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RESEARCH ARTICLE

ANTIBIOTIC SENSITIVITY PATTERN OF BACTERIAL PATHOGENS IN RAJEEV GANDHI CANCER HOSPITAL, DELHI

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ABSTRACT

We performed a retrospective, comparative study to evaluate efficacy outcomes of empiric Elores (ceftriaxone/sulbactam/EDTA) therapy compared with the meropenem, imipenem and piperacillin/tazobactam in patients suspected of bacterial infections. Among the isolates which showed the presence of bacteria, around 36.0 % samples were of urine followed by sputum and blood which contributed to 15.7 % and 11.5 % respectively. Among the isolates, *Escherichia coli* (51.7 %) was found to be the most dominant pathogen followed by *Klebsiella pneumoniae* (29.5 %), *Pseudomonas aeruginosa* (15.0 %), *Acinetobacter baumannii* (2.3 %), and *Proteus mirabilis* (1.5 %). Higher susceptibility rates were achieved with Elores in comparison with piperacillin/tazobactam and meropenem. Susceptibility pattern for imipenem was almost same as that for Elores. Piperacillin/tazobactam resistance was high in all the tested pathogens ranging from 54.0 % (least in *P. aeruginosa*) to 100.0 % (highest in *Proteus spp.*) when compared to Elores to which low resistance was observed ranging from 19.0 % (least in *P. aeruginosa*) to 33.3 % (highest in *A. baumannii*) was observed. Overall, the results of the present study strongly advocate the superiority of Elores over piperacillin/tazobactam and meropenem and an equivalence to imipenem. Elores can be a very effective alternative to treat against the deadly multi drug resistant Gram negative bacteria, sparing penems as reserve drugs.

INTRODUCTION

Worldwide resistant bacteria are emerging as a threat to treatment of common infections in community and hospital settings. Urinary tract, gastrointestinal and pyogenic infections are the common hospital aquired infections caused by Gram negative bacteria (Kumar *et al*. 2014). β-lactam antibiotics are among the most frequently prescribed antibiotics in ICUs world-wide, which are favoured because of their efficacy, broad spectra and low toxicity. Pressures which are generated by indiscriminate use of the beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases such as the ESBLs, AmpC β-lactamases and metallo-β-lactamases (MBLs) which have emerged as the most worrisome resistance mechanism which poses a therapeutic challenge to the health care settings (Deshmukh *et al*., 2011).

ESBL are bacterial enzymes that hydrolyse cephalosporins for example: cefuroxime, cefotaxime, ceftriaxone and ceftazidime (Lavilla *et al*., 2008). The prevalence of ESBL among clinical isolates varies among geographic areas (Paterson and Bonomo, 2005; Shrestha *et al*., 2006). ESBLs are most commonly produced by *Klebsiella* spp. and *E. coli* but may also occur in other Gram-negative bacteria such as *Pseudomonas* spp., and *Proteus* spp. (Goussard *et al*., 1999). Multiple surveys have shown that the highest ESBL rates for *E. coli* and *Klebsiella* spp. occur in India (≥ 80%) and China (≥ 60%) (Hoban *et al*., 2011; Chaudhuri *et al*., 2011; Hsueh *et al*., 2010).

Carbapenems have been the most successful β-lactam antibiotics used in treatment of infections caused by β-lactam resistant Gram-negative bacteria. However, there have been reports of resistance to carbapenems (Yano *et al*., 2001; Kurokawa *et al*., 1999). The clinical utility of these antimicrobials is under threat with emergence of carbapenemases, particularly the class B metalloβ-lactamases (MBLs). Resistance to carbapenem due to the production of metallo-beta-lactamases (MBL) in Gram-negative organisms is an increasing international public health problem (Cornaglia *et al*., 2007). MBLs can hydrolyze most β-lactams except for monobactams and confer a broad-spectrum β-lactam resistance to bacterial host, which is not reversible by conventional therapeutic β-lactamase inhibitors. The prevalence of MBLs has been increasing worldwide, not among *P. aeruginosa* but also, amongst other Gram-negative bacteria as well (Walsh *et*

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al., 2005). There are several mechanisms for carbapenem resistance such as lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux mechanisms (Walsh *et al*., 2002). In India, the prevalence of MBLs ranges from 7.5% to 71% (De *et al*., 2010). The carbapenems available for use in India are imipenem and meropenem (Gupta *et al*., 2006). In India, resistance to meropenem varies from 37 to 42 % in *Pseudomonas* spp (Chaudhary and Payasi, 2013; Gupta *et al*., 2006) and upto 89% in *A. baumannii* (Karthika *et al*., 2009).

