Comparative Assessment of Antibiotic Susceptibility Pattern of Gram Negative Pathogens Isolated from Intensive Care Unit Patients in Pune

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Authors’ contributions

This work was carried out in collaboration between both the authors. Author NM designed the study and wrote the protocol. Author SA performed the experiments, wrote the first draft of the manuscript and managed the literature searches. Both authors managed the analysis of the study, read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/18199

Editor(s):
(1) Preeti Bharaj, Department of Microbiology and Immunology, University of Texas Medical Branch (UTMB), Galveston USA.

Reviewers:
(1) Gonsu Kamga Hortense, University of Yaounde 1, Cameroon.
(2) Enty Tjoa, Catholic Atma Jaya University, Indonesia.

Complete Peer review History: http://sciencedomain.org/review-history/10593

Received 8th April 2015
Accepted 1st July 2015
Published 18th August 2015

ABSTRACT

Introduction and Aim: Extended spectrum β-lactamases (ESBLs) and Metallo-β-lactamases (MBLs) production is one of the main means of the resistance developed by Gram negative bacteria against β-lactam antibiotics. The present study was carried out to evaluate the incidences of ESBL and MBL producers in gram negative bacteria isolated from Ruby Hall Clinic, Pune, Maharashtra, India and to evaluate the efficacy of drugs against these bacteria.

Methodology: 254 different samples collected from various sources were screened for the presence of bacterial pathogens. The pathogens were identified using selective media technique. The ESBL and MBL producer's screening and the antimicrobial susceptibility testing (AST) of pathogens towards a new drug; Elores (ceftriaxone + sulbactam with adjuvant ethylenediaminetetraacetic acid, EDTA) in comparison with commonly used antibiotics like meropenem, imipenem, piperacillin-tazobactam and cefoperazone-sulbactam was carried out according to CLSI guidelines.

Results: Among 254 samples collected, 200 samples showed the presence of bacterial infections

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with Klebsiella sp. (39%) as the most predominant pathogens followed by, E. coli (32%) and Pseudomonas sp. (16.5%), Acinetobacter sp. (12.5%). Of the identified pathogens, 61% (122/200) were found to be ESBL producers and 4.5% (9/200) were MBL producers. Nearly, 3.5% (7/200) pathogens were both ESBL and MBL producers. However another significant number (66 isolates) of pathogens were identified as non-ESBL/ non-MBL producers. Further, our data showed that, Elores was highly susceptible (87 to 100%) followed by imipenem-cilastatin (30 to 67%), meropenem (33 to 68%), cefoperazone-sulbactam (24 to 70%) and piperacillin-tazobactam (4 to 81%) against Gram negative bacteria.

**Conclusion:** The results of the present study concludes, that Elores is an useful option to treat the infections caused by carbapenemase producing multi-drug resistance Gram negative bacteria.

**Keywords:** Elores; gram negative bacteria; ESBL; MBL; antimicrobial resistance.

### 1. INTRODUCTION

Nosocomial infections caused by multi drug resistant bacteria are the leading causes of morbidity and death among the hospitalized patients and poses a major burden on the patients and public health system of any country [1]. Among all infections, infections caused by Gram negative bacteria are responsible for a large portion of device associated infections [2]. Gram-negative bacteria are responsible for more than 30% of hospital-acquired infections and more than 40% of infections in patients in intensive care units [3,4].

Third generation β-lactam antibiotics, carbapenems like imipenem, meropenem and combinations of β-lactam antibiotics with β-lactamase inhibitors have been widely used in the treatment of Gram negative bacterial infections [5]. However, Gram-negative organisms particularly, Klebsiella sp., Enterobacter sp., Pseudomonas aeruginosa and Acinetobacter sp. are showing rising rates of resistance to these therapies [6-8].

Production of β-lactamas (ESBLs and MBLs), is the main mechanism adapted by pathogens to counter the β-lactam antibiotics [9,10]. ESBLs are the enzymes produced by Gram-negative bacteria that have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group [11]. ESBLs are usually plasmid-mediated β-lactamases and since 1983 the number of ESBL variants has been constantly growing; at present more than 300 different ESBL variants are known [12].

Metallo-β-lactamases (MBLs) are other class of broad substrate spectrum enzymes which can also hydrolyze most of β-lactam antibiotics except monobactams. MBLs are resistant to inhibition by β-lactam inhibitors and can be inactivated by agents like EDTA [13,14]. IMP and VIM types are the most commonly distributed MBLs in Gram-negative pathogens like Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii [15,16]. Along with these, newly discovered NDM-1 β-lactamase provides an example of the potential for dissemination of MBLs.

