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Research Article

COMPARATIVE ANTIMICROBIAL EFFICACY EVALUATION OF A NEW PRODUCT ELORES AGAINST MEROPENEM ON GRAM-NEGATIVE ISOLATES

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ABSTRACT

Background and objective: Increased resistance of Gram-negative bacteria towards most of the available antibiotics, especially beta-lactam antibiotics is a prime difficulty for the treatment of infections caused by these pathogens. In view of the fact that there is a continuous increase in the antibitic resistance and the limited available therapeutic options, we aimed the present work to evaluate the antibiotic susceptibility pattern of 847 isolates towards meropenem and Elores (ceftriaxone+sulbcatam+and adjuvant ethylenediaminetetraacetic acid).

Methods: A total of 1180 clinical samples were collected from patients suspected of bacterial infection between January 2014 to June 2014. These samples were subjected for bacterial identification. Antibiotic susceptibility testing were carried out according to the recommendations of Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: Among the samples which showed the presence of bacteria, around 29.04% samples were of sputum followed by urine and blood which contributed to 21.95% and 12.51%, respectively. Escherichia coli (39.55%) was found to be the most dominant pathogen, followed by Pseudomonas aeruginosa (19.12%), *Klebsiella pneumoniae* (12.39%), *Proteus mirabilis* (8.50%), *Klebsiella oxytoca* (8.26%), *Acinetobacter baumannii* (5.31%), *Morganella morganii* (3.77%), *Serratia marcescens* (2.24%). The susceptibility of Elores was comparable with meropenem in some of the organisms, but Elores displayed higher susceptibility in *E. coli*, *A. baumannii*, *K. pneumoniae*, *P. mirabilis*, *K. oxytoca*, *M. morganii* and *S. mercescens* which might be due to presence of metallo-beta lactamases in these isolates.

Conclusion: Overall, the results of this study strongly advocate the equivalance of Elores with meropenem and can be of very effective alternative to treat against the deadly multi drug resistant Gram-negative bacteria.

Keywords: *Elores, Gram*-negative bacteria, Nosocomial infections, Antimicrobial Resistance, Susceptibility.

INTRODUCTION

Resistant bacteria are emerging worldwide as a threat to favourable outcomes of treatment of common infections in community and hospital settings. Urinary tract, gastrointestinal and pyogenic infections are the common hospital acquired infections caused by Gram-negative bacteria [1]. Hospital-acquired infections (HAIs) are a significant cause of increased morbidity and mortality in hospitalized patients. In addition, HAIs, a cause of prolonged hospital stay, are inconvenient for the patient, and constitute an economic burden on health care. It is estimated that 80% of all hospital deaths are directly or indirectly related to HAIs [2].

Till now, among the various factors of resistance, extended spectrum β-lactamase (ESBL) production by Gram-negative bacteria was considered as the most important threat to clinical therapeutics [3]. Increasing prevalence (66.8-71.5%) of infections due to ESBL positive bacteria has been observed in various studies [3-5]. This increased rate has led to a unregulated increase in the usage of β-lactamase inhibitor/β-lactam combinations, monobactams and carbapenems. Carbapenems like meropenem possess stability against hydrolysis by ESBL and AmpC chromosomal β-lactamase enzymes and are often reserved to treat the most serious infections [6-8]. Meropenem has been effectively used in bacterial meningitis; skin and soft tissue infections, bone and joint infections (BJIs); serious gastrointestinal infections; septicemia; febrile neutropenia; nosocomial pneumonia; cystic fibrosis-associated respiratory infections; and serious urinary tract infections [6,9,10]. However, in the past few years, carbapenem resistance among the members of the *Enterobacteriaceae* family has been reported increasingly throughout the world and India [11-16]. Carbapenem resistance has been reported to be associated with 40-50% of mortality and morbidity and observed to carry genes showing high levels of resistance to several other antimicrobials, restricting very limited therapeutic options [17,18]. Besides, carbapenem resistance in *Enterobacteriaceae*, it has also been reported frequently in lactose non-fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacer* spp. [13,15,19,20]. In India, resistance to meropenem varies from 37% to 42% in *Pseudomonas* spp. [13,19] and up to 89% in *Acinetobacter baumannii* [20]. Overall, in India, the prevalence of carbapenemases, responsible for carbapenem resistance, ranged from 7.5% to 89% [20,21].

