

Original Research Article

Prevalence and susceptibility analysis of carbapenem resistant Gram negative pathogens in super specialty tertiary care center, Mumbai, India

ABSTRACT

Aim: Taking into account the rise in mortality rate due to multidrug resistant gram negative bacteria, we purposed to analyze the susceptibility behavior of 74 carbapenem resistant clinical isolates towards common antibiotic classes [sulphomide, β -lactam (BL), β -lactam and β -lactamase inhibitor combination (BL-BLI), aminoglycoside, cephalosporin, quinolone, peptide and glycylycylcline] with a novel antibiotic-adjuvant entity (AAE) [CSE-1034; ceftriaxone, sulbactam and disodium edetate].

Place and duration: The different clinical samples were collected from the infected patients admitted to Super Speciality tertiary care center, Mumbai (India) between January 2015 to December 2015.

Methodology: Collected specimens were further subjected to bacterial identification and susceptibility examination.

Results: 74 carbapenem resistant clinical isolates with MIC>16 against imipenem and meropenem were used in this study. *Klebsiella pneumoniae* (47.30%) was found to be the most common pathogen among all clinical isolates followed by *Acinetobacter baumannii* (%), *Escherichia coli*(%) and

Pseudomonas aeruginosa (<18%) while *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae* and *Pseudomonas luteola* possessed <5% prevalence (Authors are expected to give precise % of used isolates). Antibigram data

suggested colistin as the most susceptible (100% each) drug against all resistant isolates followed

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24 by tigecycline (60-100%) (State precise %). While CSE-1034 was determined as third most susceptible drug and

when compared with rest of the antibiotics (sulphomide, BL, BL-BLI, aminoglycoside, cephalosporin and quinolones) showed 18.16, 31.39, 7.7, 37.5 and 50% enhanced antimicrobial activity against *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *E. cloacae*. (Authors are expected to be precise with the antimicrobial used in the classes of the antibiogram for further studies and references).

Keywords: Antibiotic, Clinical isolates, CSE-1034, Prevalence, Susceptibility, Resistance.

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30 1. INTRODUCTION

Approximately, 71.8% infections in India have been caused by gram negative bacteria, with *Klebsiella* species scoring for 16.4%, *Pseudomonas* species 13.6%, *E. coli* 11.8%, *Enterobacter* spp. (species) 11.4% and *Acinetobacter* spp. 10% [1]. To treat these infections a variety of antibiotics have

been used in the past but currently the trend is changing with their efficacy being seriously compromised by inappropriate and irrational use. This has led to the emergence of bacteria that are resistant to many commonly used antibiotics and doctors are left with no option (Not referenced). Hence, there is an

urgent need for the evaluation of resistant microbes that have surpassed the development of antibiotics. 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 (Not referenced). 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 (Not referenced). Earlier

studies have identified three particular problematic gram negative pathogens which are excessively obsessed with resistance includes *Enterobacteriaceae*, *Acinetobacter* spp. and *Pseudomonas* spp.

47 [2].