Different research groups from India have reported the prevalence of ESBL producers to be between 28% to 84% [17-19]. Because of increasing incidence of ESBLs, a high resistance rate was seen in Gram negative organisms [20,21].

In India prevalence of MBLs range from 7 – 65% [22], with a recent study reporting 34% occurrence [23]. Interestingly, a study by Hu et al. [24] demonstrated the least susceptible of Enterobacteriaceae family to imipenem and meropenem, with only 6.5 and 1.3 %, respectively. In various studies, across the world varying resistance (4-60%) has been seen towards these drugs [25].

The above stated points strongly advocate the alarming situation of the rising resistance towards the routinely used antibiotics and demand the need of new antibiotic agents to overcome the situation. The continuous surveillance of antibiotic resistance trends in bacteria isolated from hospital-acquired infections is essential for the selection of adequate initial empiric therapy [26,27]. The laboratory-based antibiogram is efficacious as a guide for the rational selection of antimicrobial therapy, and to alert health-care providers to the presence of unusual or emerging antimicrobial resistance mechanisms. Thus the present study was aimed to monitor the prevalence trend of various pathogens and type of resistance along
with antibiotic susceptibility profile of the new antibiotic entity Elores in comparison with imipenem, meropenem, piperacillin-tazobactam and cefoperazone-sulbactam.

2. MATERIALS AND METHODS

2.1 Sample Collection

Different clinical samples such as blood, pus, sputum, urine, swab, central line, endotracheal tube (ETT) section, broncho-alveolar lavage (BAL), bed sore swab, catheter, Foley catheter (FC) tip, nasal swab, pleural cavity (PC)/pleural fluid (PF), stool, wound and wound swabs were collected from 254 (Two hundred and fifty four) patients suspected of bacterial infection at Ruby Hall clinic Pune, India during the period of January 2014 to June 2014. The collection and processing of the samples were done as per Clinical and Laboratory Standards Institute (CLSI) guidelines using a common standard operating procedure (SOP) by all laboratories.

2.2 Isolation and Identification of Pathogens

All the samples were collected aseptically in sterile containers. Urine samples collected in sterile universal container were directly inoculated to the respective selective media. Other liquid specimens such as pus, sputum, and broncho-alveolar fluids collected in sufficient amount were inoculated on the different selective and non-selective culture media as per the standard microbiological techniques. The study was aimed to assess the susceptibility pattern of only four common pathogens (E. coli, Klebsiella sp. Pseudomonas sp. and Acinetobacter sp.) responsible for various Gram negative infections and were isolated using different selective media. Details of the culture media used for the isolation of these pathogens from various clinical samples are given in Table 1. Blood samples collected in brain heart infusion (BHI) broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37°C and then subcultured on to 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 characteristics according to Bergey’s Manual of Determinative Bacteriology [28].

2.3 Screening of Isolates for ESBL and MBL Production

Screening of isolates for ESBL production was performed as per CLSI guidelines [29]. Isolates exhibiting zone size ≤25 mm with ceftriaxone (30 μg), ≤22 mm for ceftazidime (30 μg) and ≤27 mm with cefotaxime (30 μg) were considered as possible ESBL producer. ESBL production was confirmed by disk potentiation test using ceftazidime (30 μg) and cefotaxime (30 μg) antibiotic disks with and without clavulanic acid (10 μg) and by double disc susceptibility test (DDST) (CLSI 2013). Similarly, phenotypic detection of MBL among clinical isolates was carried out using imipenem (10 μg) and imipenem (10 μg) + EDTA (750 μg) discs as described by [30].

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

| Pathogen              | Selective media                          |
|-----------------------|------------------------------------------|
| E. coli               | Eosine Methylene Blue (EMB) agar medium   |
| Klebsiella sp.        | MacConkey's agar medium                  |
| Pseudomonas sp.       | Citrimide agar                           |
| Acinetobacter sp.     | Leeds Acinetobacter agar base medium     |

2.4 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the CLSI guidelines [29]. Meropenem disk (10 μg), imipenem disk (10 μg), piperacillin-tazobactam (100 μg/10 μg), cefoperazone-sulbactam (75 μg/30 μg) and ceftriaxone+sulbactam+EDTA disk (45 μg) were procured from Himedia (Mumbai, India) and used in the study. Inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller-Hinton broth (MHB) from isolated colony of pathogens selected from 18–24 hour 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 (MHA) plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60° over the agar surface. After 15 minutes, antibiotic discs were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from
center to center. Within 15 minutes of the disc application, the plates were incubated in inverted position for 16-18 hrs aerobically at 37°C. Sensitivity of isolated organisms against antibiotics were reported as sensitive (S) or resistant (R) based on the breakpoints.