To overcome this serious threat of antibiotic resistance against carbapenems and to preserve carbapenems for future generations, one has to look for other alternative antibiotic options or the existing antibiotics with added potentiators to treat the infections caused by these multi drug resistant (MDR) strains. These antibiotic adjuvant entities have been reported to break resistance cycle and overcome different resistance mechanisms adopted by bacteria [21-24]. Considering all these aspects, the present work focuses to study the susceptibility pattern of the Gram-negative bacteria and to evaluate the efficacy of new antibiotic adjuvant entity - ceftriaxone+sulbactam+with adjuvant ethylenediaminetetraacetic acid (EDTA) (Elores) in comparison to meropenem among Gram-negative pathogens.

METHODS

Sample collection

Different clinical samples such as blood, pus, sputum, urine, abdominal fluid, bile, semen, swab, tissue, brancho alveolar fluid and endotracheal section were collected from 1180 patients suspected of bacterial

infection at various hospitals of western Uttar Pradesh and Gujarat state of India, during the period of January 2014 to June 2014. The collection and processing of the samples were done as per a common SOP by all laboratories.

Isolation and identification of microbes

All the samples were collected aseptically in sterile containers. Urine samples collected in the sterile universal container were directly inoculated to the respective selective media. Other liquid specimens such as pus, sputum, abdominal fluid, bile semen and brancho alveolar fluids collected in sufficient amount were inoculated on the different selective and non-selective culture media as per the standard microbiological techniques. Details of the culture media used for the isolation of pathogens from various clinical samples are given in Table 1. Blood samples collected in brain heart infusion broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37°C and then subcultured onto the selective and non-selective media. All the media were incubated aerobically overnight at 37°C. The organisms were identified on the basis of colony morphology, Gram-staining, motility, and biochemical reactions. Biochemical reactions were performed by inoculating the bacterial colony in a nutrient broth at 37°C for 2-3 hrs.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute guidelines [18]. Meropenem disk (10 µg) and Elores disk (45 µg) were procured from Hi-media (Mumbai, India) and used in the study. Inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller-Hinton broth (MHB, Hi-Media, Mumbai, India) from the isolated colony of pathogens selected from 18 to 24 hrs agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a Mueller-Hinton agar plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60°C over the agar surface. After 3-5 minutes, antibiotic discs were applied and pressed down to ensure complete contact with the agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates are then inverted and incubated for 16-18 hrs aerobically at 37°C within 15 minutes of disc application. The sensitivity of isolated organisms against antibiotics were reported as sensitive (S) or resistant (R) based on the breakpoints.

RESULTS AND DISCUSSION

A total 1180 different clinical samples were collected from different hospitals and processed for isolation of pathogenic bacteria according to common standard operating procedure. 11 types of clinical samples involved in the study included urine, pus, sputum, blood, abdominal fluid, bile, semen, swab, tissue, brancho alveolar fluid and endotracheal section. Out of total samples analyzed, 847 (71.77%) samples showed the presence of infection while in 333 (28.22%) samples no growth of organisms was observed in the culture medium (Table 2).

Among the samples (n=847) which showed the presence of pathogens, around 29.04% samples were of sputum, followed by urine (21.95%) and blood (12.51%) samples. Swab, pus, endotracheal section, abdominal fluid, bile and semen samples contributed between 4% and 8%, however samples from tissue and brancho alveolar fluid had a lesser share in total number of pathogen containing samples with percentile share 0.80 and 0.70, respectively (Table 2).

Morphological and biochemical characterization of the samples (n=847) showing bacterial growth revealed the presence of 13 different Gram-negative organisms (Gram-positive organisms are not included in the study). The detailed profile of various organisms collected from various clinical samples is shown in Fig. 1. The identified bacteria include *Escherichia coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Klebsiella oxytoca*, *A. baumannii*, *M. morgannii*, *Serratia marcescens*,

