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Prevalence and Susceptibility Analysis of Gram Negative Pathogens in Super Specialty Tertiary Care Centers, Pune in India from January 2018- January 2019

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Background and Objective: The encumbrance of antimicrobial resistance worldwide is substantive and likely to rise without any appropriate treatment which 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 758 clinical isolates collected from Pawana Hospital and Accord SDH, Pune (India), towards Elores (a novel antibiotic and adjutant entity of ceftriaxone, sulbactam and disodium edetate) and other antibiotics (imipenem, meropenem and piperacillin + tazobactam).
Methods: The clinical samples collected from outpatients and inpatients during a period of one year (January, 2018 to January, 2019), from Pawana Hospital and Accord SDH, Pune (India) and were further subjected to bacterial identification. Antibiotic susceptibility testing was executed in accordance with the recommendations of Clinical Laboratory Standards Institute (CLSI) guidelines.
Results: Out of 758 collected samples, urine samples contributed 69.41 and 37.63% among Enterobacteriaceae and Non-Enterobacteriaceae followed by pus (20.25 and 19.35%) and sputum (4.2 and 19.89%) while <4% and <12% Enterobacteriaceae and Non-Enterobacteriaceae isolates were collected from rest of the specimen. *E. coli* were found most prevalent (50.13%) along with 19.13 and 16.09% prevalence of *Pseudomonas* spp. and *Klebsiella* spp. whereas rest of the

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pathogens were <7% present. The antibacterial activity of Elores (87.80%) was observed superior to carbapenem drugs (meropenem; 62.67% and imipenem; 60.95%) and far better to piperacillin + tazobactam (48.42%) against 758 clinical pathogens. Antibiogram profile depicted Elores as most susceptible (95.10%) drug towards Enterobacteriaceae isolates which was approximately 31-50% more sensitive than other test drugs. Similar pattern was obtained for Non-enterobacteriaceae isolates (62.90%) where Elores contributed approximately 1-5% higher activity. However, Elores also conquered 79.51-86.59% resistance among meropenem, imipenem and piperacillin + tazobactam resistant pathogens.

Conclusion: Susceptibility profile data revealed the equivalence of Elores (Antibiotic-adjuvant entity; AAE) with carbapenem drugs (meropenem and imipenem) and superiority over piperacillin + tazobactam against clinical pathogens. Elores was also found active towards meropenem, imipenem and piperacillin + tazobactam resistant pathogens. Therefore, Elores, a resistance breaker, can be used as an efficient treatment alternate towards infections caused by resistant pathogens.

Keywords: Elores; clinical isolates; susceptibility.

1. INTRODUCTION

Antibiotic resistance is a significant health, social and economic problem at this time which has become a biological risk, increases morbidity and mortality of mankind [1]. It has been estimated that by 2050, 10 million lives a year will be at risk due to emergence of the infections raised by antimicrobial resistant pathogens [2]. Nowadays some of the infectious diseases have become virtually untreatable due to these resistant super bugs which are a matter of deep concern to the clinicians. Though, dealing with gram positive resistant pathogens is important concern but presently the continuous spread of resistant gram negative bacteria is currently the most imperative emerging issue. Antibiotic resistance issue is perturbed for the several reasons including treatments options are limited, resistance has spread widely on several fronts like horizontal gene transfer, pose significant challenges for infection control, associate with increased mortality and economic cost. Three particular problematic gram negative pathogens which are excessively obsessed with resistance are identified includes Enterobacteriaceae, Acinetobacter spp. and Pseudomonas spp. [3]. Earlier reports have revealed 66-80% resistance among gram negative bacteria against cephalosporin drugs and ß lactam- ß lactamase inhibitor (BL-BLI) antibiotics [4,5,6,7]. Recently, resistance was also observed up to 50-61% among clinical isolates towards third generation cephalosporins [8] and resistance of 17 to 38% resistance among gram negative pathogens towards aminoglycosides [9].

Rise in fluoroquinolone resistance among Enterobacteriaceae causing community acquired or healthcare associated urinary tract infections and intraabdominal infections, exceeding 50% was also reported in some parts of the world, particularly in Asia [10]. Resistance to imipenem and other carbapenems have also been studied globally among clinical bacterial isolates [11,12]. Mechanism behind antimicrobial resistance in these microorganism includes production of extended spectrum ß lactamases (ESBL) and metallo ß lactamase (MBL), changes in membrane permeability, over-expression of efflux pump and production of biofilms etc. [13,14,15,16].