3. RESULTS AND DISCUSSION

Different clinical samples (n = 254) were collected from ICU of Ruby Hall Clinic Pune, India and were processed for the isolation of pathogenic bacteria. Bacteria were isolated according to Bergey’s manual from 10 different types of samples wiz; urine, ETT section, pus, swab, sputum, stool, blood, catheter, central line and peritoneal fluid. 200 samples out of 254 samples tested showed the presence of the bacterial infections, where as the remaining 54 samples did not show growth of any pathogens (Table 2). Among the 200 culture positive samples, 88 (44%) samples were of urine, 39 (19.5%) samples were of ETT section followed by pus, swab and sputum samples which contributed 21 (10.52%), 15 (7.5%) and 10 (5%) respectively. Stool and blood samples contributed 8 (4%) and 7 (3.5%) respectively, while samples from catheter, central line and peritoneal fluid had an equal contribution of 4 (2 %) each (Table 2).

Colonie morphology and biochemical characterization of the bacteria isolated from the culture positive samples demonstrated the presence of four types of Gram negative pathogens. The identified bacteria include *Klebsiella sp.*, *E. coli*, *Pseudomonas sp.* and *Acinetobacter sp.* However the Gram positive pathogens detected in the samples were not included in the study. Among the isolated pathogens, *Klebsiella sp.* (39%) was found to be the most predominant pathogens followed by, *E. coli* (32%) and *Pseudomonas sp.* (16.5%). However *Acinetobacter sp.* (12.5%) was the least contributing bacteria among all the samples (Fig. 1). *Klebsiella sp.* (43.18%) and *E. coli* (43.18%) were the predominant pathogens responsible for urinary tract infections followed by *Pseudomonas sp.* (13.63%). Along with urine samples, *Klebsiella sp.* was also a predominant pathogens in ETT section (35. 89%), swab (46.66%) and blood (42.85%) samples (Table 3).

The isolated pathogens were subjected to screen for ESBL and MBL productions. Among the isolates, 61% (122) pathogens were found to be ESBL producers. Similar prevalence of ESBL producers (51.78%) were reported earlier [31]. However lower ESBL producer prevalence rates were reported in earlier studies by Taneja et al. [32]. Hawser et al. [33] reported the increasing trends of ESBL producers in the Asia-Pacific region over a period from 2003 (15%) to 40% in 2007. This rising trend of ESBL producers, especially in Asian sub-continent is a strong indicative representation of the increased resistance towards the routinely used antibiotics. The overall prevalence of MBL producers was 4.5% (9) which is significantly low as compared to ESBL producers. Even small population of 3.5 % (7) clinical isolates produced both ESBL and MBL. However a significant portion (66 isolates) of pathogens were identified as non-ESBL/ non-MBL producers (Fig. 2). The clinical isolates producing maximum number of ESBLs belonged to *Klebsiella sp.* (66) followed by *E. coli* (43) and a small portion of ESBL producers also belonged to *Acinetobacter sp.* (8) and *Pseudomonas sp.* (5). 4 isolates each of *Acinetobacter sp.* and *Pseudomonas sp.* produced MBL and 5 *Klebsiella sp.* bacteria produced both ESBL as well as MBL (Fig. 2).

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

| S. no. | Clinical samples | Total | Number of samples showing growth of pathogens | Number of samples not showing growth of pathogens |
|--------|------------------|-------|---------------------------------------------|-----------------------------------------------|
| 1      | Urine            | 103   | 88                                          | 15                                            |
| 2      | ETT section      | 54    | 39                                          | 15                                            |
| 3      | Pus              | 23    | 21                                          | 2                                             |
| 4      | Swab             | 18    | 15                                          | 3                                             |
| 5      | Sputum           | 16    | 10                                          | 6                                             |
| 6      | Stool            | 11    | 8                                           | 3                                             |
| 7      | Blood            | 10    | 7                                           | 3                                             |
| 8      | Catheter         | 7     | 4                                           | 3                                             |
| 9      | Central Line     | 6     | 4                                           | 2                                             |
| 10     | Peritoneal fluid | 6     | 4                                           | 2                                             |
| Total  |                  | 254   | 200                                         | 54                                            |
Fig. 1. Prevalence of various pathogens

