ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 4, Issue 9, pp 347-351, September 2015

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

RETROSPECTIVE ANALYSIS OF ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE PATTERNS AGAINST NOSOCOMIAL GRAM NEGATIVE PATHOGENS IN FORTIS MEMORIAL RESEARCH INSTITUTE GURGAON

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ARTICLE INFO

Article History:

Received 23th, August, 2015
Received in revised form 31th, August, 2015
Accepted 20th, September, 2015
Published online 28th, September, 2015

Key words:

Elores, nosocomial infections, ESBL, MBL, antibiotic resistance

ABSTRACT

Antibiotic resistance is an alarming problem globally, especially in developing nations like India. This study was aimed to study the susceptibility pattern of nosocomial gram negative microbes towards meropenem, piperacillin+tazobactam, amikacin and ceftriaxone+sulbactam+EDTA (Elores) in Fortis Hospital, Gurgaon, India. A total of 129 clinical isolates from various clinical specimens were collecte. All the samples were processed under strict quality control measures and identified as per standard microbiological methods. Susceptibility study was done by the disc diffusion method according to the procedure of Clinical Laboratory Standard Institute guidelines. Among 129 samples tested, 85 samples showed the presence of infection and 44 were sterile. Among the isolates, *E. coli* (43.52%) was found to be the most dominant pathogen followed by *K. pneumoniae* (20%), *A. baumannii* (9.41%), *P. aeruginosa* (9.41%). However, other Gram negative bacteria accounted for a cumulative share of 17.64%. Among the tested antibiotics, Elores was the most effective against all the tested pathogens with 87 to 100 % susceptibility. Results of the meropenem were comparable to *Elores against P. aeruginosa* (100% susceptibility), and other gram negative bacteria (93.35), except *K.pnemoniae, E.coli and A. baumannii.* The susceptibilities of meropenem against *A. baumannii*, *E. coli* and *K. pneumoniae* were 62.5, 37.8 and 35.3%. respectively. The susceptibilities of piperacillin+tazobactam and amikacin varied 29 to 64% and 47 to 83 %, respectively. Susceptibility to pathogens isolated from blood, sputum, urine, endotracheal secretion and broncho alveolar lavage showed poor response to all drugs studied except Elores. On the basis of our results we conclude that Elores is more effective than other tested antibiotics routinely used to treat gram negative bacterial infections.

INTRODUCTION

Gram-negative bacterial infections (GNBI) pose the greatest threat in terms of nosocomial or Hospital transmitted contagions diseases owing to higher incidences of about 5-10% in most developed nations and in developing nations like India. One in four patients admitted, acquire surgical site infections (SSIs), urinary tract infections (UTIs), pneumonia and blood stream infections (BSIs) (Saranya, 2009). According to the National Nosocomial Infection Surveillance System, each year, 65-80% of the cases were associated with Gram negative pathogens like *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii and with and Pseudomonas aeruginosa* (Gaynes *et al.*, 2005).

The most common therapies to treat GNBI are β- lactam antibiotics which include penicillins like amoxicillin, extended-spectrum cephalosporins such as ceftriaxone and cefepime, monobactams like aztreonam and carbapenems like imipenem, meropenem, ertapenem, and doripenem (Gilbert *et al.*, 2009). A study conducted by GARP (2011)- India Working Group has reported the increased and irrational antibiotic usage in India corroborating the fact that the usage of cephalosporins was significantly high (60%)

contemplating in the development and spread of resistant bacteria which were earlier vulnerable.

The most widespread cause of resistance to β- lactam antibiotics is the production of β- lactamase enzyme, which have the ability to hydrolyze the β-lactam chemical bond that distinguishes β-lactam antibiotics from other antibacterial agents, thereby rendering the molecules incapable of killing bacteria (Bush and Jacoby, 2010). Besides production of various β-lactamases, other mechanisms such as drug efflux systems, antibiotic-modifying enzymes, outer-membrane protein changes, and antibiotic-target modification also contribute to the resistance (Livermore, 2003; Chopra *et al.*, 2008).

Carbapenems have served as an important antimicrobial class for the treatment of ESBL producing pathogens. However, the emergence of novel β-lactamases with direct carbapenem hydrolyzing activity has contributed to an increased prevalence of carbapenem resistant *Enterobacteriaceae* (CRE). CRE are particularly problematic given the high mortality associated with infections caused by CRE (Patel *et*

al., 2008). Globally, resistance to meropenem varies from 10 to 66.7 % in *Pseudomonas* spp., *A. baumannii* and Enterobacteriaceae (Chaudhary and Payasi, 2013; Gupta *et al*., 2006; Karthika *et al*., 2009; Grundmann *et al*., 2010). Recently, Hu *et al.,* (2012) demonstrated the least susceptible of *Enterobacteriaceae* family to imipenem and meropenem, with only 6.5 and 1.3 %, respectively.

