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RESEARCH ARTICLE

ALARMINGLY RISING B-LACTAMASE-MEDIATED MEROPENEM RESISTANCE IN NOSOCOMIAL INFECTIONS IN INDIAN HOSPITALS

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ABSTRACT

Growing insensitivity to multiple antibiotic groups particularly beta-lactams has been a concern for past decade. The concerns of antibiotic resistance, lack of new antibiotics and limited therapeutic options led us to compare the susceptibility of a new antibiotic adjuvant entity Elores (Ceftriaxone+sulbactam+ethylenediaminetetraacetic acid, EDTA) with meropenem among Gram negative organisms isolated from >1100 clinical samples obtained from various hospitals of India during past six months. Out of total samples analyzed 923 samples showed the presence of infection and 281 samples were sterile. *E. coli* (44.2%) was found to be the most dominant pathogen followed by *P. aeruginosa* (18.2%), *K. pneumoniae* (10.9%), *A. baumannii* (9.0%), *M. morganii* (4.9%), *P. mirabilis* (4.8%), *S. marcescens* (3.0%), *K. oxytoca* (3.8%) and *E. cloacae* (1.2%). Higher success rates have been achieved with Ceftriaxone+sulbactam+EDTA in comparison to meropenem. Use of meropenem in the light of alarmingly rising resistance (9 to 62 %) warrants restricted use and re-evaluation of the therapies where penems are used in high doses and to evaluate ceftriaxone+sulbactam+EDTA as alternative. Results of meropenem was comparable to ceftriaxone+sulbactam+EDTA against *M. morgannii*, *P. aeruginosa*, and *S. mercescens* but in enterobacters ceftriaxone+sulbactam+EDTA exhibited around 3% to 33% higher susceptibility. We conclude that ceftriaxone+sulbactam+EDTA is much more effecive against most of the multidrug resistant (MDR) pathogens and can be of a better option to treat against these pathogens.

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INTRODUCTION

Hospital-acquired infections are a major challenge to patient safety. It is estimated that in 2002, a total of 1.7 million hospital-acquired infections occurred (4.5 per 100 admissions) and almost 99,000 deaths resulted from or were associated with a hospital-acquired infection (Klevens *et al.,* 2007), making hospital-acquired infections the sixth leading cause of death in the United States (Kung *et al.,* 2008). Carbapenems, a class of broad-spectrum antibiotics that includes imipenem and meropenem possess stability against hydrolysis by ESBL and AmpC chromosomal β-lactamase enzymes and are often reserved to treat the most serious infections (Ayalew *et al.,* 2003; Zhanel *et al.,* 2007; Brink *et al.,* 2004). Although both meropenem and imipenem have shown activity against aerobic gram-negative bacilli, aerobic gram-positive cocci but meropenem is more active against gram-negative pathogens (Ayalew *et al.,* 2003; Shah and Narang, 2005), Meropenem has been effectively used in bacterial meningitis skin, soft tissue, and joint infections; serious gastrointestinal infections, septicemia, febrile neutropenia, nosocomial pneumonia, cystic fibrosis–associated respiratory infections, and serious urinary tract infections (Ayalew *et al.,* 2003; Merrem, 2007; Shah and Narang, 2005). However in past few years, carbapenem resistance among the members of the Enterobacteriaceae family has been reported increasingly throughout the world and India (Francis *et al.,* 2012; Hu *et al.,* 2012; Gupta *et al.,* 2006; Grundmann *et al.,* 2010; Varaiyam *et al.,* 2008; Chaudhary and Payasi, 2013). Carbapenem resistant Enterobacteriaceae (CRE) can be defined as Enterobacteriaceae that are resistant to carbapenems, ertapenem, meropenem, imipenem or doripenem (Nair and Vaz, 2013). It 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 (Toolkit, 2012).