Fig. 2. Prevalence of ESBL and MBL production among different pathogens

Antibiogram profile for all the pathogens isolated from various clinical samples is presented in Figs. 3 and 4. The most predominant pathogen showed highest (73.07%) susceptibility towards Elores followed by cefoperazone-sulbactam (51.28%), imipenem (44.87%), meropenem (33.33%) and showed least (23.07%) sensitivity towards piperacillin-tazobactam. Elores was also effective towards *E. coli* demonstrating superior (81.25%) susceptibility as compared to piperacillin-tazobactam, cefoperazone-sulbactam, imipenem and meropenem to which *E. coli* showed 71.87%, 70.31%, 67.18% and 35.93% susceptibilities respectively (Fig. 3). Similar results were also reported from a recent study carried out by Sahu et al. [34], where they have demonstrated higher susceptibilities of *Klebsiella* sp., *E. coli*, *Acinetobacter* sp. and *Pseudomonas* sp. towards Elores. According to a previous study conducted in India for the treatment of skin and skin structure infection (SSSIs) and bone and joints infections (BJIs) more than 80% of the studied patient were clinically cured with ceftriaxone+sulbactam+EDTA(Elores) [35]. The most predominant pathogen *Klebsiella* sp. showed highest resistance towards piperacillin-tazobactam (76.93%) followed by meropenem (66.67%), imipenem (55.13%), cefoperazone-sulbactam (48.72%) and the least resistance was observed towards Elores (26.93%).
However, *E. coli* showed highest resistance towards meropenem (64.07%) followed by imipenem (32.80%), cefoperazone sulbactam (29.69%), piperacillin-tazobactam (28.13%) and the least resistance towards Elores (18.75%). Similar trends of least resistance towards Elores was also observed in *Acinetobacter sp.* and *Pseudomonas sp.* pathogens (Fig. 4). Earlier Parveen et al. [36] studied carbapenem susceptibilities among nosocomial *Klebsiella sp.* isolated from south India and reported meropenem resistance in about 43.6% isolates. Our results for the piperacillin-tazobactam resistance testing were comparable to the study reported by Mohanty et al. [37], where they have demonstrated nearly similar resistance rates for piperacillin-tazobactam towards *E. coli* (28.13 %) and *Pseudomonas sp.* (18.19 %). However, contradictory to our results they reported very low (12.5 %) resistance of *Klebsiella sp.* towards piperacillin-tazobactam. These overall comparative data sheds light on the increasing trend of the antibiotic resistance over the years and advocate the requirement of new novel therapeutic options and Elores has got the potential to be that new therapeutic options.

Table 3. Prevalence of individual pathogens in different samples

| S. no. | Samples            | Total samples | Clinical isolates |  |
|-------|-------------------|---------------|-------------------|---|
|       |                   | Clinical isolates | Clinical isolates |  |
|       |                   | *Klebsiella sp.* | *Pseudomonas sp.* | *E. coli | *Acinetobacter sp.* |
| 1     | Urine             | 88            | 38 (43.18)        | 12 (13.63) | 38 (43.18) | 0 (0) |
| 2     | ETT Sec           | 39            | 14 (35.89)        | 10 (25.64) | 2 (5.12)  | 13 (33.33) |
| 3     | Pus               | 21            | 6 (28.57)         | 3 (14.28)  | 6 (28.57) | 6 (28.57) |
| 4     | Swab              | 15            | 7 (46.66)         | 4 (26.66)  | 2 (13.33) | 2 (13.33) |
| 5     | Sputum            | 10            | 6 (60)            | 2 (20)     | 2 (20)    | 0 (0)    |
| 6     | Stool             | 8             | 0 (0)             | 0 (0)      | 8 (100)   | 0 (0)    |
| 7     | Blood             | 7             | 3 (42.85)         | 2 (28.57)  | 2 (28.57) | 0 (0)    |
| 8     | Catheter          | 4             | 2 (50)            | 0 (0)      | 2 (50)    | 0 (0)    |
| 9     | Central           | 4             | 0 (0)             | 0 (0)      | 0 (0)     | 4 (100)  |
| 10    | peritoneal fluid  | 4             | 2 (50)            | 0 (0)      | 2 (50)    | 0 (0)    |
|       | Total             | 200           | 78                | 33         | 64        | 25       |

*Note: The values in the parenthesis indicate the percent incidences of individual pathogen in the sample group*

![Fig. 3. Susceptibility pattern of Gram negative pathogens isolated](image-url)
4. CONCLUSION

Study resulted in generation of representative status of the prevalence resistance pattern of different pathogens from West zone part of India and is found to be alarmingly high for imipenem, piperacillin-tazobactam, cefoperazone-sulbactam and meropenem. Infections with Acinetobacter sp. are one of the major concerns to treat because of their intrinsic and acquired resistance which is easily broken by Elores (evidenced by 100% susceptibility results). Elores displayed significant susceptibility against these pathogens. With the support of the previous reports, present study advocates the superiority of Elores over other routinely used antibiotics to treat Gram negative pathogens. Further this study also will be of great usefulness for the clinicians in general and of the region in particular to help make them chose correct antibiotic and ensure the judicious use of the same for their patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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