ARTICLE INFO

Article History:
Received 5th, May, 2015
Received in revised form 12th, May, 2015
Accepted 6th, June, 2015
Published online 28th, June, 2015

Key words:
Gram negative bacteria, Drug resistance, ESBL, micro-broth dilution, Elores

ABSTRACT

In the present study, we attempted to find the resistance pattern to antibacterial agents among extended spectrum beta-lactamases (ESBL) and carbapenemase positive isolates, obtained from different clinical specimens at Gandhi Medical College Hospital, Hyderabad, India. A total of 299 isolates consisting of 250 ESBL and 49 carbapenemase producing isolates were recovered from various samples collected from intensive care units (ICU) and wards. Antibiotic susceptibility study was done by the disc diffusion method according to the Clinical Laboratory Standards Institute guidelines. Out of 299 isolates, 281 (93.9%) were of Enterobacteriaceae family and 18 (6.0%) were from non-Enterobacteriaceae. Of Enterobacteriaceae family, 184 (65.5%) were E. coli and 97 (34.5%) were K. pneumoniae. Among non-Enterobacteriaceae, 9 of each were Acinetobacter spp and P. aeruginosa. The most prevalent pathogen was E. coli followed by K. pneumoniae, and equal prevalence of Acinetobacter spp and P. aeruginosa. Ceftriaxone+sulbactam+disodium edetate (Elores) was the most effective drug showing 100% susceptibility to P. aeruginosa followed by E. coli (88.4%), K. pneumoniae (78.0%), Acinetobacter spp (66.6%). The comparator drugs showed low sensitivity up to 55.0%. Carbapenemase producers showed 100% resistance to Meropenem. However, Elores showed sensitivity ranging from 50.0% to 58% in carbapenemases producing Ecoli, K. pneumoniae and P. aeruginosa. This study provides important data for clinicians to plan the appropriate treatment regimen. As Elores showed better activity against both Enterobacteriaceae and non-Enterobacteriaceae family pathogens, it may be a useful option to treat the infections caused by these organisms.

INTRODUCTION

Gram-negative bacteria particularly Escherichia coli, Klebsiella pneumoniae of Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter spp of non-Enterobacteriaceae are thought to be of particular concern as they are considered to be responsible for life threatening infections. These are predominant pathogens in hospital setting, accounting for 70% of all Gram negative pathogens causing health care associated infections (Sievert et al., 2013).

According to a study conducted in 1265 intensive care units in 75 countries, the prevalence of Gram negative bacteria was in 62% of patients (Vincent et al., 2009). Gram negative organisms cause a variety of infections in humans (Boucher et al., 2009; Rice, 2008). They frequently cause infections like intra-abdominal infections (IAIs), urinary tract infections (UTIs), meningitis, otitis media, nosocomial pneumonia and bacteremia (Sievert et al., 2013). Development and use of antibiotics to treat such infections is one of the greatest advances in modern medicine (Kohler et al., 1999). Among various classes of antibiotics, β-lactam antibiotics are widely used to treat bacterial infections accounting for over 50% of all antibiotics in use (Jalalpour and Ebadi, 2012). However, Gram-negative organisms are very adaptive and develop resistance to antibiotics by many mechanisms such as, production of β-lactamases and metallo-β-lactamases, modifications of cell wall and target sites and reduced accumulation of β-lactam antibiotics inside the bacterial cells (Kumar et al., 2006; Sarojamma and Ramakrishna, 2011). These resistance mechanisms against antibiotics have caused more than 50% increase in MDR species (Rosenthal et al., 2009).