48 Sharma **et al.** (Not italicize), (2007) performed a susceptibility study of ceftriaxone, cefotaxime,
 amoxicillin,
 49 cephalixin, ciprofloxacin, gentamicin on *E. coli* and found 80.9, 82.9, 97.9, 89.4, 74.5 and 74.5 %
 50 resistant, respectively ten years ago [3]. Since then susceptibility has been continuously declining.
 51 The rate of resistance of other *Enterobacteriaceae* to commonly used antibiotics is now constantly
 52 above 70% [4]. Likewise *P. aeruginosa* resistant rate is also increasing and reached up to 40%
 53 whereas resistance rates are equally on the rise in *A. baumannii* (70%) [5]. Other studies also
 54 highlighted a high resistance rates of gram negative against cotrimoxazole (83.5%), ceftriaxone
 55 (83.1%), ceftazidime (80.8%), cefotaxime (78.1%), cefoperazone+sulbactam (30-66%) amoxyclav
 56 (80.4%), piperacillin (77.6%) and meropenem (32-67%) [6,7].
 57 The broad spectrum antibiotics such as novel β -lactam and β -lactamase inhibitor (BL-BLI)
 58 combinations and penems considered superior over BL-BLI are supposed to treat variety of serious
 59 infections caused by these multidrug resistant pathogens. A hike in the resistance among gram
 60 negative pathogens was observed against these antimicrobial agents in recent past
 (Not referenced).
 61 Ampicillin/sulbactam resistance (86-100%) was seen by Patil **et al.**, (2013) whereas Wasihun **et al.**,
 62 (2015) reported the resistance (80-100%) against amoxicillin/clavulanic acid among gram negative
 63 bacteria [8,9]. Similarly a rise in resistance was reported with piperacillin+tazobactam (up to 60%),
 64 cefoperazone+sulbactam (30-70%) and meropenem (70-98%) [10,11] in gram negative bacteria.
 65 Thus, the spread of multidrug resistance by gram negative bacteria is now seen as a globalized
 66 threat.
 67 In view of the above data, the increasing rate of the antibiotic resistance and its impact on treatment
 68 failure compelled us to screen alternative approaches by which the increasing mortality rate because
 69 of failure of drug therapy can be controlled. The concept of adjuvant addition to antibiotic (CSE-
 70 1034) has been recently introduced and a great success has been reported. Hence, we aimed to
 71 compare the susceptibility profiles of different drug classes such as sulphomide, BL, BL-BLI,

aminoglycoside, cephalosporin, quinolone, peptide and glycylicline with a novel antibiotic adjuvant entity (CSE-1034) towards different carbapenem resistant gram negative bacteria isolated from various clinical samples.

2. MATERIALS AND METHODS

2.1 Sample collection

Different clinical samples such as urine, blood, wound, sputum, tracheal secretion and pus were collected from suspected patients having bacterial infection during a period of one year (January to December 2015), from Super Specialty tertiary care center, Mumbai (India). The collection and processing of the samples were done as per a common standard operating procedure of hospital.

2.2 Isolation and identification of microbes

All the samples were collected aseptically in sterile containers. All the clinical specimen were collected in sufficient amount and inoculated on the different selective and non-selective culture media as per the standard microbiological techniques (Not referenced). Enterobacteriaceae-N280 and *Pseudomonas* group-N281 cards (bioMérieux, France) were used for the detection of *Enterobacteriaceae* and *Pseudomonas spp.* while *A. baumannii* was screened out by using leeds acinetobacter agar base medium as a selective media.

2.3 Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by Broth microdilution method using Vitek 2 as recommended by the CLSI guidelines [12]. The disc diffusion method was used to determine the susceptibility of CSE-1034 towards clinical isolates. The discs of CSE-1034 (45 µg) were obtained from HiMedia, India.

3. RESULTS AND DISCUSSIONS

In 1 year study period numerous gram positive and negative clinical isolates were collected. Out of which, 74 carbapenem resistant gram negative isolates were used in this study. The extreme

prevalence of clinical isolates was observed in urine samples (37.84%) followed by sputum and tracheal secretion (13.51% each) then wound and pus (12.16% each) and blood (10.81%) samples (Table 1).

Table 1. A profile of clinical samples used as a source for the isolation of carbapenem resistant isolates.

