
Total	**4204**	**2413**	**57.39**

Table 2: Prevalent organisms in 2413 Gram negative rods

Organisms	Number	Percentage
Escherichia coli	1039	43.0
Pseudomonas aeruginosa	667	27.6
Klebsiella pneumoniae	490	20.3
Proteus species	62	2.6
Acinetobacter species	40	1.7
Providencia species	35	1.5
Enterobacter species	34	1.4
Citrobacter species	9	0.37
Salmonella species	8	0.33
Morganella species	8	0.33
Salonella typhi	6	0.25
Hafnia species	4	0.17
Serratia species	4	0.17
Aeromonas species	4	0.17
Yersinia atypical	1	0.04
Fusobacteria species	1	0.04
Xanthomonas species	1	0.04
Total	**2413**	**100.0**

Table 3: Distribution of various Gram negative rods in different samples

Organisms	High vaginal swab	Sputum	Pus	Urine	Blood	Total
Escherichia coli	130	30	227	662	30	1039
Pseudomonas aeruginosa	51	40	290	260	26	667
Klebsiella pneumoniae	68	56	171	189	6	490
Proteus species	11	1	33	13	4	62
Acinetobacter species	11	—	12	16	1	40
Providencia species	3	3	11	18	—	35
Enterobacter species	4	1	12	13	4	34
Citrobacter species	1	—	2	6	—	9
Salmonella species	—	—	6	—	2	8
Morganella species	1	—	7	—	—	8
Salonella typhi	—	—	—	—	6	6
Hafnia species	1	—	—	3	—	4
Serratia species	—	1	2	1	—	4
Aeromonas species	—	—	1	—	3	4
Yersinia atypical	—	—	1	—	—	1
Fusobacteria species	—	—	1	—	—	1
Xanthomonas species	—	—	1	—	—	1
Total	**281**	**132**	**777**	**1141**	**82**	**2413**

Table 4: Sensitivity pattern of Gram negative rods

	Carbapenems										β-lactam β-lactamase inhibitor combinations									
Isolates	**Meropenem**					**Imipenem**					**Cefoperazone/Sulbactum**					**Piperacillin/Tazobactum**				
	S	**I**	**R**	**Total**	**%**	**S**	**I**	**R**	**Total**	**% R**	**S**	**I**	**R**	**Total**	**% R**	**S**	**I**	**R**	**Total**	**% R**
Klebsiella pneumoniae	13	—	—	13	0	197	1	3	201	1.5	8	—	2	10	20	137	6	1	144	0.69
Escherichia	16	—	—	16	0	265	6	9	280	3.2	23	2	—	25	0	225	7	19	251	7.56
Enterobacter spp						8	—	1	9	11						6	1	—	7	0
Proteus mirabilis						39	—	7	46	15	3	—	—	3	0	39	2	1	42	2.38
Pseudomonas aeruginosa	156	18	15	189	7.9	227	13	29	269	10.8	7	—	—	7	0	135	13	12	160	7.5
Providencia spp						29	—	—	29	0	3	—	—	3	0	21	7	—	28	0
Acinetobacter spp						12	—	3	15	20	3	—	—	3	0	7	1	2	10	20
Total				**218**					**849**					**51**					**642**	

Key: R=Resistant, S=Sensitive, I=Indeterminate

less than 1% of the total Gram-negative rods. (Table-2).

Escherichia coli 622/1039 (59.8%), Klebsiella pneumoniae 189/490 (38.5%), Acinetobacter spp 16/40 (40%) and Providencia spp 18/35 (51.4%) were most commonly isolated from urine samples.

Pseudomonas aeruginosa 290/667 (43.5%) and Proteus spp. 33/62 (53.2%) were most commonly isolated from pus samples. (Table-3).

Out of 849 Gram-negative rods tested against imipenem, 1.5% isolates of Klebsiella pneumoniae, 3.2% of Escherichia coli, 11.0 % of Enterobacter spp, 15% of Proteus mirabilis, 10.7% of Pseudomonas aeruginosa and 20% of Acinetobacter were resistant to imipenem. While none of Providencia spp. were found resistant to it.

