ORIGINAL ARTICLE

Prevalence and Susceptibility Analysis of Carbapenem Resistant Gram Negative Pathogens in Tertiary Care Hospital, Mumbai

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ABSTRACT

Background and objective: Antibiotic resistance has risen perilously in all parts of the world. The lack of appropriate therapeutics to encounter resistant pathogens has enhanced the urge for the development of either new antibiotics or adjuvant therapy with antibiotics. Thus, we aimed to study a comparative antibiogram pattern of 102 clinical isolates towards Elores (a *novel antibiotic* and *adjutant entity of ceftriaxone, sulbactam* and *disodium edetate)* and other antibiotics (*colistin, piperacillin/tazobactam, tigecycline, cefoperazone+sulbactam and cefepime+tazobactam).* Methods: The clinical samples collected from infective patients admitted to Tertiary care Hospital, Mumbai (India) between June 2016 to December 2016 was further subjected to bacterial identification. Antibiotic susceptibility testing was executed in accordance with the recommendations of Clinical Laboratory Standards Institute (CLSI) guidelines. All isolates included in this study were resistant to *carbapenems*. Results: Out of 4212 collected samples, *carbapenem* resistant isolates were recovered from 102 clinical samples in which urine samples contributed 51.96% followed by sputum and tracheal secretion which added 8.82% each while rest of the clinical specimen contributed 30.4%. *Klebsiella pneumoniae* (61.76%) was most predominant among all the resistant clinical isolates followed by *Escherichia coli* (18.63%), *Acinetobacter baumannii* (14.71%), *Serratia marcescens* (2.94%) and *Enterobacter cloacae* (1.96%). These 102 clinical isolates which were found resistant towards *imipenem and meropenem* were included in this study and further processed for antimicrobial susceptibility analysis with respect to other antibiotics. Data suggested, the antibiogram profile of Elores was extremely higher (100% susceptible) towards clinical pathogens over rest of the antibiotics such as *piperacillin/tazobactam* (0-6.7%), *cefoperazone+sulbactam* (0-20%), *cefepime+tazobactam* (0-33.3%) and *tigecycline* (34.7-100%) but it was found comparable to colistin (87.3-100%) except *S. marcescens* which shows inherent resistance to colistin. Conclusion: Susceptibility profile data revealed the equivalence of Elores (Antibiotic-adjuvant entity; AAE) with colistin and strong superiority over other antibiotics including *β-lactam and β-lactamase inhibitors combination* (BL-BLI) and *protein synthesis inhibitors*. Hence, Elores can be treated as a most efficient treatment option towards infections caused by *carbapenem* resistant pathogens.

Key words: Antibiotic, clinical isolates, elores, prevalence, susceptibility, resistance

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INTRODUCTION

The role of antibiotics is not limited to save patients from infection but also has role in surgery which includes chemotherapy treatment, organ transplants, cardiac surgery, rheumatoid arthritis, diabetes etc.[1-3] In fact antibiotics have helped to decrease the morbidity and mortality rates in developing countries thus extending the human life span.[4,5] But unfortunately in recent decades a medical threat *i.e.,* resistance against antibiotics has been observed towards most of the antibiotics which has become the major issue in combating against clinical pathogens.[6] Overuse of antibiotics, inappropriate processing, extensive agricultural use and lack of regulatory barriers are the major causes for antibiotic resistance.[7] At present, antibiotic resistance has become a global threat that may pass from one species of bacteria to another through various genetic methods including horizontal gene transfer.[8]According to the studies *E. coli* has been reported with a huge rise in resistance against many antibiotic like *carbapenems*, third generation cephalosporin and *fluoroquinolone* and up to 66.6%, 83% and 85% respectively.[9,10] In North-east India, *E. coli* have also been found resistant to *doripenem, a carbapenem antibiotic*, which exhibited 78.57% resistance.[11] Likewise, *K. pneumoniae* clinical isolates have also displayed resistance at a very high scale *i.e.,* 80%, 73% and 52% towards third generation cephalosporin, fluoroquinolone and *carbapenems* respectively.[12] Extended-spectrum beta-lactamases (ESBL) carrying *K. pneumoniae* clinical isolates have also shown resistance towards *cefoperazone* and *cefepime* up to 70-100% compared to the ESBL non-producer (0-25%).[13,14] A recent study from Mumbai, India also depicted the similar antibiotic resistance in *A. baumannii* which depicted enhanced resistance in *A. baumannii* due to the routine use of antibiotics including tigecycline, piperacillin and colistin.[15] Acienetobacter species isolated from blood, pus, urine and sputum were also found to exhibit 58.34% resistance towards *piperacillin/tazobactam*.[16]

Based on the present data of antibiotic resistance, there is a keen requirement of either new antibiotics or adjuvant therapy along with antibiotics which should have the potential to prevent this medical threat by treating infectious diseases and help mankind. Herein, we aimed to study the susceptibility pattern of Elores (a novel antibiotic-adjuvant entity of *ceftriaxone, sulbactam* and *disodium edetate*) in comparison to the different classes of antibiotics polymixins (colistin), BL-BLI (*piperacillin/tazobactam, cefoperazone+sulbactam and cefepime+tazobactam*) and protein synthesis inhibitors (tigecycline) against *carbapenem* resistant pathogens.

