

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

Dhruv K. Mamtara^{1*}, Sanjith Saseedharan², Pallavi Bhalekar³, Surekha Katakdhond⁴

¹Consultant Microbiologist and infection control officer, S. L. Raheja Hospital, A fortis associate,
under aegis of Diabetic Association of India, Mumbai- 400016

²Head Critical care, S. L. Raheja Hospital, Mumbai

³Microbiology technical supervisor, S. L. Raheja Hospital, Mumbai

⁴Microbiology Technician, S. L. Raheja Hospital, Mumbai

Corresponding author

Dr. Dhruv K. Mamtara

Consultant Microbiologist and Infection Control Officer

S. L. Raheja- A Fortis Associate Hospital

Police Colony, Mahim, Mumbai, Maharashtra 400016

Email ID: dkmamtora692@gmail.com

ABSTRACT

Aim:

Taking into account the rise in mortality rate due to multi-drug resistant gram negative bacteria, we purposed to retrospectively analyze the susceptibility behavior of 74 carbapenem resistant clinical isolates towards common antibiotic classes [Sulphomide, β -lactams (BL), β -lactam/ β -lactamase inhibitor combination (BL/BLI), aminoglycosides, cephalosporins, quinolone, peptide and glycylyccline] and a novel antibiotic-adjuvant entity, CSE-1034 [Ceftriaxone/Sulbactam/disodium edetate].

Materials and methods:

To characterize the anti-bacterial susceptibility pattern, a retrospective, observational analysis of antibiogram data obtained from different clinical samples in Super Speciality tertiary care center, Mumbai (India) between January 2015 to December 2015 was performed.

Results:

74 carbapenem resistant clinical isolates with MIC>16 against imipenem and meropenem were analyzed in this study. *K. pneumoniae* (47.30%) was found to be the most common pathogen among all clinical isolates followed by *A. baumannii* (17.57%), *E. coli* (14.86%) and *P. aeruginosa* (10.81%). Antibiogram data suggested colistin as the most susceptible (100% each) drug against all carbapenem-resistant isolates followed by tigecycline (60-100%). CSE-1034 was determined as third most susceptible drug. The susceptibility rates of *K. pneumoniae*, *A. baumannii*, *E. coli* and *P. aeruginosa* to CSE-1034 were 81.8%, 57.1%, 69.2% and 75%. The susceptibility to aminoglycosides varied from zero for *A. baumannii* to 63.6% for *E.coli* in case of amikacin and 8.57% for *K. pneumoniae* and 37.5% for *P aeruginosa* in case of gentamicin. The susceptibility to all other antibiotics tested was very poor.

Conclusion:

From this study, it can be concluded that along with colistin and tigecycline, CSE-1034 and aminoglycosides can be considered for patients infected with carbapenem resistant strains. Moreover, CSE-1034 or aminoglycosides can be good alternates in carbapenem resistant bloodstream and urinary tract infections as tigecycline is not preferred agent in these infections.

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

58

59 INTRODUCTION

60 Approximately, 71.8% infections in India are caused by gram negative bacteria, and the rising
61 antimicrobial resistance has complicated the treatment of these bacterial infections. Carbapenems serve
62 as therapies of last resort for MDR Gram-negative bacterial infections including those caused by
63 extended spectrum β -lactamases strains. However, the emerging carbapenem resistance worldwide
64 among clinical isolates of *Enterobacteriaceae* and non-fermentative Gram negative bacilli facilitated
65 by either clonal expansion of carbapenem-resistant isolates or horizontal spread of carbapenemase
66 genes is of particular concern when treating patients infected with these pathogens^{1 2 3 4}. The rising
67 emergence of carbapenem-resistant infections is a menace to patients, particularly to those with
68 debilitating conditions, serious infections, underlying diseases or medical interventions^{1 2}. Various
69 factors responsible for escalating carbapenem resistance rates include the increased dependence and
70 selective pressure on penem family as treatment option for the rapidly increasing number of infections
71 by extended-spectrum β -lactamase (ESBL) strains worldwide^{5 6}. Other factors include poor infection
72 control practice and the lack of proper anti-microbial stewardship programs in many hospitals^{5 6}.

73

74 Carbapenem resistance is complex and can be mediated by several mechanisms, including
75 production of enzymes called as carbapenemases. Though carbapenemase production is the main
76 mechanism of carbapenem-resistance, AmpC β -lactamases, porin mutations/loss, expression of efflux
77 pumps, and/or alterations in penicillin-binding proteins may also confer carbapenem resistance^{5 6 7 8 9}
78 . Since carbapenemases have the ability to hydrolyze penicillins, cephalosporins, besides carbapenems,
79 Gram-negative bacteria carrying a carbapenemase-encoding gene frequently exhibit resistance to
80 virtually all β -lactams including cephalosporins, quinolones and aminoglycosides, leaving few or, in
81 some cases, no optimal therapeutic options⁷. However, as carbapenem resistant strains are reported to
82 exhibit other resistance mechanisms also, making them specifically resistant to carbapenems whereas
83 sensitivity to other antibiotics is retained.