Sr. no.	Name of clinical samples	Number of pathogens (%)
1	Urine	28 (37.84)
2	Blood	8 (10.81)
3	Wound	9 (12.16)
4	Sputum	10 (13.51)
5	Tracheal secretion	10 (13.51)
6	Pus	9 (12.16)
	Total	74

Eight different carbapenem resistant gram negative bacteria with MIC >16 against imipenem and meropenem as per CLSI guidelines were obtained in this study including *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *C. freundii*, *E. aerogenes*, *E. cloacae* and *P. luteola*. Table 2 portrayed an outlook of various clinical pathogens isolated from different clinical samples. Among eight clinical isolates, *K. pneumoniae* (47.30%) isolates have shown highest prevalence in all clinical specimens followed by *A. baumannii* (17.57%) and *E. coli* (14.86%). Moini [et al.](#), (2015) have revealed the prime occurrence of *K. pneumoniae* (46.4%) which favors our present data [13]. Ezech [et al](#) (Not italicize)., (2016)

showed 19.59% presence of *A. baumannii* during their study which confirms our prevalence value [14]. Present report revealed the existence of *E. coli* i.e. 14.86% which is lower than what is usually reported but is in accordance with Kibret and Abera, (2011) who determined 14.2% prevalence of *E. coli* [15]. Our data displayed 10.81% and 4.05% prevalence of *P. aeruginosa* and *C. freundii* which coincides with a few studies who demonstrated 8.2 and 3.4% presence of *P. aeruginosa* and *C. freundii* in all clinical samples [16]. Present study also depicted less than 3% prevalence of *E. aerogenes*, *E. cloacae* and *P. luteola* which is in agreement with other studies (Give reference value) [17]. Table 2 depicted the prevalence of clinical pathogens in different clinical specimen. *K. pneumoniae* was found predominantly in urine (67.8%) and sputum (50%) samples. Similar data was supported by Makkar et al., (2015) who also obtained prime ratio of *K. pneumoniae* in urine samples (Give reference value) [18].

Table 2. Prevalence of different carbapenem resistant clinical isolates in different samples.

Clinical isolates (%)									
Clinical samples	No. of isolates	<i>E. coli</i> (%)	<i>K. pneumoniae</i> (%)	<i>A. baumannii</i> (%)	<i>P. aeruginosa</i> (%)	<i>C. freundii</i> (%)	<i>E. aerogenes</i> (%)	<i>E. cloacae</i> (%)	<i>P. luteola</i> (%)
Urine	28	5 (17.8)	19 (67.8)	3 (10.7)	2 (7.1)	2 (7.1)	0	2 (7.1)	1 (3.5)
Blood	8	1 (12.5)	3 (37.5)	1 (12.5)	0	1 (12.5)	0	0	0
Wound	9	1 (11.1)	3 (33.3)	2 (22.2)	0	0	1 (11.1)	0	0

Sputum	10	2 (20)	5 (50)	1 (10)	0	0	0	0	0
Tracheal secretion	10	1 (10)	3 (30)	5 (50)	1 (10)	0	0	0	0
Pus	9	1 (11.1)	2 (22.2)	1 (11.1)	5 (55.5)	0	0	0	0
Total	74	11 (14.86)	35 (47.30)	13 (17.57)	8 (10.81)	3 (4.05)	1 (1.35)	2 (2.70)	1 (1.35)

A. baumannii exhibited prime occurrence in tracheal secretion (50%), wound (22.2%) samples and up to 12% in other samples. Present study was in accordance with Jaggi [et al.](#), (2012) who reported the supreme prevalence of *A. baumannii* in tracheal secretion (57%) among all the clinical samples [19].

Present study (continuous repetition of connective words) demonstrated, *E. coli* were predominant in sputum (20%), urine (17.8%) and up to

12% in other clinical samples. Banu [et al.](#), (2011) documented the high prevalence of *E. coli* in sputum (29%) [20] whereas Mehta [et al.](#), (2012) observed high percentage of *E. coli* (40%) in urine [21].

Data also suggested *P. aeruginosa* strains have shown their high presence in pus (55.5%) samples followed by tracheal secretion (10%) and urine samples (7%) which is in accordance with Kaur [et al.](#), (2016) who also showed high prevalence of *P. aeruginosa* in pus (37%) samples among 118 clinical samples [16]. The least common pathogens including *C. freundii*, *E. aerogenes* and *E. cloacae* which were found prevalent in blood (12.5%), wound (11.1%) and urine (7.1%) respectively whereas *P. luteola* isolates were present in urine (3.5%) samples only.