MATERIALS AND METHODS

Sample collection

Various clinical samples including urine, wound, endotracheal tube (ET) secretion, central line, tissue, cerebrospinal fluid (CSF), tracheal secretion, ICD fluid, ascitic fluid, bile, sputum, pus, high vaginal swab and pleural fluid were collected from patients at Tertiary care Hospital, Mumbai (India), during the period of June 2016 to December 2016. The collection and processing of the samples were done as per SOP.

Isolation and identification of microbes

All the clinical samples were collected aseptically from the infected body sites of the patients and inoculated on the various selective and non-selective culture media as per the standard microbiological techniques. Different selective culture media were used for the isolation of microorganisms such as sheep blood agar, sheep chocolate agar,

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MacConkey agar medium. Organisms were categorized based on colony morphology, gram staining and identification was done using Vitek 2 system (Biomerieux).

Antibiotic susceptibility testing

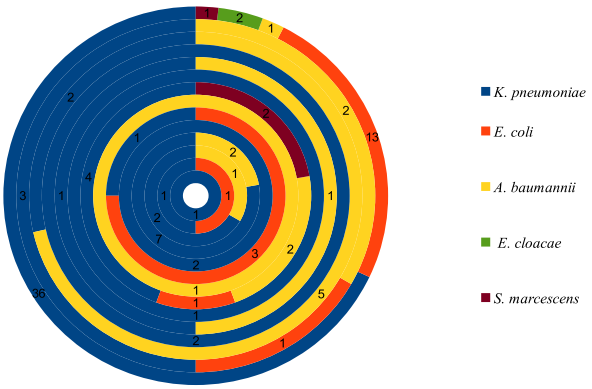
The clinical isolates which were resistant or showed intermediate susceptibility against imipenem and meropenem were included in this study and further antimicrobial susceptibility study was performed on theses isolates by Vitek-2 system and Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines.[17] In brief, inoculum of 0.5 McFarland standards turbidity was prepared in saline from isolated colony of pathogens selected from 18-24 hour agar plates. A sterile cotton swab was dipped into the inoculum and streaked three times on the dried surface of a Mueller-Hinton agar (MHA) plate. After 5 minutes, antibiotic discs were applied and pressed down to check absolute contact with agar surface. The discs were apportioned in a minimum distance of 24 mm from the centre. The plates were then incubated for 16-18 hrs aerobically at 37°C. The discs of Elores (45 µg) and *cefepime+tazobactam* (40 µg) were obtained from HiMedia, India and used in the study. Colistin, *piperacillin-tazobactam*, *cefoperazone+sulbactam* and tigecycline were tested by MIC using Vitek 2 system.

RESULTS AND DISCUSSION

Out of the collected clinical isolates recovered from the various clinical specimens, 102 carbapenem resistant gram negative bacteria were used in this study in which the highest occurrence of resistant pathogens was found in urine samples (51.96%) followed by tracheal secretion and sputum (8.82% each), ET secretion (5.88%), ascitic fluid (3.92%), pus (2.94%) whereas clinical samples of central line, tissue and high vaginal swab exhibited less prevalence (1.96% each) and samples collected from CSF, ICD fluid and pleural fluid contributed the least prevalence *i.e.*, 0.98% each [Table 1].

On the basis of morphological and biochemical screening five different pathogens were isolated included *K. pneumoniae, E. coli, A. baumannii, E. cloacae* and *S. marcescens*. Figure 1 depicts the detailed side view of the various *carbapenem* resistant clinical pathogens isolated from various clinical samples. Among all the five pathogens, *K. pneumoniae* (61.76%) was found to be most prevalent in all the clinical samples followed by *E. coli* (18.63%) and *A. baumannii* (14.71%) while *E. cloacae* (1.96%) and *S. marcescens* (2.94%) were least prevalent [Figure 2]. Akter *et al.* Revealed through their study that in recent few years *K. pneumoniae* has become a major and frequent opportunistic pathogen in hospital-associated infections.[18] Chaudhary *et al.* Have shown extreme prevalence of *K. pneumoniae* (63.1%) which favours our present study.[19] Our data demonstrated 18.63% prevalence of *E. coli* which is in accordance with Chaudhary and Payasi who determined 18.6% presence of *E. coli* among all clinical specimens.[20] Our present data showed the 13.7% prevalence of *A. baumannii* which is supported by Darvishi who demonstrated 13.3% occurrence of *A. baumannii* in clinical infectious samples.[21] Kumar *et al.* in their study observed 0.35% E. cloacae and 2.24% *S. marcescens* prevalence in clinical samples which confirms our present report.[22]