As it is a well known fact that antibiotic resistance may vary by region or by hospitals and the selection of antibiotics for the treatment of infections is based on the knowledge of antibiotic susceptibility profiles against the organisms. It is, therefore a clinician's prerogative to effectively analyze the antimicrobial susceptibility patterns in the geographic region/hospital for therapeutic regimen, however such type of studies are subjected to the Hospital Policies and may vary in the Indian spectrum.

To tackle this alarming situation pertaining to the antibiotic resistance due to improper prescription of antibiotics, there is a desperate need of conducting surveillance study in hospitals. Thus in view of these aspects, the present work was aimed to study the susceptibility pattern of nosocomial gram negative microbes towards meropenem, piperacillin+tazobactam, amikacin and ceftriaxone+sulbactam+EDTA (Elores) in Fortis Memorial Research Institute, Gurgaon, India. This study will bring into focus the efficiency of drugs which are routinely used to treat the infections caused by MDR strains in Fortis hospitals.

MATERIALS AND METHODS

Sample Collection

Different clinical samples such as blood, pus, sputum, urine, endotracheal secretion , swab, semen, tissue, abdominal fluid, brancho alveolar fluid, bile, stool and pleural fluid were collected from 129 (one hundred twenty nine) patients suspected of bacterial infection at Fortis Hospital, Gurgaon, India from November 2013 to June 2014. Figure 1 shows the percentile number of respective samples among the total samples showing growth of pathogens.

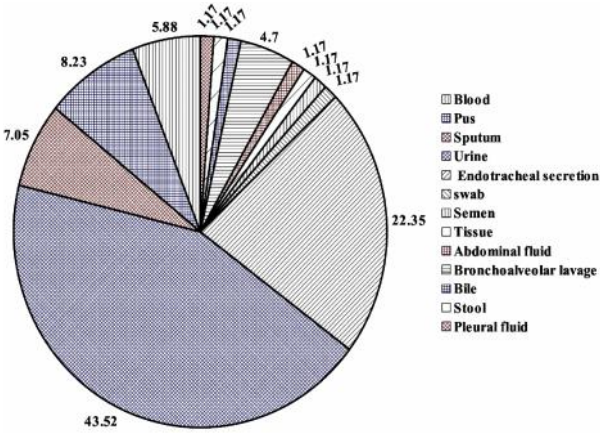

Figure 1 Percentile number of respective samples among the total samples showing growth of pathogens

Isolation and Identification of Microbes

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, 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 (BHI) broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37°C and then sub-cultured on to the selective and non-selective media. All the media were incubated aerobically overnight at 37°C and the pathogens were identified by standard laboratory procedures including Gram's staining, motility, colony characters and biochemical reactions (Collee *et al.,* 1996).

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

Pathogens	Selective media
E. coli	Eosine Methylene Blue (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 and S. fonticola	Caprylate- Thallous agat (CT agar)
E. cloacae	Hicrome coliform agar modified medium
Salmonella paratyphi A	Bismuth Sulfite agar
Shigell boydii	Xylose lysine deoxycholate agar (XLD)

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) (2013). Meropenem disk (10 µg), piperacillin+tazobactam (110 µg), amikacin (30 µg) and ceftriaxone+sulbactam+EDTA (Elores) disk (55 µg) were procured from Himedia (Mumbai, India) and used in the study. Inoculum of 0.5 McFarland standards turbidity was prepared in a nutrient broth 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° C over the agar surface. After 3-5 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. The plates are then inverted and incubated for 16-18 hrs aerobically at 37° C within 15 minutes of disc application. Sensitivity of isolated organisms against antibiotics were reported as sensitive (S) or resistant (R) based on the breakpoints.

RESULTS AND DISCUSSION

Out of 129 clinical samples received from Fortis Hospital

Gurgaon during the study period, there were 7 blood samples, 12 pus samples, 10 sputum samples, 48 urine samples, 28 endotracheal secretion samples, 8 broncho alveolar lavage samples, 3 samples each from semen and abdominal fluid, 2 samples were from tissue and a single sample was collected from swab. Among 129 samples analyzed, only 85 samples showed the presence of infections, while 44 samples did not show the presence of growth and were considered as sterile (Table 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	Blood	7	5 (5.88)	2
2	Pus	12	7 (8.23)	5
3	Sputum	10	6 (7.05)	4
4	Urine	48	37 (43.52)	11
5	Endotracheal secretion	28	19 (22.35)	9
6	swab	1	1 (1.17)	0
7	Semen	3	1(1.17)	2
8	Tissue	2	1(1.17)	1
9	Abdominal fluid	3	1(1.17)	2
10	Broncho alveoler lavage	8	4(4.70)	4
11	Bile	3	1(1.17)	2
12	Stool	3	1(1.17)	2
13	Pleural fluid	1	1(1.17)	0
	Total	129	85	44

Note: The values in the parenthesis indicate the percentile number of respective samples among the total samples showing growth of pathogens.