Table 1: Selective culture medium used for isolation of different pathogens

Pathogen	Selective media
E. coli	EMB agar medium
A. baumannii	Leeds acinetobacter agar base medium
K. pneumoniae and *K. oxytoca*	Hicrome Klebsiella selective agar base medium
P. mirabilis	EMB agar and Mcconkey's agar
P. aeruginosa	Citrimide agar
S. marcescens	CT agar
E. cloacae	Hicrome coliform agar modified medium
M. morganii	Blood agar and Mcconkey's agar
Salmonella Typhi	Xylose-Lysine Deoxycholate agar
S. boydii	EMB agar and Mcconkey's agar
B. cepacia	*B. cepacia* agar base

E. coli: Escherichia coli, A. baumannii: Acinetobacter baumannii, K. pneumonia: Klebsiella pneumoniae, K. oxytoca: Klebsiella oxytoca, P. mirabilis: Proteus mirabilis, P. aeruginosa: Pseudomonas aeruginosa, S. marcescens: Serratia marcescens, E. cloacae: Enterobacter cloacae, M. morganii: Morganella morganii, S. typhi: Salmonella typhi, S. boydii: Shigella boydii, B. cepacia: Burkholderia cepacia, EMB: Eosine Methylene Blue, CT: Caprylate - Thallous

Table 2: A profile of clinical samples used as a source of the pathogenic isolates

Serial no	Clinical samples	Total	Number of samples showing growth of pathogens	Number of samples not showing growth of pathogens
1	Sputum	368	246 (29.04)	122
2	Urine	214	186 (21.95)	28
3	Blood	158	106 (12.51)	52
4	Swab	113	69 (8.14)	44
5	Pus	75	65 (7.67)	10
6	Endotracheal section	65	48 (5.66)	17
7	Abdominal fluid	57	44 (5.19)	13
8	Bile	47	36 (4.25)	11
9	Semen	62	34 (4.01)	28
10	Tissue	10	7 (0.80)	3
11	Brancho alveolar fluid	11	6 (0.70)	5
	Total	1180	847	333

The values in the paranthesis indecate the percentile number of respective samples among the total samples showing growth of patho

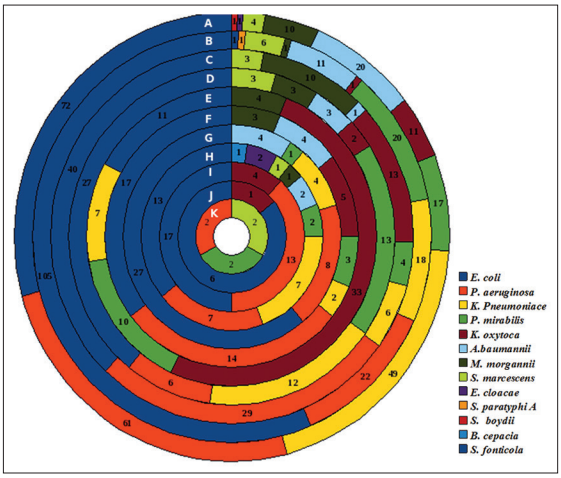

Fig. 1: Profile of different clinical isolates isolated from various samples, A: Sputum, B: Urine, C: Blood, D: Swab, E: Pus, F: Endotracheal section, G: Abdominal fluid, H: Bile, I: Semen, J: Tissue, K: Brancho alveolar fluid

Enterobacter cloacae, *Salmonella paratyphi A*, *Shigella boydii, Serratia. fonticola* and *Burkholderia cepacia* in decreasing order of prevelance.

Among the isolates, *E. coli* (39.55%) was found to be the most dominant pathogen. Similar results with high rates of *E coli* (54.9%) infections were reported by Sikka *et al*. [25]. *P. aeruginosa* (19.12%), *K. pneumoniae* (12.39%) also contributed significantly to the isolated pool of pathogens followed by *P. mirabilis* (8.50%), *K. oxytoca* (8.26%), *A. baumannii* (5.31%), *M. morgannii* (3.77%), *S. marcescens* (2.24%). A similar prevalence of *Klebsiella* sp. (22.08%) and *M. Morgannii* (1.95%) isolated from tertiary care hospital was also reported by Patel *et al*. [26]. However, the isolates like *E. cloacae* (0.35 %), *S. fonticola* (0.11%), *S. paratyphi A* (0.11%), *S. boydii* (0.11%) and *B. cepacia* (0.11%) contributed non-significantly (Fig. 2).