Overuse of antibiotics is considered as the prime reason for hike in antimicrobial resistance. Many countries like BRICS (Brazil, Russia, India, China and South Africa) have accounted for 3/4th of total usage of antibiotic in the world [17]. Likewise ß lactam antibiotics usage reports for 50% of global antibiotic consumption [6]. Considering the rise in the predominance of multidrug resistant pathogens globally, there is a need to study the prevalence and susceptibility profile of various pathogens. Surveillance studies provide important information that allows for the identification of trends in pathogen incidence and antimicrobial resistance, including identification of emerging pathogens at national and global levels so there is a need of routine surveillance because it ensures the accurate information in order to establish, modifies the treatment guidelines and guides the clinicians for the prescription of appropriate empirical antimicrobial therapy. In view of the above data, the increasing rate of the antibiotic resistance and its impact on treatment failure compelled to screen alternative approaches by which the increasing mortality rate because of failure of drug therapy can be controlled. The concept of adjuvant addition to antibiotic (Elores) has been recently introduced

and a great success has been reported. Hence, we aimed to compare the susceptibility profiles of different drugs such as imipenem, meropenem and piperacillin + tazobactam with Elores towards different clinical bacteria isolated from various clinical samples at Pawana Hospital and Accord SDH, Pune (India).

2. MATERIALS AND METHODS

2.1 Sample Collection

Different clinical samples such as bronchoalveolar fluid, body secretion, urine, blood, pus swabs and sputum specimen were collected from patients in a period of one year (January, 2018 to January, 2019), from Pawana Hospital and Accord SDH, Pune (India).

2.2 Isolation and Identification of Microbes

All the samples were collected aseptically in sterile containers in sufficient amount and inoculated on the different selective and non-selective culture media as per the standard microbiological techniques (Table 1). The collection and processing of the samples were done as per a common Standard Operating Procedures.

2.3 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was done by Kirby– Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [18]. In brief, 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

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

Sr. no.	Pathogen	Selected media
1	Acinetobacter spp.	Leeds Acinetobacter agar base medium
2	Citrobacter spp.	Eosine Methylene Blue (EMB) agar medium
3	E. coli	EMB agar medium
4	Enterobacter spp.	EMB agar medium
5	Klebsiella spp.	Hicrome Klebsiella selective agar base medium
6	Morganella spp.	MacConkey medium
7	Pseudomonas spp.	Citrimide agar
8	Proteus spp.	EMB agar and Mcconkey's agar
9	Providencia spp.	MacConkey medium

Table 2. Comparative zone diameters used for interpreting as susceptible, intermediate or resistant

Drugs	Microorganisms	Zone of diameter (mm)		
		Susceptible	Intermediate	Resistance
Elores (ceftriaxone/sulbactam/EDTA)	Enterobacteriaceae	≥23	20-22	≤19
Pip+Taz		≥ 21	18–20	≤ 17
Imipenem		≥23	20-22	≤19
Meropenem		≥23	20-22	≤19
Elores	Pseudomonas spp.	≥ 21	14-20	≤ 13
Pip+Taz		≥ 21	15–20	≤ 14
Imipenem		≥19	16-18	≤15
Meropenem		≥19	16-18	≤15
Elores	Acinetobacter spp.	≥ 21	14-20	≤ 13
Pip+Taz		≥ 21	18–20	≤ 17
Imipenem		≥22	19-21	≤18
Meropenem		≥18	15-17	≤14

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. Antibiotics sensitivity disc of Elores (45 µg) were obtained from Abtek while rest of the antibiotic discs such as piperacillin-tazobactam (110 µg), meropenem (10 µg) and imipenem (10 µg) were obtained from HiMedia, India. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI) as well as in-house for Elores.

3. RESULTS AND DISCUSSION

Infections caused by gram negative bacteria are a matter of concern worldwide. Due to extensive use of antibiotics and lapses in effective infection control measures, resistance among clinical pathogens is rising. For overcoming resistance, it's significant to monitor the change in susceptibility pattern among isolates towards antibacterial agents in the different hospitals of the world.

In the present study, numerous gram negative clinical isolates (n= 758) were collected from Pawana Hospital and Accord SDH, Pune in a period of one year. Out of which, 572 were Enterobacteriaceae and 186 were Non-Enterobacteriaceae. The extreme prevalence of clinical isolates was observed in urine samples (69.41% Enterobacteriaceae and 37.63% Non-Enterobacteriaceae) followed by pus (20.45% Enterobacteriaceae and 19.35% Non-Enterobacteriaceae), sputum (4.2% Enterobacteriaceae and 19.89% Non-Enterobacteriaceae). Table 3 depicts the prevalence percentage of clinical pathogens among different clinical samples. Present results were correlated and also noted similar findings [19,20].