84 Knowledge of these specific phenotypes can open the route for re-exploring abandoned treatment
85 options for these carbapenem-resistant infections which are often restricted to only polymyxins and
86 mainly include colistin. Hence, we aimed to evaluate the susceptibility profiles of different drug
87 classes towards different carbapenem resistant gram negative isolates from various clinical samples.

88

89

90 **MATERIALS AND METHODS**

91 **Sample collection**

92 This retrospective study was carried on carbapenem resistant bacterial isolates obtained from
93 clinical samples of patients suspected of bacterial infections during a period of one year (January to
94 December 2015), from Super Specialty tertiary care center, Mumbai (India). Different clinical samples
95 used for pathogen isolation were urine, blood, wound, sputum, tracheal secretion and pus. The
96 collection and processing of the samples were done as per a common standard operating procedure of
97 hospital.

98 **Isolation and identification of microbes**

99 All the clinical specimens were collected aseptically in sterile containers and inoculated on different
100 selective and non-selective culture media as per the standard microbiological techniques.
101 Enterobacteriaceae-N280 and Pseudomonas group-N281 cards (bioMérieux, France) were used for the
102 detection of *Enterobacteriaceae* and *Pseudomonas spp.* while *A. baumannii* was screened by using
103 leeds acinetobacter agar base medium as a selective media.

104 **Antibiotic susceptibility testing**

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

108 **RESULTS**

109 In this 1 year study period, a total of 181 different clinical samples were collected from the
110 patients and processed for pathogen isolation. The different clinical samples processed were urine
111 (37.84%), sputum and tracheal secretions (13.51% each), wound and pus (12.16% each) and blood
112 (10.81%). Of the total 181 samples analyzed, carbapenem resistant gram negative isolates were
113 obtained from 74 (40.8%) samples and used further in this study (Table 1). Eight different carbapenem
114 resistant gram-negative species with MIC>16 against imipenem and meropenem as per CLSI
115 guidelines were *E. coli* (n=11), *K. pneumoniae* (n=35), *A. baumannii* (n=13), *P. aeruginosa* (n=8), *C.*
116 *freundii* (n=3), *E. aerogenes* (n=1), *E. cloacae* (n=2) and *P. luteola* (n=1). For details, refer to Table no
117 2.

118 The distribution pattern of carbapenem resistant strains varied in different clinical samples
119 processed. Carbapenem-resistant *E. coli* were observed predominantly in urine (45.45%), *A.*

120 *baumannii* exhibited prime occurrence in tracheal secretions (34.48%) and *P. aeruginosa* strains
121 showed their high presence in pus (62.5%) samples. The least common pathogens including *C. freundii*
122 was mainly isolated from urine (66.6%), *E. aerogenes* from wound (100%) whereas *E. Cloacae* and *P.*
123 *luteola* were isolated from urine (100%).

124

125 The susceptibility profile of these carbapenem resistance bacterial strains to various classes of
126 antibiotics is shown in detail in Table 3. Overall, all the carbapenem resistant clinical isolates were
127 reported to be 100% susceptible to colistin followed by tigecycline which possessed second highest
128 activity against *K. pneumoniae* (90.91%), *A. baumannii* (60%), *E. coli* (84.62%), *C. freundii* (100%)
129 and *E. cloacae* (100%) whereas none of the isolates of *Pseudomonas spp.* and *E. aerogenes* were
130 reported susceptible.

131 CSE-1034 was determined the third highest susceptible drug after colistin and tigecycline. The
132 susceptibility rates of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, *C. freundii* and *E. cloacae*
133 to CSE-1034 were 81.8%, 57.1%, 69.2%, 75%, 66.6% and 100% respectively. Susceptibility to Co-
134 trimoxazole (sulphonamides) were 54.5%, 66.67%, 14.29% and 7.69% for *E. coli*, *C. freundii*, *K.*
135 *pneumoniae*, *A. baumannii* respectively. None of the other isolates were observed susceptible. Among
136 BL-BLI drugs, cefoperazone-sulbactam susceptibility observed was 61.5% for *A.baumannii*; 12.5% and
137 37.5% of *P. aeruginosa* showed susceptibility to cefoperazone-sulbactam and piperacillin-tazobactam
138 respectively whereas all other pathogens showed zero susceptibility. Amoxyclave didn't display any
139 susceptibility against any clinical isolates. Among aminoglycosides, gentamicin demonstrated average
140 susceptibility against *E. coli* (36.36%), *A. baumannii* (30.76%), *P. aeruginosa* (37.5%) and *E. cloacae*
141 (50%) whereas no other pathogen isolates were reported susceptible. Amikacin possessed 63.64%,
142 66.67%, 50% susceptibility against *E. coli*, *C. freundii*, *E. cloacae*, *K. pneumoniae* and *P. aeruginosa*.
143 Cephalosporins including cefepime, cefuroxime and ceftriaxone documented zero susceptibility against
144 all clinical isolates except cefepime which displayed 25% susceptibility against *P. aeruginosa*. Among
145 quinolones, nalidixic acid and ciprofloxacin showed 50% susceptibility against *E. cloacae* whereas
146 <16% susceptibility was observed against *A. baumannii* and *K. pneumoniae*; *P. aeruginosa* exhibited
147 25% susceptibility towards Levofloxacin.