Table 2 represented the prevalence of different clinical isolates in different samples. *K. pneumoniae* has shown maximum occurrence in sputum (77.8%) and urine (67.9%). It has been considered as the major source for hospital acquired infections especially in nosocomial diseases. Romanus and Egwu reported that among all the clinical samples studied, sputum was containing highest percentage of Klebsiella spp (47.1%).[23] The same data was supported by Shilpa *et al.* who also determined the high ratio of *K. pneumonia* isolated from sputum (23%).[24] Barakzahi *et al.* Observed the high frequency of *K. pneumoniae* in urine (50%) which is in accordance with our findings.[25]



A-Urine, B-Wound, C-ET secretion, D-Central line, E-Tissue, F-CSF, G-Tracheal secretion, H-ICD fluid, I-Ascitic fluid, J-Bile, K-Sputum, L- Pus, M-High Vaginal swab, N-Pleural fluid

Figure 1: Profile of different carbapenem resistant pathogens isolated from various samples

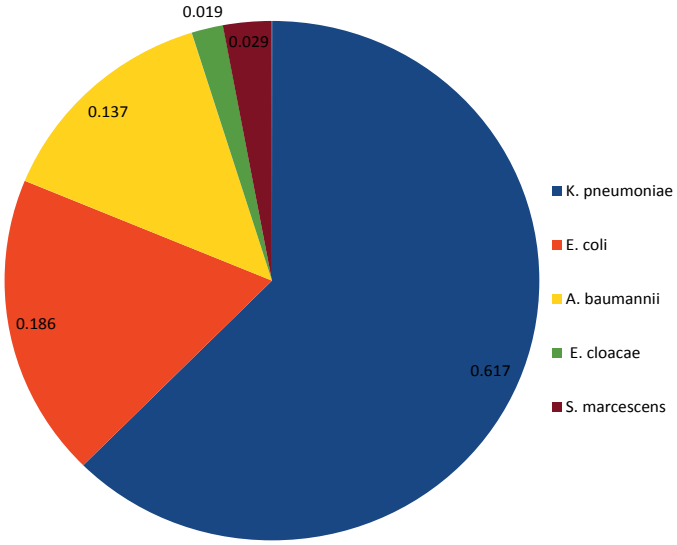


Figure 2: Prevalence of various carbapenem resistant isolates

Present data reported extreme prevalence of *E. coli* in urine (24.5%) and wound (16.7%). The prevalence of ESBL producing *E. coli* has augmented globally and have become the leading root of treatment failure in ICUs. Mehta *et al.* Reporting high prevalence of *E. coli* in urine (40%).[26] Kibret *et al.* Have shown 18.7% prevalence of *E. coli* in wound samples.[27] On the other hand *A. baumannii* was the most prevalent in ET secretion (71.4%) followed by tracheal secretion (22.2%), wound (22.2%) and sputum (22.2%). *A. baumannii* can be found in respiratory, urinary, gastrointestinal tract and wound samples and displays a particular penchant for the ICU patients (Towner). Jaggi *et al.* During their study, isolated maximum isolates of *A. baumannii* from respiratory secretions (57.4%).[28] Kaur *et al.* Also reported the supreme prevalence of *A. baumannii* in ET secretion and sputum.[29]

S. marcescens were observed in tracheal secretion (22.2%) while *E. cloacae* were found in urine (3.7%). Vetter *et al.* reported a nosocomial eruption of **S. marcescens** in respiratory samples predominantly from suspected patients in ICU which are acknowledged as a causative agent of intense nosocomial and surgical site infections[30] and Leski *et al.* Displayed the presence of *E. cloacae* in urine samples of the patients which coincides with our findings.[31]

Table 2 depicts the prime prevalence (50-100%) of *K. pneumoniae* in the clinical samples including urine, central line, CSF, bile, sputum,

Table 1: A profile of clinical samples used as a source of the carbapenem resistant pathogens