Out of 642 Gram-negative rods tested against piperacillin/tazobactam, 0.69% isolates of Klebsiella pneumoniae, 2.38% of Proteus mirabilis, 7.5% of Escherichhia coli & Pseudomonas aeruginosa each, 20% of Acinetobacter spp while none of Enterobacter & Providencia spp, were resistant to it.

To meropenem, only 7.9% isolates of Pseudomonas aeruginosa wee resistant. None of Klebsiella pneumoniae and Escherichia coli isolates was resistant to it.

While in the case of cefoperazone/sulbactam, 20% of Klebsiella pneumoniae were resistant but none of Escherichia coli, Enterobacter, Proteus mirabillis, Pseudomonas aeroginosa, Providencia and Acinetobacter were found resistant to it. (Table-4).

DISCUSSION

Over the past several decades, the frequency of antimicrobial resistance and its association with serious infectious diseases has increased at alarming rates. The emergence of resistance to antimicrobial agents is a global public health problem, particularly in pathogens causing nosocomial infections.[13] Antimicrobial resistance results in increased illness, deaths and health-care costs.[14]

In the last decade there has been a major shift in the etiology of nosocomial infections.[15] The trend is from more easily eradicated pathogens towards more resistant ones, with fewer options left for treatment.[16] Gram negative pathogens resistant to β-lactam antibiotics have emerged as a major part of this disturbing trend.[17] Inducible beta lactamases have been responsible for multiple β-lactam resistance among the isolates of Enterobacteriacae and Pseudomonas aeruginosa. The increased incidence of infection due to these organisms is the result of frequent use of broad spectrum β-lactam agents.[18],[19]

Both imipenem and piperacillin/tazobactam have been considered very promising antibiotics in the management of commonly encountered Gram-negative infections in the hospital settings. The resistance to imipenem is present in bacteria that produce β-lactamases with specific carbapenemase activity which is found only in a very small population of organisms suggesting that the spread of resistance by such a mechanism is slow. Wide spread resistance to imipenem has not been observed among common Gram negative and Gram positive aerobes even after three years of extensive clinical use. In various multi-center studies conducted in different clinical settings piperacillin/tazobactam has shown very high index of sensitivity against resistant nosocomial pathogens.[20,21]

The previous studies showed a different trend of sensitivity towards carbapenems and β-lactam β-lactamase inhibitor combinations, where majority of the MDR isolates of Escherichia coli and Klebsiella pneumoniae were found resistant to piperacillin/tazobactam showed high index of sensitivity to imipenem.[22,23]

However, over a period, shifting trends have been observed in the susceptibility of carbapenems (imipenem, meropenem) and β-lactam β-lactamase inhibitor combinations (piperacillin/tazobactam, cefoparazone/sulbactam) at our institute and there is a development of considerable resistance by MDR Gram negative bacilli against these two groups of antibiotics. Escherichia coli, pseudomonas aeruginosa, Klebsiella pneumoniae and Acinetobacter were the most common organisms which showed the presence of resistance to multiple drugs. Klebsiella pneumonia showed a resistance rate ranging from 0-1.5% for carbapenems and 0.69-20% for beta-lactam beta-lactamase inhibitor combinations. Escherichia coli showed a resistance ranging from 0-3.2% for carbapenems and 0-7.56% for β-lactam β-lactamase inhibitor combinations. Pseudomonas aeruginosa showed a resistance ranging from 7.9-10.8% for carbapenems and 0-7.56% for beta-lactam beta-lactamase inhibitor combinations. While Acinetobacter showed an equal resistance (20%) to both.

CONCLUSION

The resistance pattern of carbapenems and β-lactam β-lactamase inhibitor combinations show that both these groups of antibiotics are effective in treating serious life threatening infections caused by Gram-negative bacilli.

Clinical trials to determine their in vivo efficacy may determine the choice for empirical therapy in life threatening infections by Gram negative bacilli.

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