Frequency of isolation of pathogenic organisms from various specimens is depicted in Table 3. *E. coli* was the most prevalent pathogen among of the samples accounting for 29.26% in sputum, 56.45% urine, 37.73% in blood, 39.13% in swab, 35.41% in endotracheal section, 61.36% in abdominal fluid, 36.11% in bile and 50% in semen samples (Table 3). Similar results were observed by Mehta *et al*. [27] reporting high prevalence (41%) of *E. coli* among the urine samples collected from urinary tract infection patients. Patel *et al*. [26] reported a high prevalence of *E coli* among sputum (45.83%) which is in well accordance with results of the present study. Contradictory to our results, Patel *et al*. [26] reported very high prevalence of *E. coli* in endotracheal secretion (88.88%). *P. aeruginosa* accounted for 24.79% in sputum, 27.35% in blood, 29.16% in endotracheal section and 38.23% in semen samples (Table 3). *K. pneumoniae* contributed for 19.91% in sputum samples and *K. oxytoca* contributed for 50.76% in pus samples (Table 3). Sikka *et al*. [25] also reported considerable prevalence of (9.9%) *K. pneumoniae* in nosocomial sputum samples.

Antibiogram profile for all the pathogens isolated from various clinical samples is presented in Figs. 3 and 4. The susceptibility of the three most predominant pathogens *E. coli and K. pneumoniae* toward Elores (83.88% and 81.90%, respectively) was high when compared towards

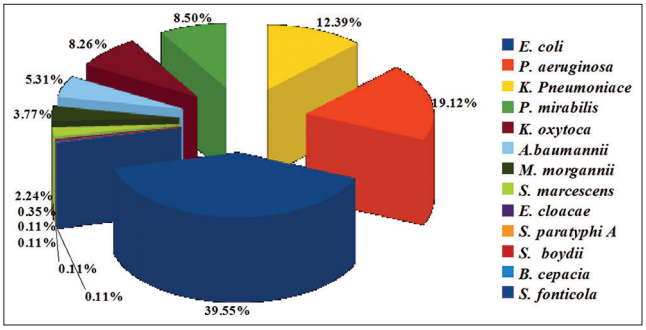

Fig. 2: Prevalence of various pathogen

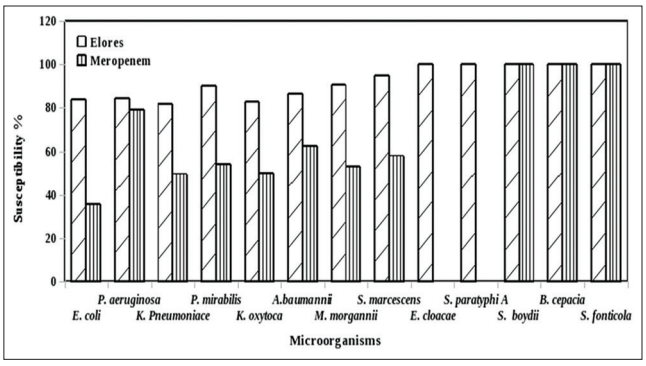

Fig. 3: Susceptibility pattern of Gram-negative pathogens isolated across India

Table 3: Prevalence of different clinical isolates in different samples

Samples	Number of isolates	Clinical isolates %											
		E. coli	*A. baumannii*	*K. pneumoniae*	*K. oxytoca*	*P. aeruginosa*	*P. mirabilis*	*M. morganii*	*S. marcescens*	*S. fonticola*	*E. cloacae*	*S. paratyphi A*	*S. boydii*	*B. cepacia*
Sputum	246	29.26	8.13	19.9	4.47	24.79	6.91	4.06	1.62	0	0	0	0.4	0
Urine	186	56.45	5.91	9.67	0.53	11.82	10.75	0.53	3.22	0.53	0	0.53	0	0
Blood	106	37.73	0.94	5.66	12.26	27.35	3.77	9.43	2.83	0	0	0	0	0
Swab	69	39.13	4.34	17.39	2.89	8.69	18.84	4.34	4.34	0	0	0	0	0
Pus	65	1.53	0	10.76	50.76	0	15.38	6.15	0	0	0	0	0	0
Endotracheal section	48	35.41	8.33	4.16	10.41	29.16	6.25	6.25	0	0	0	0	0	0
Abdominal fluid	44	61.36	9.09	9.09	0	18.18	2.27	0	0	0	0	0	0	0
Bile	36	36.11	4.54	19.44	0	19.44	4.54	2.77	2.77	0	4.54	0	0	2.77
Semen	34	50	0	0	11.76	38.23	0	0	0	0	0	0	0	0
Tissue	7	85.71	0	0	14.28	0	0	0	0	0	0	0	0	0
Brancho alveolar fluid	6	0	0	0	0	33.33	33.33	0	33.33	0	0	0	0	0