136 Antibigram profile of carbapenem resistant pathogens obtained from clinical specimen is
137 presented in Table 3 and 4.

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139 **Table 3.** Susceptibility pattern of carbapenem resistant clinical isolates.

		Susceptibility (%)							
Clinical isolates		<i>E. coli</i> (n=11)	<i>K. pneumoniae</i> (n=35)	<i>A. baumannii</i> (n=13)	<i>P. aeruginosa</i> (n=8)	<i>C. freundii</i> (n=3)	<i>E. aerogenes</i> (n=1)	<i>E. cloacae</i> (n=2)	<i>P. luteola</i> (n=1)
Drug classes	Drugs								
AAE	CSE-1034	81.8	57.1	69.2	75	66.67	0	100	0
Sulphomides	Co-trimoxazole	54.5	14.29	7.69	0	66.67	0	0	0
BL	Ampicillin	0	0	0	0	0	0	0	0
BL-BLI	Amoxyclave	0	0	0	0	0	0	0	0
	Piperacillin-tazobactam	0	0	0	37.5	0	0	0	0
	Cefoperazone-sulbactam	0	0	61.5	12.5	0	0	0	0
Aminoglycoside	Gentamicin	36.36	8.57	30.76	37.5	0	0	50	0
	Amikacin	63.64	25.71	0	37.5	66.67	0	50	0

Cephalosporin	Ceftriaxone	0	0	0	0	0	0	0	0
	Cefuroxime	0	0	0	0	0	0	0	0
	Cefepime	0	0	0	25	0	0	0	0
Quinolone	Nalidixic acid	0	2.86	15.38	0	0	0	50	0
	Ciprofloxacin	0	2.86	15.38	25	0	0	50	0
	Levofloxacin	0	0	0	25	0	0	0	0
Peptide	Colistin	100	100	100	100	100	100	100	100
Glycylcycline	Tigecycline	90.91	60	84.62	0	100	0	100	0

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142 **Table 4.** Resistance pattern of carbapenem resistant clinical isolates.

(Table 4 is reverse of table 3; not necessary again)

Resistance (%)									
		<i>E. coli</i> (n=11)	<i>K. pneumoniae</i> (n=35)	<i>A. baumannii</i> (n=13)	<i>P. aeruginosa</i> (n=8)	<i>C. freundii</i> (n=3)	<i>E. aerogenes</i> (n=1)	<i>E. cloacae</i> (n=2)	<i>P. luteola</i> (n=1)
Drug classes	Drugs								
AAE	CSE-1034	18.2	42.9	30.8	25	33.33	100	0	100
Sulphomides	Co-trimaxazole	45.5	85.71	92.31	100	33.33	100	100	100
BL	Ampicillin	100	100	100	100	100	100	100	100

BL-BLI	Amoxyclave	100	100	100	100	100	100	100	100
	Piperacillin-tazobactam	100	100	100	62.5	100	100	100	100
	Cefoperazone-sulbactam	100	100	38.5	87.5	100	100	100	100
Aminoglycoside	Gentamicin	63.64	91.43	69.24	62.5	100	100	50	100
	Amikacin	36.36	74.29	100	62.5	33.33	100	50	100
Cephalosporin	Ceftriaxone	100	100	100	100	100	100	100	100
	Cefuroxime	100	100	100	100	100	100	100	100
	Cefepime	100	100	100	75	100	100	100	100
Quinolone	Nalidixic acid	100	97.14	84.62	100	100	100	50	100
	Ciprofloxacin	100	97.14	84.62	75	100	100	50	100
	Levofloxacin	100	100	100	75	100	100	100	100
Peptide	Colistin	0	0	0	0	0	0	0	0
Glycylcycline	Tigecycline	9.09	40	15.38	100	0	100	0	100