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Besides, carbapenem resistance in Enterobacteriaceae, it has also been reported frequently in lactose non-fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacer* spp. (Gupta *et al.,* 2006; Varaiyam *et al.,* 2008; Chaudhary and Payasi, 2013; Karthika *et al.,* 2009). In India, resistance to meropenem varies from 37 to 42 % in *Pseudomonas* spp. (Gupta *et al.,* 2006; Chaudhary and Payasi, 2013) and upto 89% in *A. baumannii* (Karthika *et al.,* 2009). Overall, in India, the prevalence of carbapenemases, responsible for carbapenem resistance, ranged from 7.5% to 89% (Chaudhary and Payasi, 2013; Karthika *et al.,* 2009; Sinha and Srinivasa, 2007; Chaudhury *et al.,* 2013).

To address this worstening problem of antibiotic resistance against carbapenems there is a urgent need to look for new antibiotic options or potentiation of existing antibiotic with antibiotic adjuvants (Sahu *et al.,* 2014). These new Antibiotic adjuvant entities (AAEs) have been reported to break resistance cycle and overcome different resistance mechanisms adopted by bacteria (Chaudhary and Payasi, 2013; Chaudhary *et al.,* 2013; Chaudhary *et al.,* 2012; Chaudhary and Payasi, 2013). Thus in view of all these aspects, the present work was aimed with an objective to identify the rise in CRE and to evaluate the susceptibility of a new antibiotic adjuvant entity (Elores) Ceftriaxone Sulbactam EDTA, among the Gram negative microbes isolated from from different hospitals across India.

MATERIALS AND METHODS

Sample collection

Different clinical samples such as blood, pus, sputum, urine, abdominal fluid, bile, semen, swab, tissue, brancho alveolar fluid and endotracheal section were collected from 1204 (one thousand two hundred and four) patients suspected of bacterial infection at various hospitals in north India region during the period of January 2014 to June 2014. The collection and processing of the samples were done as per a common standard operating procedure 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 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 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 and *K. oxytoca*	Hicrome Klebsiella selective agar base medium
P. mirabilis	EMB agar and Mcconkey's agar
P. aeruginosa	Citrimide agar
S. marcescens	Caprylate- Thallous agat (CT agar)
E. cloacae	Hicrome coliform agar modified medium

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the CLSI guidelines (CLSI, 2013). Meropenem disk (10 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 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° 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

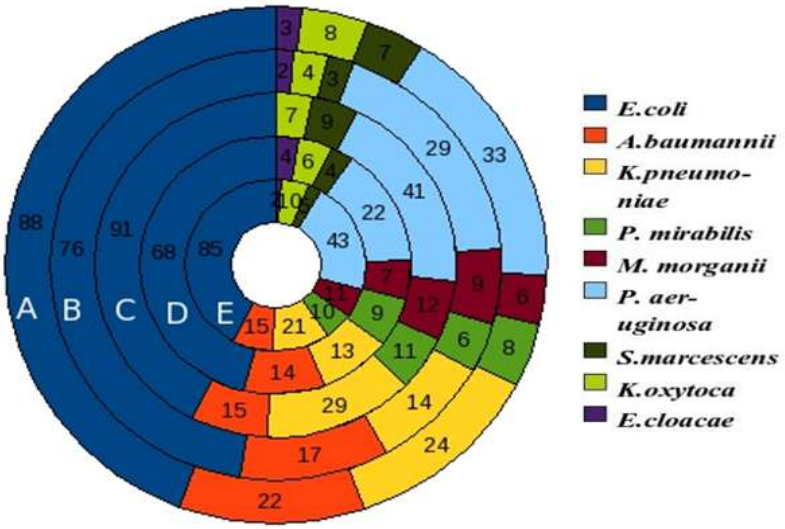
A total 1204 different clinical samples were collected from 5 centres across India and processed for isolation of pathogenic bacteria. Eleven types of clinical samples included urine, pus, sputum, blood, abdominal fluid, bile, semen, swab, tissue, Brancho alveolar fluid and Endotracheal section. Out of total samples analyzed 923 samples showed the presence of infection while in 281 samples no growth of organisms was observed in the culture medium (Table 2). Among the samples (n = 923) which showed the presence of pathogens around 35.5% samples were of urine followed by pus and sputum samples which contributed to 15.6% and 15.4% respectively. Blood, Endotracheal section, semen and abdominal fluid samples contributed to 11.6 %, 7.5 %, 4 % and 3.7% respectively. However samples from tissue, brancho alveolar fluid, bile and swab had a lesser share in total number of pathogen containing samples with percentile share of 2.7, 1.95, 1.19 and 0.8 respectively (Table 2).