To overcome this serious threat of antibiotic ressistance against carbapenems and cephalosporins, one has to look for other alternative antibiotic options or the existing antibiotics with added potentiators to treat the infections caused by these MDR strains. 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 adjuvent entity – ceftriaxone+sulbactam+EDTA (Elores) in comparision to piperacillin tazobactam, meropenem and imipenem among these pathogesns.

MATERIALS AND METHODS

Sample collection

A total of 652 clinical samples which consisted of blood, pus, sputum, urine, abdominal fluid, bile, swab, tissue, bronchial secretion were collected from Rajiv Gandhi Cancer Institute of India (Delhi) during the period of September 2014 to December 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 asceptically 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 bronchial secretion 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 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 reactions. Biochemical reactions were performed by inoculating the bacterial colony in a nutrient broth at 37°C for 2–3 hours.

Table 1 Selective culture medium used for isolation of different pathogens

Pathogen	Selective media
E. coli	Eosine Methylene Blue (EMB) agar medium
A. baumannii	Leeds acinetobacter agar base medium
K. pneumoniae	Hicrome Klebsiella selective agar base medium
Proteus spp.	EMB agar and Mcconkey's agar
P. aeruginosa	Citrimide agar

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines (2014). All the discs, meropenem (10 µg), imipenem (10), Elores (45 µg) and Piperacillin/tazobactam (110 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, Hi-Media, Mumbai, India) 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 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

A total 652 different clinical samples of urine, pus, sputum, blood, abdominal fluid, bile, swab, tissue, bronchial secretion and Foley's catheter tip cultures were collected from Rajiv Gandhi Cancer Institute Delhi, India and processed for isolation of pathogenic bacteria. Out of total samples analyzed, 261 samples showed the presence of infection while in 391 samples no growth of organisms was observed in the culture medium (Table 2).

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

Sr. No.	Clinical samples	Total	Number of samples showing growth of pathogens	Number of samples not showing growth of pathogens
1	Abdominal fluid	29	7 (2.7)	22
2	Urine	230	94 (36)	136
3	Bronchial secretion	43	14 (5.3)	29
4	Bile	43	25 (9.6)	18
5	Blood	75	30 (11.5)	45
6	Foley's tip catheter	29	5 (2.0)	24
7	Infected tissue	8	1 (0.4)	7
8	Swab	43	16 (6.1)	27
9	Pus	56	28 (10.7)	28
10	Sputum	96	41 (15.7)	55
	Total	**652**	**261**	**391**

Morphological and biochemical characterization of the samples (n=261) showing bacterial growth revealed presence of 5 different Gram negative organisms. The detailed profile of various organisms collected from various clinical samples is shown in Figure 1. The identified bacteria include *E. coli, K. pneumoniae, P. aeruginosa, A. baumannii,* and *P. mirabilis,* in decreasing order of prevelance. Among the isolates, *E. coli* (51.7 %) was found to be the most dominant pathogen. Similar results with high rates of *E coli* infections (54.9 %) , (68.8 %)

and (49.2 %) were reported earlier (Sikka *et al*., 2012; Dash *et al*., 2013; Patil *et al*., 2013). *K. pneumoniae* (29.5 %), and *P. aeruginosa* (15.0 %), also contributed significantly to the isolated pool of pathogens followed by *A. baumannii* (2.3 %), and *P. mirabilis* (1.5 %). Similar prevalence of *Klebsiella sp.* (19.5 %) and *P. aeruginosa* (9.2 %) was reported by Patel *et al*., (2008), which is in accordance with study reported by Ali *et al*. (2004) where prevalance of *K. pneumoniae* was (21%) followed by *P. aeruginosa* (19.2%) and *A. baumannii* (4.4 %). *E. coli* was the most prevalent pathogen among most of the samples accounting for 22.0 % in sputum, 70.0 % urine, 53.3 % in blood, 44.8 % in swab, 43.0 % in abdominal fluid, 24.0 % in bile and 44.4 % in bronchial secretions. Similar results were observed by Mehta *et al*. (2012) reporting high prevalence (41 %) of *E. coli* among the urine samples collected from urinary tract infection patients. Mulvey *et al*. (2005) also reported *E. coli* strains in a high rate from urine (77.5%) which is in well accordance with results of the present study.