148

149 DISCUSSION

150 Of the 74 Carbapenem resistant strains identified, a marginally higher number of strains were

151 isolated from urine samples (45.16%) closely followed by blood (42.1%) and sputum (40%). Nair et al.
 152 ¹⁰ has also reported that majority of the carbapenem isolates were detected in urine samples (46%) out
 153 of total 57 carbapenem resistant isolates obtained from 465 Enterobacteriaceae isolates screened.
 154 Although not mentioned, the high prevalence of carbapenem resistance in uropathogens reported in this
 155 study could be simply because of higher number of urine clinical samples processed initially, thus
 156 creating a bias. The overall prevalence of carbapenem resistance in the screened strains was found to be
 157 40.8%. This is similar to or little higher than the carbapenem resistance rates obtained in different
 158 studies from other parts of India. Wattal et al. ¹¹ have reported a Carbapenem resistance rate ranging
 159 from 2% to 80% in various multi drug resistant organisms including *E. coli*, *Klebsiella spp.*,
 160 *Pseudomonas spp.* and *Acinetobacter spp.* in a tertiary care hospital in Delhi. Gupta et al. ¹² reported a
 161 carbapenem resistance rate varying from 17 to 22% among Enterobacteriaceae while Datta et al. ¹³
 162 have reported carbapenem resistance of 7.87% among Enterobacteriaceae strains in a study conducted
 163 in a tertiary care hospital in North India. In consistent with the global distribution pattern, *K.*
 164 *pneumoniae* (47.30%) was the predominant carbapenem-resistant species reported followed by *A.*
 165 *baumannii* (17.57%) and *E. coli* (14.86%). *K. pneumoniae* isolates are the most common
 166 carbapenemase including MBLs producing Enterobacteriaceae reported worldwide, from United
 167 States, South and Central America, the Middle East, China Italy and Greece ^{14 3 7 15}. In a study based
 168 in India, Oberai et al. ¹⁶ have also reported MBL producing *K. pneumoniae* as the most common
 169 carbapenem resistant Gram-negative isolate. Similarly kind of prevalence pattern has been reported by
 170 Kazi et al. also ¹⁷. Moreover, our results have shown that the carbapenem resistant clinical pathogens
 171 showed highest susceptible to colistin (100%). However, polymyxins are always drug of last choice for
 172 the physicians and fall out of favor among many clinicians due to adverse nephrotoxic and neurotoxic
 173 effects. Moreover, in recent years increased use of polymyxins including colistin with rising rates of
 174 carbapenem resistant infections has lead to outbreaks of colistin-resistant carbapenem-resistant
 175 infections through the world. To prevent further rise in colistin resistance, there is a high need to
 176 explore alternate options to reduce the excessive consumption of colistin. Although, the susceptibility
 177 reported to tigecyclin is very good, tigecyclin cannot be considered as a drug of choice in urine and
 178 blood infections because of its inability to maintain the minimum MIC required for its anti-bacterial
 179 activity and the major mode of its excretion being through the biliary route. As CSE-1034 (AAE) was
 180 determined the third highest susceptible drug and is reported to effective in urinary and blood
 181 infections, it can be a drug of choice over tigecyclin in blood and urine infections which are the

182 predominant source of isolated pathogens in this report. A good number of previous studies have
183 reported the greater susceptibility of CSE-1034 (the novel antibiotic adjuvant entity) against different
184 gram negative pathogens. In an antimicrobial susceptibility pattern study, Sahu et al. (2014) has
185 reported that 100%, 64% and 63% of ESBL producing *A. baumannii*, *K. pneumoniae* and *E. coli* and
186 89%, 60%, 42% and 41% of MBL producing isolates of *A. baumannii*, *E. coli*, *P. aeruginosa* and *K.*
187 *pneumoniae*, respectively were susceptible to CSE-1034 ¹⁸