Morphological and biochemical characterization of the samples (n=923) showing bacterial growth revealed presence of 9 different Gram negative organisms (Gram positive organisms are not included in the study). The detailed profile of various organisms collected from various centres is shown in Figure 1.

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	368	328 (35.5)	40
2	Blood	166	107 (11.6)	59
3	Sputum	232	142 (15.4)	90
4	Pus	150	144 (15.6)	6
5	Endotracheal section	86	69 (7.5)	17
6	Semen	63	37 (4)	26
7	Abdominal fluid	41	34 (3.7)	7
8	Tissue	41	25 (2.7)	16
9	Brancho alveolar fluid	29	18 (1.9)	11
10	Bile	16	11 (1.2)	5
11	Swab	12	8 (0.9)	4
	Total	1204	923	281

Note: The values in the paranthesis indecate the percentile number of respective samples among the total samples showing growth of pathogens



A - Goenka Research Institute and Dental college Ahmedabad
B - Saraswati Mediacal and Dental College and Hospital Lucknow
C - I.T.S. Research Centre, Hospital and Dental college, Gaziabad
D - Kothiwal College and Research Centre, Moradabad
E - Medical Microbiology, Manipal University

Figure 1. Profile of different clinical isolates isolated from various centres

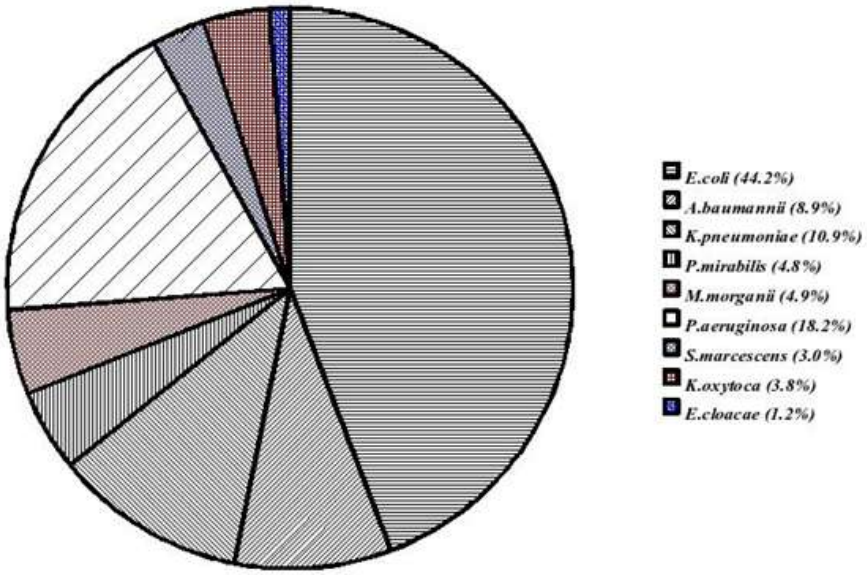


Figure 2. Prevalence of various pathogen

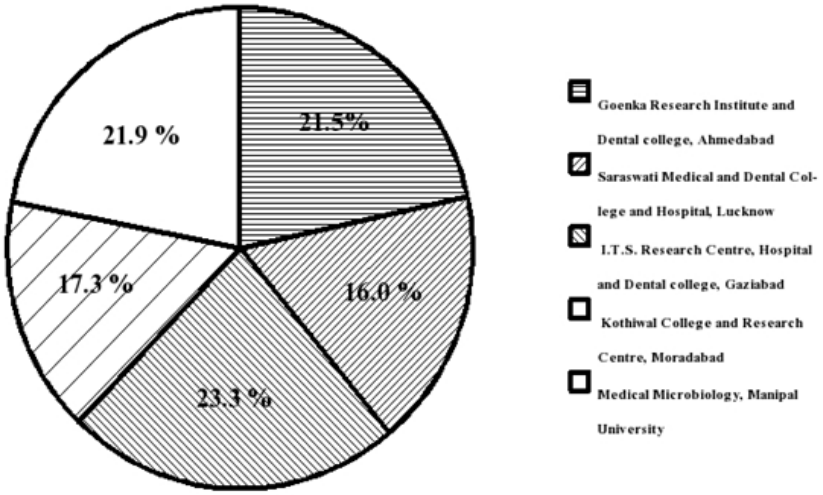
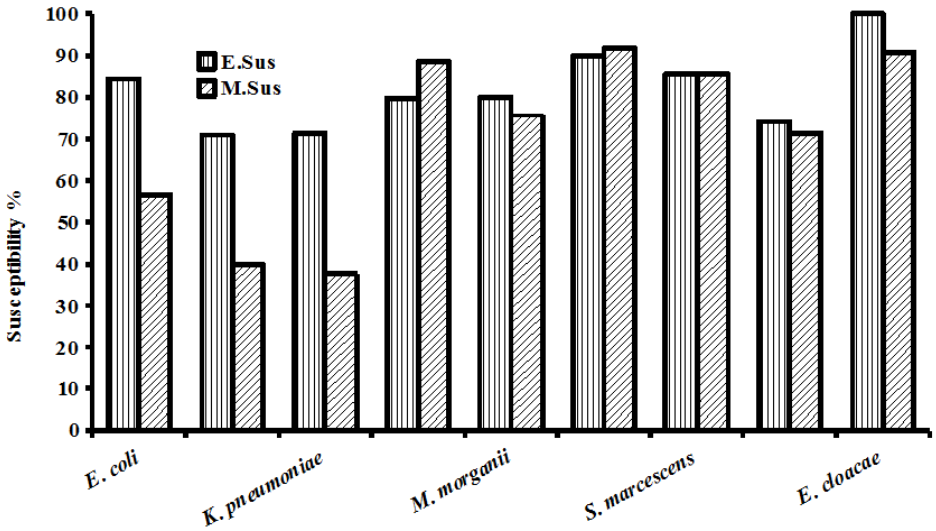
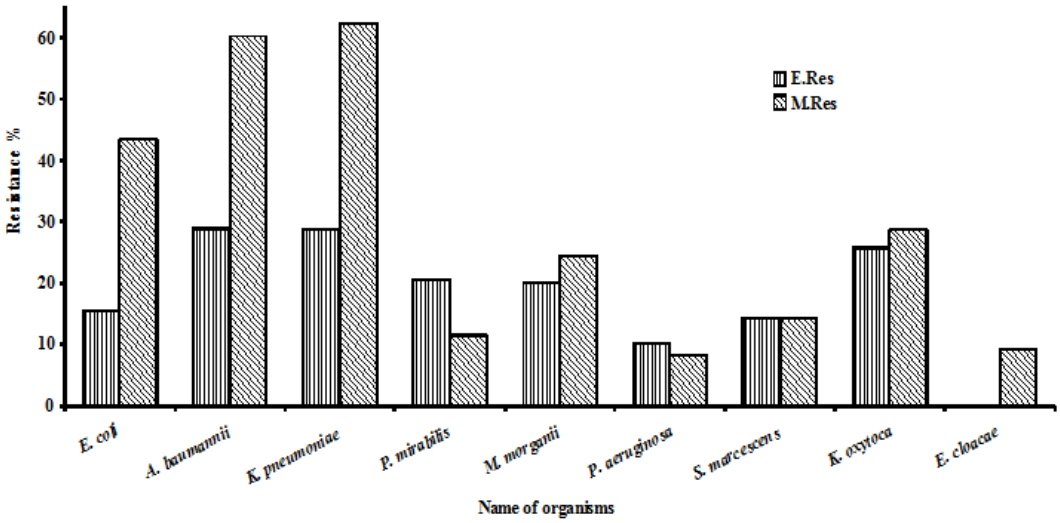