On the basis of morphological and biochemical screening most prevalent pathogens (50.13%) were *E. coli*, *Pseudomonas* spp. (19.13%), *Klebsiella* spp. (16.09%), Enterobacter spp. (6.07%) and Acinetobacter spp. (5.41%). Many studies have also revealed that gram negative bacteria as a major opportunistic and frequent pathogens and are extremely prevalent in hospital-associated infections which favors recent study [17,21]. Clinical pathogens like Proteus spp., Morganella spp., Providencia spp. and Citrobacter spp. were found least prevalent <2% (Fig. 1). Incidences of these isolates in clinical specimens corroborates with earlier studies [22,23,24,25].

Antibiogram profile of all the pathogens obtained from clinical specimens is presented in Fig. 2. Present data suggests higher activity of Elores (87.80%) against clinical isolates and found 26-40% superior over other tested drugs such as meropenem (62.67%), imipenem (60.95%) and piperacillin+tazobactam (48.42%) (Fig. 2). Similar pattern was observed towards

Table 3. A profile of clinical samples used as a source of the pathogenic isolates

Sr. no.	Specimen	Enterobacteriaceae (%)	Non-Enterobacteriaceae (%)
1	Bronchoalveolar fluid	0.70	8.6
2	ET secretion	3.32	11.83
3	Ascitic fluid	0.17	-
4	Fluid Appendicular Abscess	0.17	-
5	CSF	0.17	-
6	Peritoneal fluid	0.52	-
7	Permanent catheter	0.17	0.54
8	Tissue	0.17	0.54
9	Blood	0.35	0.54
10	Bed sore swab	0.17	-
11	Vaginal swab	-	0.54
12	Wound swab	-	0.54
13	Pus	20.45	19.35
14	Sputum	4.2	19.89
15	Urine	69.41	37.63
Total (n)	758	572	186

Enterobacteriaceae isolates where Elores was the most susceptible drug (95.10%) followed by meropenem (63.11%), imipenem (60.83%) and piperacillin+tazobactam (45.45%) (Fig. 3). On the other hand, Non-Enterobacteriaceae isolates were most sensitive towards Elores (62.90%) which was comparable to the carbapenem drugs (meropenem and imipenem; 61.29% each) and found 5.37% more active than pipercillin+tazobactam (57.53%). Data also revealed that 20.97% of the Non-Enterobacteriaceae isolates were falling into intermediate range for Elores which was 13-18% higher than rest of the test drugs (Fig. 4).

Earlier studies also reported >60% sensitivity of imipenem and meropenem drugs against gram negative isolates which supports present data [26,27]. Likewise, similar results (22-78%) were observed by for gram negative pathogens against piperacillin+tazobactam [28].

Fig. 5 depicts the resistance breaking efficacy of Elores towards meropenem (79.51%), imipenem (82.69%) and pipercillin+tazobactam (86.59%) resistant clinical isolates. Many authors have also observed the greater susceptibility (70-95%) of Elores against various Enterobacteriaceae and Non-enterobacteriaceae isolates [24,29,30].

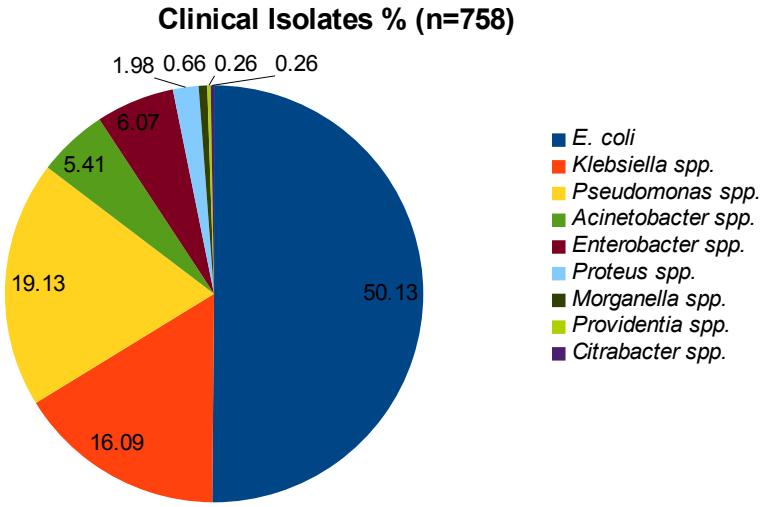

Fig. 1. Prevalence percentage of clinical pathogens among different clinical samples