were imipenem resistant (Gupta *et al*., 2006). Similarly, overall meropenem resistance was about 30% in a study by Mulla S *et al* (2011) and 31.81% by Mahajan G *et al* (2011). However, Parveen *et al*. (2010) reported the high meropenem resistance trends (43.6 %) in *K. pneumoniae* isolated from south India. Recently another report from Srinivasan and Madhusudhan (2014) showed that *E.coli* and *Klebsiella* spp. were highly resistant to meropenem (77% and 50% respectively). Petro *et al*. (2014) reported that (48.7%) *K. pneumoniae*, were susceptible to piperacillin/tazobactam whereas (34.0%) of the *Pseudomonas* spp were susceptible to piperacillin-/tazobactam. The results of the present study also revealed Elores susceptibility patterns observed in *A. baumannii* (66.7 %) and *Proteus* spp. (75 %). On the other hand *A. baumannii* showed (66.7%) resistant against piperacillin/tazobactam and meropenem, whereas resistant to imipenem (33.3 %) was same as that for Elores. However *Proteus* spp. were completely resistance to piperacillin/tazobactam.

Table 3 Prevalence of different clinical isolates in different samples

Samples	Number of isolates	Clinical isolates %				
		A. baumannii %	*E. coli* %	*Klebsiella sp.* %	*P. aeruginosa* %	*Proteus sp.* %
Abdominal fluid	7	0	43	43	14.3	0
Urine	94	10	70.2	18	8.5	1.1
Bronchial secretion	14	0	57.1	19.1	14.2	0
Bile	25	8	24	52.0	16.0	0
Blood	30	3.3	53.3	30.0	13.3	0
Foley's tip catheter	5	0	60.0	40.0	0	0
Infected tissue	1	0	100	0	0	0
Swab	16	0	43.8	25	18.7	12.5
Pus	28	0	57.1	17.9	21.5	3.6
Sputum	41	14.3	22.0	46.3	26.8	0

Similarly, *E. coli* were isolated in a high frequency from urine (78.5%) in a study carried out in Canadian hospitals (Kaye *et al*., 2004). Jameel *et al*. (2012) reported high occurrence of *E. coli* in blood samples (52.9%) same as reported in the present study (53.3 %). *Klebsiella spp.* contributed for 46.3 % in sputum samples, 30.0 % in blood samples and 18% in urine samples (Table 3). Subha *et al*. (2003) also reported considerable prevalence (42.8%) *Klebsiella spp.* in nosocomial sputum samples, (28.6%) from blood and (28.6%) from urine specimens. *P. aeruginosa* accounted for (26.8) % in sputum, (13.3) % in blood, (8.5) % in urine, (21.5) % in pus samples, abdominal fluid (14.3) %, and bronchial secretion (14.2) % (Table 3). Rajkumari *et al*. (2014) reported that the most common sample from which *P. aeruginosa* was recovered was from urine samples (29.0 %), followed by tracheal aspirates (24.4 %), pus/wound swabs (20.0%), blood (8.0 %), bronchalveolar lavage (8.0 %), tissues (1%), and sputum (0.1%).

Antibiogram profile for all the pathogens isolated from various clinical samples is presented in Figure 3 and 4. The susceptibility of the three most predominant pathogens *E. coli*, *P. aeruginosa* and *Klebsiella spp.* towards Elores (80.46 %, 81.0 % and 74.2 % respectively) was better when compared towards meropenem (78.0 %, 67.0 % and 62.0 % repectively), and imipenem (79.5 %, 72.0 % and 70.0 % repectively) but was very high when compared with piperacillin/tazobactam (30.4 %, 46.0 %, 26.0 % repectively). In a study performed in India overall 36.4% of nonfermenters were resistant to imipenem and 42% of *P. aeruginosa* and 18.5% *A. baumanni*