Sr. No.	Name of clinical samples	Number of Pathogens (%)
1	Urine	53 (51.96)
2	Wound	6 (5.88)
3	Endotracheal tube (ET) Secretion	7 (6.86)
4	Central Line	2 (1.96)
5	Tissue	2 (1.96)
6	Cerebrospinal fluid (CSF)	1 (0.98)
7	Tracheal secretion	9 (8.82)
8	ICD fluid	1 (0.98)
9	Ascetic fluid	4 (3.92)
10	Bile	2 (1.96)
11	Sputum	9 (8.82)
12	Pus	3 (2.94)
13	High vaginal swab	2 (1.96)
14	Pleural fluid	1 (0.98)
	Total	**102**

Table 2: Prevalence of different carbapenem resistant isolates in different samples

Samples	Clinical isolates					
	No. of isolates	*K. pneumoniae (%)*	*E. coli (%)*	*A. baumannii (%)*	*E. cloacae (%)*	*S. marcescens (%)*
Urine	**53**	36 (67.9)	13 (24.5)	1 (1.9)	2 (3.7)	1 (1.9)
Wound	**6**	3 (50)	1 (16.7)	2 (33.3)	0	0
ET Secretion	**7**	2 (28.6)	0	5 (71.4)	0	0
Central Line	**2**	2 (100)	0	0	0	0
Tissue	**2**	1 (50)	0	1 (50)	0	0
CSF	**1**	1 (100)	0	0	0	0
Tracheal secretion	**9**	4 (44.4)	1 (11.1)	2 (22.2)	0	2 (22.2)
ICD fluid	**1**	0	0	1 (100)	0	0
Ascitic fluid	**4**	1 (25)	3 (75)	0	0	0
Bile	**2**	2 (100)	0	0	0	0
Sputum	**9**	7 (77.8)	0	2 (22.2)	0	0
Pus	**3**	2 (66.7)	0	1 (33.3)	0	0
High vaginal swab	**2**	1 (50)	1 (50)	0	0	0
Pleural fluid	**1**	1 (100)	0	0	0	0
Total	**102**	**63**	**19**	**15**	**2**	**3**

pus and pleural fluid where Elores (AAE) exhibited 100% susceptibility against *K. pneumoniae* as compared to the other antibiotics (*tigecycline, piperacillin/tazobactam, cefoperazone+sulbactam* and *cefepime+tazobactam*). Likewise Elores contributed similar output (100% sensitivity) in the case of *A. baumannii* which displayed high prevalence (50-100%) in ICD fluid, ET secretion and tissue samples. From the present study it is evident that Antibiotic adjuvant therapy is superior to other commonly used classes of antibiotics (protein synthesis inhibitors, BL-BLI) in *carbapenem* resistant strains [Table 3].

Susceptibility pattern for isolated pathogens from clinical samples was analyzed and the data suggested that all the five pathogens (*K. pneumoniae, E. coli, A. baumannii* and *E. cloacae* and *S. marcescens*) were found susceptible with 100% antimicrobial activity towards Elores which was comparable to colistin to which clinical isolates such as *K. pneumoniae* (87.3%), *E. coli* (100%), *A. baumannii* (100%) and E. cloacae (100%) also displayed high susceptibility. However few studies have shown that colistin is a toxic drug with adverse renal and neurological effects which is not considered safe to the patients. [32] Earlier studies have also documented the greater susceptibility of Elores, the novel antibiotic adjuvant entity, against various clinical pathogens.[22,33,34]

In the present study we observed *carbapenem* resistant *K. pneumoniae* exhibited a very high resistance against commonly used drugs such as *cefepime+tazobactam* (92.1%), *piperacillin+tazobactam* (98.4%), *cefoperazone+sulbactam* (100%) and tigecycline (46.1%) [Table 3]. Further, *carbapenem* resistant *E. coli* showed second highest resistance against *cefepime+tazobactam* (84.2%), *piperacillin+tazobactam* (100%) and *cefoperazone+sulbactam* (100%). Similarly, *carbapenem* resistant *A. baumannii* expressed resistance towards *cefepime+tazobactam* (66.7%), *piperacillin+tazobactam* (93.3%) and *cefoperazone+sulbactam* (40%). Furthermore, least prevalent *carbapenem* resistant E. cloacae were determined to be 100% resistant against *cefepime+tazobactam*, piperacillin+tazobactam, *cefoperazone+sulbactam*. Data also suggested that *carbapenem* resistant *S. marcescens* expressed resistance against *cefepime+tazobactam* (66.6%), *piperacillin+tazobactam* (100%) and *cefoperazone+sulbactam* (100%). *S. marcescens* is inherently resistant to colistin.

