COMPARATIVE ANTIMICROBIAL EFFICACY EVALUATION OF A NEW PRODUCT ELORES AGAINST MEROGEN 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 antibiotic 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+sublactam+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 morgani (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. marcescens which might be due to presence of metallo-beta lactamases in these isolates.

Conclusion: Overall, the results of this study strongly advocate the equivalence 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 beta-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 beta-lactamase inhibitor/ beta-lactam combinations, monobactams and carbapenems. Carbapenems like meropenem possess stability against hydrolysis by ESBL and AmpC chromosomal beta-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 (BJs); 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 Acinetobacter 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 carbapenem 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+sublactam+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, branco 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.

Antimicrobial 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 Eloxol 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 18-16 hrs aerobically at 37°C within 15 minutes of disc application. The sensitivity of isolated bacteria 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. morganii, Serratia marcescens,

### Table 1: Selective culture media used for isolation of different pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Selective media</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>EM agar medium</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>Leeds acinetobacter agar base medium</td>
</tr>
<tr>
<td>K. pneumoniae and K. oxytoca</td>
<td>Hicrome Klebsiella selective agar base</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>EM agar and Mcconkey’s agar</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>Citrimide agar</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>CT agar</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>Hicrome coliform agar modified medium</td>
</tr>
<tr>
<td>M. morganii</td>
<td>Blood agar and Mcconkey’s agar</td>
</tr>
<tr>
<td>Salmonella Typhi</td>
<td>Xylose-Lysose Deoxycholate agar</td>
</tr>
<tr>
<td>S. boydii</td>
<td>EM agar and Mcconkey’s agar</td>
</tr>
<tr>
<td>B. cepacia</td>
<td>B. cepacia agar base</td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Serial no</th>
<th>Clinical samples</th>
<th>Total (n=847)</th>
<th>Number of samples showing growth of pathogens</th>
<th>Number of samples not showing growth of pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sputum</td>
<td>368</td>
<td>246 (29.04)</td>
<td>122</td>
</tr>
<tr>
<td>2</td>
<td>Urine</td>
<td>214</td>
<td>186 (21.95)</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>Blood</td>
<td>150</td>
<td>106 (12.51)</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>Swab</td>
<td>113</td>
<td>69 (8.14)</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>Pus</td>
<td>75</td>
<td>65 (7.67)</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Endotrachal section</td>
<td>65</td>
<td>48 (5.66)</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>Abdominal fluid</td>
<td>57</td>
<td>44 (5.19)</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>Bile</td>
<td>47</td>
<td>36 (4.25)</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>Semen</td>
<td>62</td>
<td>34 (4.01)</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>Tissue</td>
<td>10</td>
<td>7 (0.80)</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>Brancho alveolar fluid</td>
<td>11</td>
<td>6 (0.70)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1180</td>
<td>847</td>
<td>333</td>
</tr>
</tbody>
</table>

The values in the parenthesis indicate the percentile number of respective samples among the total samples showing growth of pathogenic isolates.

![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](image-url)
Enterobacter cloacae, Salmonella paratyphi A, Shigella boydii, Serratia fonticola and Burkholderia cepacia in decreasing order of prevalence.

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. morganii (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...
meropenem (35.52%, and 49.52% respectively). The results of the present study also revealed >82% susceptibility of Elores in E. baumannii (86.67%), K. oxytoca (82.86%), P. mirabilis (90.28%), Morganella morgani (90.63%) and S. marcescens (94.74%). The higher susceptibility of Elores in these isolates probably due to presence of metallo-beta lactamas (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 carbapenemases 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 BJS more than 80% of the studied patient were clinically cured with ceftriaxone+ sulfbatam+ and adjuvant EDTA (Elores) [30]. By the results of the current study, it appears Elores is the most effective against 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 choose correct antibiotic and ensure the judicious use of the same for their patients.

REFERENCES


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