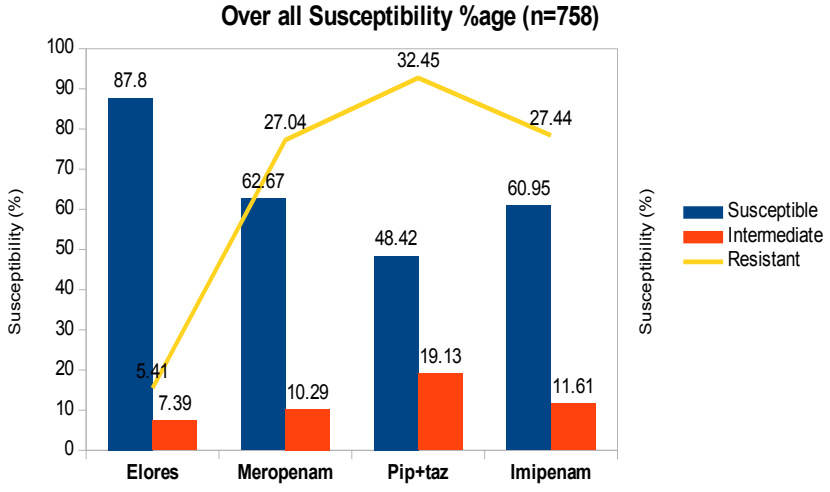

Fig. 2. Susceptibility pattern of clinical isolates towards different antibacterial agents

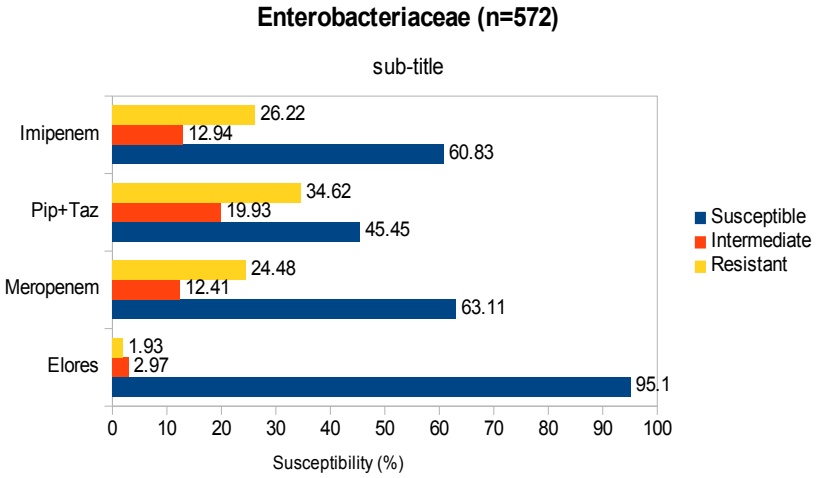

Fig. 3. Susceptibility pattern of Enterobacteriaceae towards different antibacterial agents

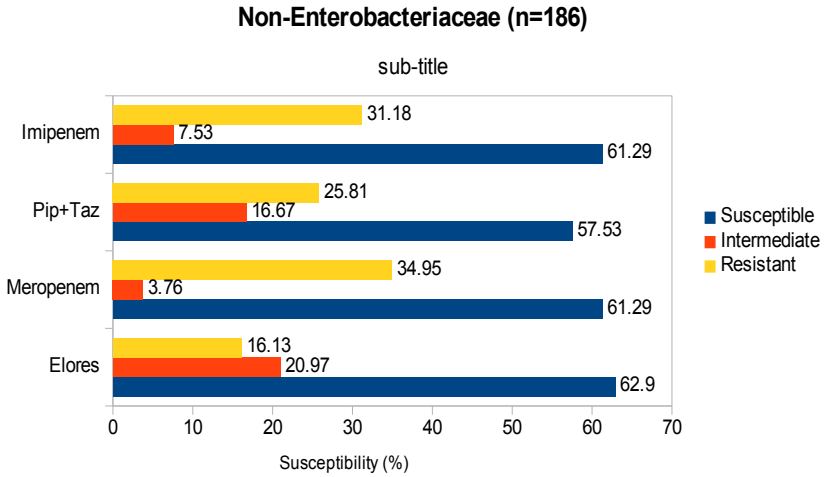

Fig. 4. Susceptibility pattern of Non-enterobacteriaceae towards different antibacterial agents