Among the samples (n = 85) which showed the growth of pathogens around 43.52% samples were of urine and 22.35% samples were of endotracheal secretion followed by pus, sputum, blood and broncho alveolar lavage samples accounted to 8.23%, 7.05%, 5.88% and 4.70% respectively. However the remaining samples like swab, semen, tissue, abdominal fluid, bile, stool and pleural fluid contributed to very small portion of the total accounting only 1.17% each (Figure 1). Morphological and biochemical characterization of the samples (n=85) showing bacterial growth revealed presence of 13 different Gram negative organisms (Gram positive organisms are not included in the study).

Among these *E. coli, K. pneumoniae, A. baumannii, P. aeruginosa* contributed a significantly higher share (82.35%). Along with these, other gram negative bacteria like *Burkholderia cepacia, Salmonella ser. paratyphi A, E. cloacae, M. morgannii, P. mirabilis, Serratia fonticola, S. marcescens, Providencia rettgeri, Shigella boydii and K. oxytoca* were also observed in the clinical samples. The detailed profile of various organisms collected from clinical samples is shown in Figure 2. *E. coli* (43.52%) was found to be the most dominant pathogen followed by *K. pneumoniae* (20%), *A. baumannii* (9.41%) and *P. aeruginosa* (9.41%). However, other Gram negative bacteria accounted for a cumulative share of 17.64%. (Figure 2). *E coli* showed high prevalence in urine samples, indicating its significant role in urinary tract infections. The high prevalence of *E coli*, followed by *K. pneumoniae* and *P. aeruginosa* in urinary tract infections was also reported by Janifer *et al.* (2009). Yinnon *et al.* (1996) reported the major role of *Klebsiella spp a*long with *E coli* in nosocomial infections, especially in gram negative sepsis responsible for a significantly high percentage (5-29%) of overall incidences. Endotracheal secretion samples were found to be the major sites of *K. pneumoniae, A. baumannii and P. aeruginosa* infections. *A. baumannii* has known as an important and common pathogen creating nosocomial pneumonia and bacteremia among patients who admitted in the intensive care unit (ICU) worldwide, followed by skin, soft tissue, and urinary tract infection, and secondary meningitis over the past few decades (Gaynes and Edward, 2005; Kanafani *et al.*, 2003; Paul *et al.*, 2005; Wisplinghoff *et al.*, 2004).

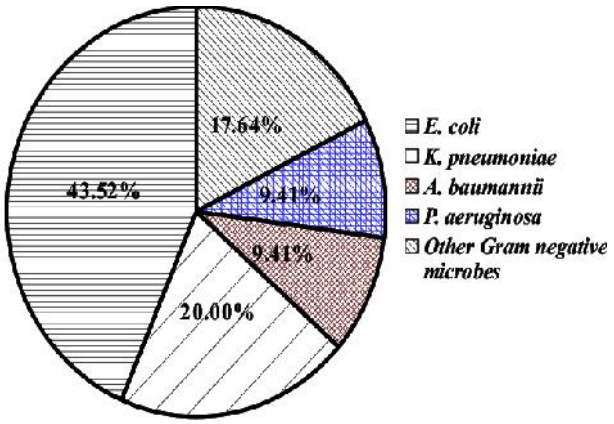

Figure 2 Prevalence of various pathogens in the clinical samples

Antibiotic resistance profile for all infectious organisms isolated from various clinical samples was established using 4 different antibiotics and is depicted in Figure 3 and 4. The susceptibility of Elores (94.6%) was highest against the most prevalent pathogen *E. coli*, followed by amikacin (83.8%) and piperacillin+ tazobactam (64.9%). However low susceptibility patterns were observed for meropenem (37.8%). Interestingly, Elores showed better response to pathogens isolated from blood, sputum, urine, endotracheal secretion and broncho alveolar lavage compared to all studied d rugs. South African susceptibility data from the private sector indicate that resistance to piperacillin+tazobactam in selected bacteraemic