The resistance to the antimicrobials has been increasing over the years and varies from country to country. In past few years, antibiotic resistance in Enterobacteriaceae (E. coli and K. pneumoniae) and non-Enterobacteriaceae (Acinetobacter spp and P. aeruginosa) has been recognized increasingly, which led to increased morbidity and mortality in India as well as other parts of world (Ko et al., 2006; Wani et al., 2009; Rahbar et al.,...
This increased resistance has limited the choice of antibiotics. However, the choice of antibiotics for the treatment of infections caused by these organisms is usually based on the knowledge of antibiotic susceptibility profile. Monitoring of antibiotic susceptibility pattern in hospitals at periodic intervals would be the best way to make awareness among the clinicians about the susceptibility of drugs in a particular hospital to treat the patients, to provide safe and effective empirical treatment.

In the light of above background, present study attempted to find resistance patterns to antibacterial agents among isolates obtained from different clinical specimens at Gandhi Medical College Hospital, which is a leading public hospital in Hyderabad, India with capacity of 1200 beds and a daily outpatient turn over of more than 1000 patients.

MATERIALS AND METHODS

Clinical isolates collection

This prospective study was undertaken in the Department of Microbiology of Gandhi Medical College Hospital, Hyderabad, India. The clinical isolates (n=299) of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter spp.* phenotypically show resistance to the 3rd generation cephalosporins in routine disk diffusion method were subjected for ESBL and carbapenemase production. These organisms were isolated from various clinical specimens like urine (200), pus (65), blood (20), sputum (8), tracheal secretion (2), endotracheal tube (2), vaginal swab (1) and peritoneal fluid (1), during the period of March 2013 to November 2013, collected from various wards and intensive care unit (ICU) (Table 1). All the samples were processed and identified as per standard laboratory bacteriological methods.

ESBL and carbapenemase production detection

ESBL production among the clinical isolates was confirmed according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2012). Briefly, lawn culture of the organism was made and discs of 3rd generation cephalosporins (ceftazidime and cefotaxime) alone and in combination with clavulanic acid were placed with 25mm apart. A ≥ 5 mm increase in a zone diameter for either antimicrobial agents tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organisms.

Carbapenemase production was detected by Modified Hodge test, where MH agar plate was inoculated with *E. coli* ATCC 25922. Meropenem disk was placed at the center, test strains were streaked from the edge of the disk to the edge of the plate. Clover leaf like zone of inhibition was indicative of carbapenemase production. Quality control was maintained by using *E.coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC BAA 1706 and *Klebsiella pneumoniae* ATCC BAA 1705 strains.

Antibiotic susceptibility testing

All the isolates were further subjected to antimicrobial susceptibility testing using discs of Elores (ceftiraxone+subbactam with adjuvant disodium edetate (CSE); 45µg), cefoperazone+subbactam (CFS: 45µg), piperacillin+tazobactam (PT, 110 µg) and meropenem (MR, 10 µg). All the discs were obtained from Hi Media, Mumbai, India.

RESULTS

Clinical isolates collection

A summary of clinical isolates which were recovered from different specimens is depicted in Table 1. Of the 299 isolates, 184 (61.5%) were *E. coli* and 97 (32.4%) were *K. pneumoniae* belonging to Enterobacteriaceae family and 18 (6.0%) isolates were of non-Enterobacteriaceae, 9 (3.0%) each of *P. aeruginosa* and *Acinetobacter spp.*. The highest number of *E. coli* isolates were recovered from urine 148 followed by pus 28, blood 5, and one each from sputum, ET and vaginal swab. The maximum number of *K. pneumoniae* isolates were detected in urine 43, pus 31, blood 14 and one each from sputum, tracheal secretion and peritoneal fluid. *P. aeruginosa* was mainly detected in urine (n=6) and one each from pus, tracheal secretion and ET tip. *Acinetobacter spp.* was predominantly identified in pus 5 followed by urine 3 and blood 1 (Table 1).