The antibiotic resistance rate of clinical pathogens is on a rise in the last few decades. Sharif *et al.* Demonstrated resistance in *E. coli* (54%), *A. baumannii* (37%) and *K. pneumoniae* (67%) against cefepime.[35] Similarly, Mohammadi and Feizabadi, reported high resistance in gram negative microorganisms against *piperacillin+tazobactam* (60%).[36] Abdul *et al.* Have shown the colistin susceptibility among many clinical pathogens [*E. coli* (96.2%), *A. baumannii* (92.8%) and *K. pneumoniae* (93.5%)].[37] While, Kucukates and Kocazeybek, observed that Serratia spp. (67%), Enterobacter spp. (34%), Klebsiella spp. (32%) and *E. coli* (63%) were displaying resistance towards *cefoperazone+sulbactam*.[38] Fernandez-canigia and Dowzicky (2012) demonstrated more than 90%

Table 3: Susceptibility pattern of carbapenem resistant clinical isolates

	Susceptibility (%)																	
	Antibiotic Adjuvant Entity			*β-lactam and β-lactamase inhibitor combinations*									*Polymixins*			*Protein Synthesis Inhibitors*		
Clinical isolates	Elores			Cefepime+Tazobactam			*Piperacillin+Tazobactam*			*Cefoperazone+Sulbactam*			*Colistin*			*Tigecycline*		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
K. pneumoniae (n=63)	100	-	0	6.3	1.6	92.1	1.6	-	98.4	0	-	100	87.3	-	12.7	34.7	19.2	46.1
E. coli (n=19)	100	-	0	15.8	-	84.2	0	-	100	0	-	100	100	-	0	100	-	0
A. baumannii (n=15)	100	-	0	20	13.3	66.7	6.7	-	93.3	20	20	40	100	-	0	92.9	7.1	0
E. cloacae (n=2)	100	-	0	0	-	100	0	-	100	0	-	100	100	-	0	-	-	-
S. marcescens (n=3)	100	-	0	33.3	-	66.6	0	-	100	0	-	100	0	-	100	50	-	50

of pathogens includes; *S. marcescens*, *A. baumannii* and Klebsiella spp. were found to be susceptible towards tigecycline irrespective to ESBL producers or non-producers.[39]

Carbapenems are treated as the last resort to treat serious infected patients. *Carbapenem* drugs were previously shown to have high antimicrobial activity against ESBL producing organisms[40] but recent reports revealed the non-susceptibility of many pathogens towards *carbapenem* antibiotics.[41] This may be due to the wide use of these antibiotics as empirical therapy for the treatment of infectious diseases.

Our present data displayed Elores (Antibiotic adjuvant entity) has greater susceptibility against *carbapenem* resistant isolates which was found unbeatable compared to other antibiotics as discussed above. Penems, BL-BLIs are the commonly used and popular antibiotics and exhibit antimicrobial activity by inhibiting cell wall synthesis but still clinicians face the problem of resistance throughout the world.[42] Many studies documented penems and protein synthesis inhibitors probably have raised resistance among pathogens by over expression of efflux pumps and impairment in the permeability of cell wall[43-45] whereas *β-lactam* and *β-lactamase* inhibitors have failed to prevent inactivation via MBLs thus enhancing resistance among pathogens.[36,38,42,46] On the other hand previous studies revealed that Elores (Antibiotic adjuvant entity) has proved its resistance breaking efficacy against most of the pathogens via enhanced penetration through addition of EDTA as adjuvant, biofilm breakage abilities, greater stability and ability to down regulate the over expression of efflux pump.[47-50] Previous data also demonstrated the importance of Elores in the treatment of skin and skin structure infections and bone and joint infections.[51] Therefore, Elores (Antibiotic adjuvant entity) is emerging in the present era as an impactful drug to treat various infectious diseases and combat antibiotic resistance.

CONCLUSION

In the light of above discussion, it is evident that Antibiotic Adjuvant Therapy scored over protein synthesis inhibitors and *β-lactam* and *β-lactamase* inhibitor combinations against *carbapenem* resistant pathogens due to its resistance breaking mechanisms. In comparison to the antibiotics used for investigating the antibiogram profile of clinical isolates, Elores exhibited prime susceptibility (100% each) which is found comparable to colistin and has again established a mark by getting over antibiotic resistance crisis. Hence Elores is efficient enough to target drug resistant pathogens and indicates a most effective remedy to treat the infections caused by various pathogens and can be considered as empiric choice to spare penems, protein synthesis inhibitors, BL-BLI. Colistin being a highly nephrotoxic drug should be used only when no other choice is available.

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