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145 Present data explicit colistin (peptide) as the most susceptible drug (100% each) against all clinical
 146 isolates followed by tigecycline (glycylcycline) which possessed second highest susceptibility
 147 against *K. pneumoniae* (90.91%), *A. baumannii* (60%), *E. coli* (84.62%), *C. freundii* (100%) and *E.*

cloacae (100%) whereas zero response towards *P. aeruginosa*, *E. aerogenes* and *P. luteola*. Similar results were reported by Abdul [et al.](#), (2014) who showed colistin susceptibility among many gram negative pathogens [*E. coli* (96.2%), *A. baumannii* (92.8%), *K. pneumoniae* (93.5%), *P. aeruginosa* (88%), *E. aerogenes* (90%)] [22]. Fernandez-canigia and Dowzicky (2012) demonstrated 70- 90% of pathogens includes *E. coli*, *A. baumannii*, *Klebsiella spp.* and *Enterobacter spp.* were found to be susceptible towards tigecycline irrespective to ESBL producers or non-producers which sustains our data [23].

CSE-1034 (AAE) was determined to be the third highest susceptible drug which claimed 81.8, 57.1, 69.2, 75, 66.6 and 100% antimicrobial activity against *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, *C. freundii* and *E. cloacae* respectively. Earlier studies have also highlighted the greater susceptibility of CSE-1034 (the novel antibiotic adjuvant entity) against various gram negative pathogens [18,24].

Co-trimaxazole (sulphomide) expressed 54.5 and 66.67% susceptibility towards *E. coli* and *C. freundii* along with low sensitive responses against *K. pneumoniae* (14.29%) and *A. baumannii* (7.69%) while no response against rest of the isolates. Marwa [et al.](#), (2015) found the analogous results related to co-trimaxazole which have shown 12-64% susceptibility against gram negative bacilli [25].

Ampicillin (BL) exhibited no sensitive response against gram negative bacteria. Similar findings for ampicillin resistance were seen by Patil [et al.](#), (2013) and Mama [et al.](#), (2014) who observed the resistance profile of *E. coli* (86%), *K. pneumoniae* (92.4%), *Pseudomonas spp.* (98%) and *C. freundii* (93%) against ampicillin [8,26].

Among BL-BLI drugs, cefoperazone-sulbactam possessed antimicrobial effect (61.5%) against *A. baumannii* with low susceptibility (12.5%) towards *P. aeruginosa* while piperacillin-tazobactam exhibited moderate susceptibility (37.5%) towards *P. aeruginosa* whereas both the drugs showed no

response against rest of the pathogens. Likewise, Amoxyclave didn't display any sensitive response against clinical isolates. Perim [et al.](#), (2015) reported 0-8% susceptibility percentage of amoxyclave (BL-BLI) against clinical pathogens which is in accordance with our data [27]. Likewise, earlier studies documented 0-40% sensitive responses of cefoperazone-sulbactam and piperacillin-tazobactam against various gram negative pathogens [28].

Aminoglycoside drugs includes gentamicin demonstrated average antimicrobial activity towards *E. coli* (36.36%), *A. baumannii* (30.76%), *P. aeruginosa* (37.5%) and *E. cloacae* (50%) while showed no response against other pathogens. Amikacin possessed 63.64, 66.67 and 50% susceptibility towards *E. coli*, *C. freundii* and *E. cloacae* while less than 38% sensitivity against *K. pneumoniae* and *P. aeruginosa* with no effects against rest of the pathogens. Similar results were reported by Chowdhury and Parial, (2015) who found 0-40% and 41-70% susceptibility of gentamicin and amikacin towards gram negative pathogens [29].