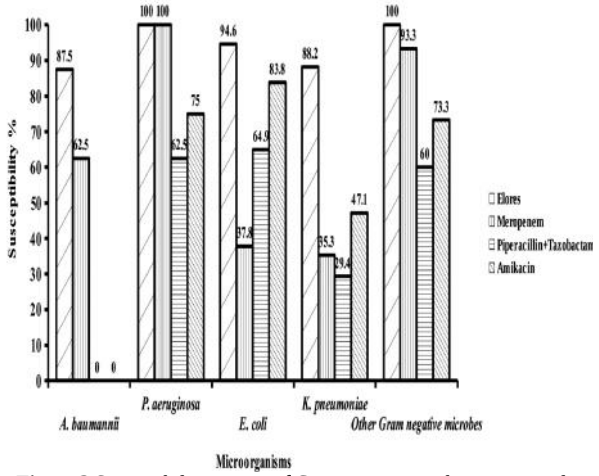

Figure 3 Susceptibility pattern of Gram negative pathogens towards antibiotics

Enterobacteriaceae ranges from 0-67% (Brink *et al.,* 2008). Sukumaran *et al.,* (2012) reported the the low resistance (1.33%) profile of *E coli* towards amikacin.

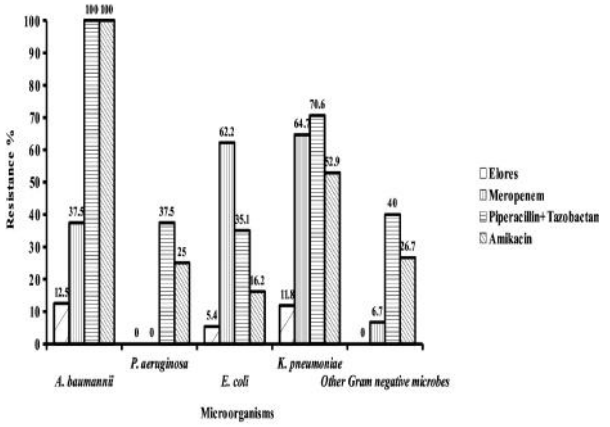

Figure 4 Resistance patterns of Gram negative pathogens isolated across India

Susceptibility of Elores against *A. baumannii* (87.5%), *P. aeruginosa* (100%) and *K. penumoniae* (88.2%) was highest when compared to other antibiotics. Meropenem also showed similar susceptibility pattern for *P. aeruginosa* (100%), but high resistance was observed in *K. penumoniae* (64.7%) and *A. baumannii* (37.5%) strains. Vahdani *et al.,* (2011) reported 19% resistance of *A. baumannii* towards carbapenem. This is the clear indication of the rising trend of carbapenem resistance. Susceptibility data for piperacillin+tazobactam against *P. aeruginosa* reported by Brink *et al.,* (2008) and Bamford *et al.,* (2011) also indicated the resistance rates of 3-60% and 21-61% in the public and private health care sectors respectively.

Piperacillin+tazobactam and amikacin were null effective against *A. baumannii* strains accounting for 100% resistance. Piperacillin+tazobactam and amikacin tested against *K. penumoniae* showed low susceptibility of 29.4% and 47.1% respectively. This rise is amikacin resistance is of great concern because, amikacin is the aminoglycosides (AG) most frequently used for *pseudomonal* infections, because *P. aeruginosa* strains with resistance to amikacin also exhibit a relatively high level of resistance to other AGs such as gentamicin, netilmicin, tobramycin and isepamicin (Torres *et al.,* 2000). Resistance towards amikacin arises through enzymatic modification of the AGs, impermeability and multidrug-active efflux systems (Poole, 2005; Miller *et al.,* 1997). Among them, inactivation of drugs by plasmid- or chromosome-encoded AG-modifying enzymes (AMEs) is the main mechanism of resistance (Poole, 2005).

For other Gram negative bacteria Elores had the highest susceptibility profile (100%) followed by meropenem (93.3%), amikacin (73.3%) and piperacillin+tazobactam (60%). Very recently, a study conducted by Sahu *et al.,* (Sahu *et al.,* 2014) also demonstrated higher susceptibility of piperacillin+tazobactam for *E. coli*, *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *E. aerugenes*, *C. freundii* and *P. vulgaris.*

CONCLUSIONS

The present study yielded a comprehensive data for the prevalence status of different gram negative pathogens towards four different antibacterial drugs. The high resistance pattern was showed by most of the pathogens against piperacillin+tazobactam and amikacin. Along with this, increased resistance of predominant nosocomial pathogens like *A. baumannii*, *K. pneumoniae*, and *E. coli* towards meropenem is a matter of frightening concern. However Elores appears to be the most susceptible to these pathogens, providing the timely and needy option to treat deadly gram negative bacteria. This work will be of great use to the clinicians to select the right antibiotic for the treatment of these multi drug resistance bacteria.

Acknowledgement

Author is thankful to Venus Medicine Research Centre, Baddi, Haryana for providing susceptibility discs.

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