Table 1 Distribution of the isolates from various clinical samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>E. coli</th>
<th>Klebsiella pneumoniae</th>
<th>Pseudomonas aeruginosa</th>
<th>Acinetobacter spp.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>148</td>
<td>43</td>
<td>6</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>Pus</td>
<td>28</td>
<td>31</td>
<td>01</td>
<td>5</td>
<td>65</td>
</tr>
<tr>
<td>Blood</td>
<td>05</td>
<td>14</td>
<td>0</td>
<td>01</td>
<td>20</td>
</tr>
<tr>
<td>Sputum</td>
<td>01</td>
<td>07</td>
<td>0</td>
<td>00</td>
<td>08</td>
</tr>
<tr>
<td>Tracheal secretion</td>
<td>00</td>
<td>01</td>
<td>01</td>
<td>0</td>
<td>02</td>
</tr>
<tr>
<td>Endotracheal tip</td>
<td>01</td>
<td>0</td>
<td>01</td>
<td>0</td>
<td>02</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>01</td>
<td>0</td>
<td>00</td>
<td>0</td>
<td>01</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>0</td>
<td>01</td>
<td>0</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>184</td>
<td>97</td>
<td>9</td>
<td>9</td>
<td>299</td>
</tr>
</tbody>
</table>

Table 2 Carbapenemase and ESBL detection among the isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Carbapenemase positive</th>
<th>ESBL positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>12</td>
<td>172</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><em>Acinetobacter spp.</em></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>250</td>
</tr>
</tbody>
</table>

ESBL and carbapenemase detection

Out of 299 isolates, 250 isolates (83.6%) were ESBL positive and 49 (16.4%) were carbapenemase positive. The detailed distribution of ESBL and carbapenemase among different isolates are depicted in Table 2.
Antimicrobial susceptibility

Antimicrobial susceptibility data of 250 ESBL isolates against CSE, CFS, PT, MR is presented in (Table 3). For E. coli 88.4% of isolates exhibited susceptibility to Elopres whereas 7.5% and 4.0% of isolates demonstrated intermediate and resistant response. Conversely, 46.5%, 45% and 54.6% isolates were susceptible to cefoperazone+sulbactam, piperacillin+tazobactam and meropenem respectively. Similarly, 37.8%, 21.0% and 23.8% isolates showed intermediate and 16.3%, 33.7% and 21.5% of E. coli isolates showed resistant to cefoperazone+ sulbactam, piperacillin+tazobactam and meropenem. Of K. pneumoniae, 78% isolates were susceptible to Elores and 50.6% to meropenem and <30% isolates were susceptible to cefoperazone+sulbactam, piperacillin+tazobactam and intermediate responses to Elopres, cefoperazone+sulbactam, piperacillin+tazobactam and meropenem were 2.7, 26.0 and 19.2 %, respectively. Approximately, 19.2% resistance was observed with Elores followed by meropenem (30.1 %), cefoperazone+sulbactam (34.2 %) and piperacillin+tazobactam (43.8 %).

Against isolates of P. aeruginosa, Elores was found to be active with 100% susceptibility followed by meropenem (50%). No isolates of P. aeruginosa were susceptible to CFS and PT. Among Acinetobacter spp., Elores was found to be more active antibacterial agent with 66.6% of isolates were susceptible to Elores and 33.3% of isolates exhibited resistant response. The susceptibility to meropenem was 33.3%. About 66.6% of the isolates were resistant to meropenem. Not any isolates of A. baumannii were susceptible to CFS and PT. High resistance was observed with piperacillin + tazobactam (100%) and CFS (100%). Elores was found to be effective on Carbapenemase producing isolates with sensitivity ranging from 50% to 58.3% of sensitivity for E.coli, K. pneumoniae and P. aeruginosa while none of the isolates of carbapenemase positive were susceptible to meropenem, cefoperazone+sulbactam and piperacillin+tazobactam (Table 4).