A. baumannii: Acinetobacter baumannii, K. pneumonia: Klebsiella pneumoniae, K. oxytoca: Klebsiella oxytoca, P. aeruginosa: Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis, M. morganii: Morganella morganii, S. marcescens: Serratia marcescens, S. fonticola: Serratia. fonticola, E. cloacae: Enterobacter cloacae, S. paratyphi A: Salmonella paratyphi A, S. boydii: Shigella boydii, B. cepacia: Burkholderia cepacia

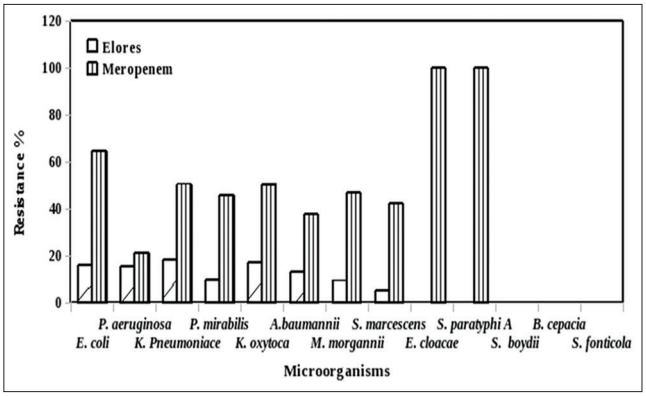

Fig. 4: Resistance patterns of Gram-negative pathogens isolated across India

meropenem (35.52%, and 49.52% repectively). The results of the present study also revealed >82% suceptibility of Elores in *A. baumannii* (86.67%), *K. oxytoca* (82.86%), *P. mirabilis* (90.28%), *Morganella morganii* (90.63%) and *S. marcescens* (94.74%). The higher susceptibility of Elores in these isolates probably due to presence of metallo-beta lactamases (MBLs) in these isolates. On the other hand the same pathogens showed higher resistance (40-50%) towards the meropenem. Both meropenem and Elores were equally effective among the less prevalent pathogens like *S. fonticola, S. boydii* and *B. cepacia*. However, *E. cloacae* and *S. paratyphi A* were completely resistant to meropenem. Very recently, Sahu *et al.* [28] also demonstrated higher susceptibility of Elores for *E. coli*, *P. aeruginosa* and *K. pneumoniae,* while Parveen *et al.* [29] reported the high meropenem resistance trends (43.6%) in *K. pneumoniae* isolated from south India. Gupta *et al.* [13] also reported high meropenem prevalence in *Pseudomonas* sp. isolated from ICU patients. Contradictory to our results, 9 years ago Gupta *et al.* [13] reported very low meropenem resistance in *E. coli* (3.5%). This difference in the meropenem resistance may be due to the increased number of pathogens producing carbapenamases over the years. Sahu *et al.* [28] also demonstrated higher susceptibility of Elores for *E. cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Proteus vulgaris.* According to a previous study conducted in India for the treatment of skin and skin structure infection and BJIs more than 80% of the studied patient were clinically cured with ceftriaxone+sulbcatam+and adjuvant EDTA (Elores) [30]. By the results of the current study, it appears Elores is the most effective agianst these MDR pathogens when compared to meropenem for *E. coli* and *K. pneumoniae* which might be because of expression of MBL genes in these pathogens to which meropenem does not respond.

CONCLUSION

This study provides the antibiotic sensitivity pattern towards the predominant Gram-negative microorganisms against meropenem and Elores suggesting that the use of Elores over meropenem should be preferred. This study will definitely be useful for the clinicians in general and of the region, in particular, to help make them cho0se correct antibiotic and ensure the judicious use of the same for their patients.

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