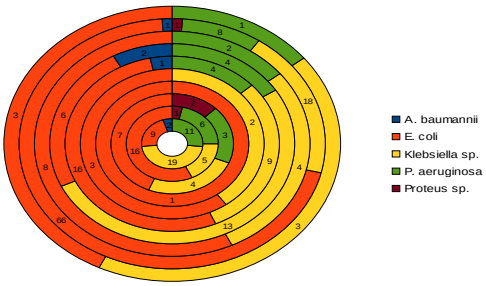

Figure 1 Profile of different clinical isolates isolated from various samples

A-Abdominal fluid; **B**-Urine; **C**-Bronchial secretion; **D**-Bile; **E**-Blood; **F**-Foley's tip catheter; **G**-Infected tissue; **H**-Swab; **I**-Pus; **J**-Sputum

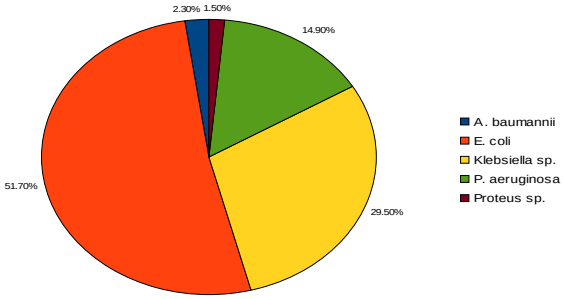

Figure 2 Prevalence of various pathogen

On the other hand the same pathogens showed (25 %) resistance to elores, meropenem and imipenem.

Several other authors also demonstrated higher susceptibility of Elores for *E. coli*, *P. aeruginosa* and *K. pneumoniae* (Chaudhary and Payasi, 2012; Sikha *et al*., 2015; Makkar *et al*.,

2015; Chaudhary and Payasi, 2014; Sahu *et al*., 2014). Also work done by (Chitnis *et al*., 2003; Laura *et al*., 2000) in cephalosporins showed that the overall resistance to various generations of cephalosporins was high on account of production of ESBLs by the bacteria involved. Hence, addition of sulbactum/EDTA to ceftraixone monotherapy significantly reduced the percentage resistance and increased the percentage susceptibility against all the organisms (Figure 4).

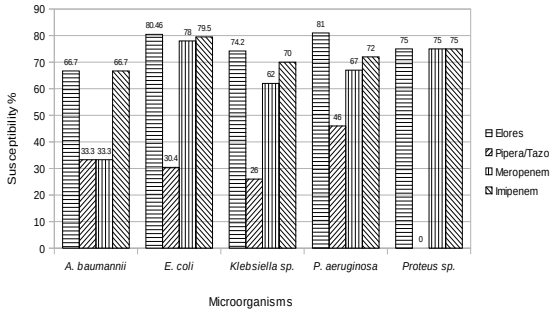

Figure 3 Susceptibility pattern of Gram negative pathogens isolated from RGCI

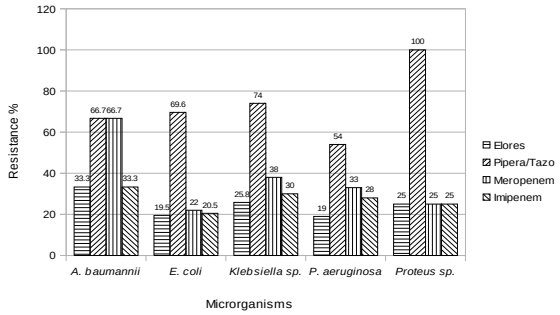

Figure 4 Resistance patterns of Gram negative pathogens isolated from RGCI

The resistance to carbapenems especially in *Pseudomonas spp*. results from reduced levels of drug accumulation or increased expression of pump efflux (Karlowsky 2003). The resistance may also be due to the production of metallo-b-lactamases (MBL) which can be chromosomally encoded or plasmid mediated (Navaneeth *et al*., 2002). By the results of the current study, it appears Elores is most effective against these multi drug resistant pathogens when compared to piperacillin/tazobactam, meropenem and imipenem.

CONCLUSION

In vitro susceptibility results of Elores appears to be very promising. Elores was found relatively more active against *P. aeruginosa, E. coli* and *Klebsiella spp.*, than imipenem piperacillin/tazobactam and meropenem. Since both Elores and imipenem were found equally sensitive in *E. coli, A. baumannii, Klebsiella spp and Proteus spp.* they may be used in life-threatening infections when susceptible but again Elores showed better susceptibility results against *P. aeruginosa* when compared with imipenem also. Therefore, Elores appears to be better choice than other comparator drug for catering to drug resistant pathogens and sparing carbapenems.

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