Moving towards cCephalosporins (cefepime, cefuroxime and ceftriaxone), documented zero sensitive

response against clinical isolates except cefepime which displayed 25% susceptibility towards *P. aeuroginosa*. Similar outputs were observed in case of cephalosporin drugs includes cefepime, ceftriaxone and cefuroxime demonstrated low sensitivity (0-25%) against clinical isolates which coincides our study [30].

Among quinolones, nalidixic acid and ciprofloxacin showed 50% each sensitive response against *E. cloacae* while <16% susceptibility towards *A. baumannii* and *K. pneumoniae* with no effect against other pathogens whereas levofloxacin exhibited low antimicrobial effect (25%) towards *P. aeuroginosa* [only](#). Earlier studies also observed the similar consequences which reported 0-50%, 20-60% and 5-40% antibacterial activity of nalidixic acid, ciprofloxacin and levofloxacin [31].

Though, carbapenem is considered the drug of last resort which is given when pathogens are resistant to primary agent of choice and critical care settings but earlier reports demonstrated the

resistance among microbes against carbapenem drugs due to the production of carbapenemase enzyme and the lesser permeability through the bacterial cell membrane [32]. Earlier studies have also revealed 65-85% resistance against carbapenem in carbapenemase producing *Enterobacteriaceae* [33]. This may be due to the wide use of these antibiotics as empirical therapy for the treatment of infectious diseases. From the results of present study, it is evident Our studies showed that the carbapenem resistant clinical pathogens are least resistible to tigecycline (%) (glycylcycline) and CSE-1034 (%) (an antibiotic and adjuvant entity) while completely (expunge) susceptible towards colistin (%) (peptide). However few studies explicit colistin as a toxic drug with adverse renal, neurological effects and tigecycline with lesser penetration which is not considered safe to the patients [34]. Data also suggested that rest of the drug classes (sulphomide, BL, BL-BLI, aminoglycoside, cephalosporin and quinolone) expressed 33-100% resistance towards carbapenem resistant isolates (Not referenced). Lesser activity of current antibacterial therapeutics is reported due to the acquisition of plasmid-mediated specific resistant genes and the production of ESBL and metallo β -lactamase (MBL) enzymes along with mutations in the efflux coding and outer membrane protein genes which accounts for easy efflux and poor penetration of antibiotics [35].

On the other hand, earlier reports advocate the resistance breaking mechanisms of CSE-1034 including enhanced penetration, inhibition of β -lactamase activity, biofilm breakage abilities, down regulation of the over expressed efflux pump coding genes which assists AAE to establish its coin over potent empirical therapeutics to prevent raising resistance among pathogens [18,36-40]. CSE-1034 has probably reduced resistivity among multi drug resistant gram negative bacteria due to the combination with adjuvant i.e. EDTA which interferes with the stability of outer membrane of microbes via chelating the cations and removing them from their binding sites thus increasing the permeability of the antibiotic hence sets forth CSE-1034 as an antibiotic resistant breaker. Hence

219 the present study is the evident that CSE-1034 (novel antibiotic and adjuvant entity) should be an

empirical choice to overcome the resistance spreading by carbapenem resistant pathogens where other drugs have failed.

4. CONCLUSION

The retrospective study indicates that CSE-1034 was 18.16, 31.39, 7.7, 37.5 and 50% more susceptible against *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa* and *E. cloacae* when compared with sulphomide, BL, BL-BLI, aminoglycoside, cephalosporin and quinolone drugs. Antimicrobial activity of CSE-1034 was found comparable to tigecycline and colistin but earlier data suggested their low penetrating abilities in urinary tract infections and nephrotoxicity respectively so they should be provided only when there is no other option left. Therefore, CSE-1034 can be advised as a choice to cure infective patients as empirical therapeutic that has the exceptional abilities to surpassing the resistance problems especially in centers where BL, BL-BLI, aminoglycoside, cephalosporin and quinolone resistance is common amongst carbapenem resistant gram negative isolates.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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