**DISCUSSION**

This study reports on rates of antimicrobial resistance among Gram negative organisms collected from a government tertiary care hospital in Hyderabad, India between March 2013 to November, 2013. This study demonstrated change in the patterns of antibiotic resistance. The antimicrobial resistance against antibiotics varies according to geographical areas and depends upon various factors such as abuse, availability and consumption of antibiotics (Mirigou et al., 2010). In general, high resistance is observed against widely used antibiotics. The worrying increase in resistance to these selected antibiotics among gram negative bacteria (GNB) may be associated with the presence of resistance determinants. The most important resistance determinants might be ESBL and MBL (Metallo beta lactamases/ carbapenemases). It has already been reported that CSE (ceftriaxone+sulbactam with adjuvant disodium edetate) is highly susceptible against the ESBL and MBL producing Enterobacteriaceae (Chaudhary and Payasi, 2012; Chaudhary and Payasi, 2013). In this study, 88.4% of E. coli isolates were susceptible to Elores which is in agreement with previous study where susceptibility to Elores found to be 92.6% (Chaudhary and Payasi, 2012). On the other hand, in this study susceptibilities to Elopres against K. pneumoniae, P. aeruginosa and Acinetobacter spp. were 78%, 100% and 66.6% respectively, which is in contrast to other studies conducted in India, which reported considerably higher susceptibility of Elopres to these isolates (Chaudhary and Payasi, 2013). The difference in susceptibility of Elores may be due to the selection of bacterial population. As with other countries, less susceptibility of piperacillin + tazobactam in Enterobacteriaceae (E. coli and K. pneumoniae) and non-Enterobacteriaceae (P. aeruginosa and Acinetobacter spp.) has been reported in India (Kumar et al., 2006; Jaggi et al., 2012; Gupta et al., 2006). In the current study, 33.7% of E. coli, 43.8% of K. pneumoniae, 100% of P. aeruginosa and 66.6% of Acinetobacter spp. isolates were resistant to piperacillin + tazobactam. The rates of Acinetobacter spp. resistance to piperacillin+ tazobactam were higher than those of reported earlier and resistance of E. coli towards piperacillin+...
tazobactam was comparable with previous study (Chaudhary and Payasi, 2012). On the whole CSE showed good activity on all ESBL producing isolates when compared to CFS, PT, MR.

Several studies have also reported meropenem resistance in Enterobacteriaceae and non-Enterobacteriaceae isolated from India and abroad (Gupta et al., 2006; Grundmann et al., 2010). In India, resistance to meropenem varies from 37 to 42 % in Pseudomonas spp. (Chaudhary and Payasi, 2013; Gupta et al., 2006). Whereas in the current study the resistance to meropenem was 66.6%. The resistance to meropenem in Acinetobacter spp was 66.6% in our study which was observed to be less than that has been reported by Karthika et al. which reported 89% of A. baumannii isolates resistant to meropenem (Karthika et al., 2009). Furthermore, our data shows 21.5% of E. coli and 33.1% of K. pneumoniae isolates were resistant to meropenem. The resistance to meropenem in these isolates probably results from reduced accumulation of drug or efflux mechanisms (Sinha and Srinivasa, 2007). The resistance may also be due to the production of metallo betalactamases (MBL) (Chaudhary and Payasi, 2013). In this study production of metallo betalactamases was detected by modified hodge test, where 49 isolates were found positive. All these isolates showed high resistance (26-100%) to CFS, PT and MR, where as resistance to Elores ranged from 4 % to 19.2% only.

Our results in the carbapenemase positive strains showed 50-58% sensitivity to CSE when compared to other three drugs indicating the usefulness of ceftriaxone+sublactam with adjuvant disodium edetate combination in treating these Gram-negative pathogens thereby suggesting it as a suitable alternative for treating patients with Gram-negative infections. Several other studies from India also confirm the high sensitivity of Elores (Chaudhary and Payasi, 2012; Chaudhary and Payasi, 2013).

**CONCLUSION**

This study provides important data which could be helpful to clinicians to choose the appropriate treatment regimen. As Elores showed high level of sensitivity against GNBs in comparison to cefoperazone+sublactam, piperacillin+tazobactam and meropenem, it may be an useful option to treat the infections caused by ESBL and MBL producing organisms.

**References**


How to cite this article:

*******