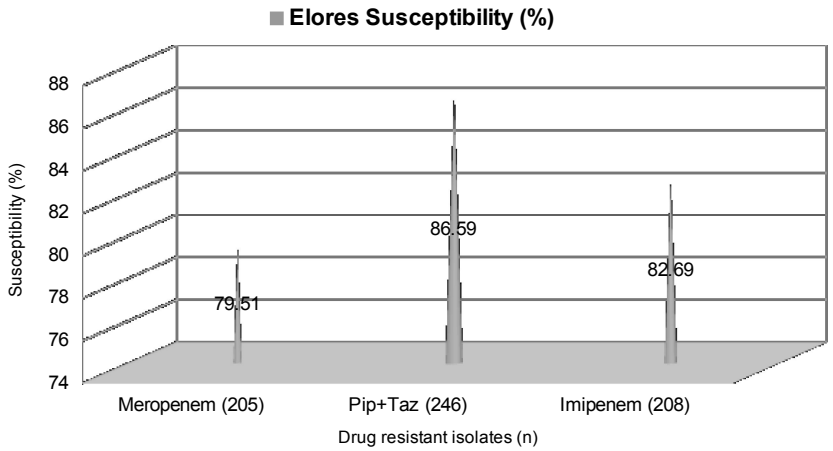

Fig. 5. Susceptibility pattern of Elores among drug resistant clinical isolates

Earlier studies documented carbapenems and BL-BLI drugs probably have raised resistance among pathogens by over expression of efflux pumps, impairment in the permeability of cell wall and production of ESBL, MBL and biofilm [13,14,15,16]. However, the higher susceptibility of Elores may be attributed to its ability to break antibiotic resistance via various mechanisms. Elores exhibited the highest permeability coefficient, enhanced penetration, greater stability and periplasmic concentration leading to higher susceptibility. Disodium edetate, a component of Elores, is a non-antibiotic adjuvant entity which helps to reduce antibiotic resistance via, down regulating over expression of efflux pumps, breaking biofilms and chelation of ions required for MBL producing bacteria. CSE-1034 has probably high susceptibility rates among multi-drug resistant Gram-negative bacteria due to the presence of antibiotic resistance breaker, i.e. EDTA which interferes with the stability of outer membrane of microbes via chelating the cations and increasing the permeability of the antibiotics. The disodium edetate present in Elores enhanced permeability of ceftriaxone and sulbactam and thereby enhancing activity against ESBL microbes synergistically. Disodium edetate chelates the divalent ions required for the activity of MBLs thus de-activating the MBLs which in turn enhanced susceptibility of Elores towards MBLs producing organisms. The down-regulation of efflux pump following treatment with EDTA is probably due to chelation of calcium ions which results in disturbance in ATP production. It has earlier been reported that ATPases that hydrolyze ATP are calcium dependent. Chelation of calcium downregulates the efflux pumps mechanism and allows ceftriaxone to remain in the bacterial periplasmic space and attach to the PBP. The addition of EDTA with ceftriaxone plus sulbactam is uniquely useful in disrupting the biofilm and synergistically eradicating organisms from the biofilm environment. EDTA chelates with divalent ions present in sessile microbial cells and EPS (extracellular polymeric substances) of biofilms thus making the membrane more porous and susceptible for antibiotics. Hence, Elores is effective in multi drug resistant gram negative pathogens [31,32,33,34].

In the light of above discussions, it is evident that Elores (a novel Antibiotic adjuvant entity) can be considered as a choice of drug in the treatment of infective diseases caused by multidrug resistant gram negative pathogens.

4. CONCLUSION

These results highlight that the antibacterial activity of Elores to be the most susceptible (95.10%) towards Enterobacteriaceae isolates, which was approximately 31-50% more sensitive than other test drugs. Similar pattern was obtained for Non-enterobacteriaceae isolates (62.90%) where Elores contributed approximately 1-5% higher activity. More than 79% shift has also been observed from imipenem, meropenem and piperacillin + tazobactam resistant isolates towards Elores susceptible. Therefore, Elores (Antibiotic Adjuvant Entity) can be used as an alternate in clinical settings to target drug-resistant bacteria due to its resistance breaking efficacy.

CONSENT

As per international standard written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard written and informed ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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