Figure 3. Total contribution of various centres



E. sus – Elores susceptibility; M. sus – Meropenem susceptibility

Figure 4. Susceptibility pattern of Gram negative pathogens isolated across India: E.Sus - Elores Susceptibility, M. Sus – Meropenem Susceptibility



E. sus – Elores susceptibility; M. sus – Meropenem susceptibility

Figure 5. Resistance patterns of Gram negative pathogens isolated across India: E.Res - Elores Resistance, M.Res – Meropenem Resistance

The identified bacteria include *E. coli, A. baumannii, K. pneumoniae, P. mirabilis, M. morganii, P. aeruginosa, S. marcescens, K. oxytoca* and *E. cloacae* in decreasing order of prevelance. Among the isolates, *E. coli* (44.2%) was found to be the most dominant pathogen followed by *P. aeruginosa* (18.2%), *K. pneumoniae* (10.9%), *A. baumannii* (9%), *M. morgannii* (4.9%), *P. mirabilis* (4.8%), *S. marcescens* (3.0%), *K. oxytoca* (3.8%) and *E. cloacae* (1.2%) (Figure 2). Figure 3 shows that maximum isolates were collected from I.T.S. Research Centre, Hospital and Dental college, Gaziabad (23.3%) follwed by Goenka Research Institute and Dental college Ahmedabad (21.9 %) Medical Microbiology, Manipal University (21.5%), Saraswati Mediacal and Dental College and Hospital Lucknow (17.3%) and Kothiwal College and Research Centre, Moradabad (16.0 %).

E. coli is the predominant pathogen responsible for the urinary tract infection followed by *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*. *E. coli* also dominates the number of isolates present in blood, abdominal fluid, semen, bile and tissue samples. *P. aeruginosa* infection was predominant in blood, sputum and endotracheal section. Whereas significant number of *A. baumanni* and *K. pneumoniae* isolates were also observed in blood, sputum and pus samples.

Antibiogram profile for all the pathogens isolated from various clinical samples is presented in Figure 4 and 5. The susceptibility of Ceftriaxone Sulbactam EDTA was 83.9% in comaprison to 54.7% of meroepnem against *E. coli.* A similar trend was observed in *K.penumoniae* and *P. aeruginosa* were >70% susceptibility was observed with Ceftriaxone+sulbactam+EDTA as against only <40% in meropenem. Very recently, a study conducted by Sahu *et al.* (2014), also demonstrated higher susceptibility of Ceftriaxone Sulbactam EDTA for *E. coli*, *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *E. aerogenes*, *C. freundii* and *P. vulgaris.* 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 (Chaudhary and Payasi, 2013). For remaining pathogens both drugs showed almost similar response pattern. Among the isolated organism, *K. pneumoniae* (62.4) showed highest resistance towards meropenem closely followed by A. *baumannii* (60.2%) and *E. coli* (45.3%). This indicates a rising trend of crabapenem resistance being observed at all centres. Earlier Parveen *et al.* (2010), studied carbapenem susceptibilities among nosocomial *K. pneumoniae* isolated from south India and reported meropenem resistance in about 43.6% isolates. Isolates like *M. morgannii* (24.4), *K. oxytoca* (17.1%) and *S. marcescens* (14.3%) showed moderate resistance towards both drugs. Ceftriaxone+sulbactam+EDTA appears to be the most susceptible to the most deadly troublesome pathogens.

In conclusion, the study resulted in generation of representative status of the prevalence resistance pattern of different pathogens across India and is found to be alalrmingly high for meropenem. Infections with *A.baumanii* are difficult to treat due to their intrinsic and acquired resistance which is easily broken by Ceftriaxone+Sulbactam+EDTA. The ratio of carbapenemases producing *E.coli* and *P. aeruginosa* is rising which may be due to biofilm produced by use of catheters. Meropenem fails to break bacterial biofilm and hence is found to be resistant as against ceftriaxone+sulbactam+EDTA which has proven efficacy against biofilm breaking [19]. This study provides the antibiotic sensitivity pattern towards the predomonant Gram negative microorganisms against meropenem and Ceftriaxone Sulbactam EDTA suggesting that use of meropenem should be restricted. This could be useful 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.

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