188

189 Similarly, another study on 515 isolates of *P. aeruginosa* has reported 97.3% and 95.1%
190 susceptibility to CSE-1034 of MBL and ESBL+MBL producing isolates resistant towards most of
191 antibiotics including piperacillin+tazobactam, doripenem, imipenem, meropenem, ceftazidime and
192 cefepime ¹⁸. The susceptibility pattern observed to other antibiotics varied from average susceptibility
193 rate of 30% for aminoglycosides, 19% to sulphonamides, 0% to BL/BLI except *P. aeruginosa* (12.5%
194 to 37.5%) and *A. baumannii* (61.5% for Cefaperazone-Sulbactam). The susceptibility rate to all
195 generations of cephalosporins was zero and for quinolones varied from 0-25% for different species.
196 Almost similar to our pattern, Shanmugan et al. ¹⁶ have reported that the resistance of carbapenem
197 resistant isolates to aminoglycoside antibiotics varied from 33% for amikacin to 94% to tobramycin.
198 The same study has reported the resistance rate of 100% to ampicillin, cotrimoxazole, all 4 generations
199 of cephalosporins and piperacillin-tazobactam ¹⁶.

200 Generally, carbapenem-resistant strains are inherently considered resistant to broader classes of
201 antibiotics because of carbapenemase production, but various other mechanisms including efflux
202 pumps, membrane impermeability, expression of class D enzymes making them specifically resistant to
203 carbapenems can't be ignored ¹⁷. A study by Mosca A et al. ¹⁸ in Italy has shown that only 84% of the
204 carbapenem resistant strains evaluated by MHT showed the production of carbapenemase clearly
205 indicating that other mechanisms of resistance also co-exist. Establishing the contribution of different
206 mechanisms of resistance to carbapenems among a collection of imipenem- and meropenem-non-
207 susceptible *P. aeruginosa* isolates, Rodríguez-Martínez et al. ¹⁹ have reported that genes encoding
208 metallo- β -lactamases or carbapenem-hydrolyzing oxacillinases were not identified in any of the
209 isolates. The main mechanisms associated with carbapenem resistance reported were loss of outer
210 membrane protein OprD, over-expression of extended-spectrum cephalosporinases (ESACs) and
211 specific efflux pumps ¹⁹. Similar to our observations, Campana et al. have reported Carbapenem-
212 resistant and cephalosporin-susceptible phenotype among *P. aeruginosa* clinical isolates in Brazil ²⁸. A

carbapenem resistant, beta-lactam susceptible phenotype is characteristic for diminished expression of OprD as carbapenems are known to enter the periplasmic space of bacteria through the OprD outer membrane porin. The OprD gene inactivation leading to porin loss probably by acquisition of mutations including insertions or deletions is known cause of imipenem resistance²⁰. Moreover, strains with OprD downregulation²¹ have reduced susceptibility to meropenem while susceptibility to other beta-lactams are not affected²². OXA-23-like and OXA-48-like are also reported to be closely related to carbapenem resistant phenotype. OXA-23 and OXA-48 which belong to class D β -lactamase, are reported to have selective carbapenem-hydrolyzing activity in *A. baumannii* and *P. aeruginosa*^{23 24 25}. Moreover, overexpression of MexAB-OprM, MexCD-OprJ and MexXY-OprM efflux systems are also involved in reduced susceptibility to meropenem, quinolones, antipseudomonal penicillins, cephalosporins, aminoglycosides whereas, ceftazidime and imipenem are not usually affected^{26 19 27}. All these studies clearly indicate that some strains are specifically resistant to carbapenems and the choice of drug in these cases could be other than colistin and tigecyclin.

It becomes imperative to mention that although carbapenem-based combination therapy is suggested for carbapenem resistant infections, its efficacy appears to be MIC dependent. Various studies have concluded that this combination therapy helps to reduce mortality in patients with carbapenem MIC ≤ 8 $\mu\text{g/mL}$, and the mortality rate shoots up if the MIC is >8 $\mu\text{g/mL}$ (Discussed in detail by Morril. et al. 2015) . The carbapenem-based combination therapy can't be considered at all for MIC $>16\mu\text{g/mL}$. Though amikacin also showed a good susceptibility pattern against many clinical isolates, only 7/32 of urinary and blood isolates were reports susceptible to amikacin compared to 21/32 reported for CSE-1034. Thus, CSE-1034 alone or in combination with other drugs can be preferred choice for all infections particularly urinary and blood infections caused by carbapenem resistant strains.

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 antibiotics³³. Hence from the present study it is evident that colistin, tigecyclin, amikacin and CSE-1034 should be an empirical choice of treatment for bacterial infections where the carbapenem resistant strains are suspected.

CONCLUSION

Overall, it can be concluded that carbapenem resistance among gram negative strains have become a common scenario in the hospitals as a consequence of excessive consumption of carbapenems. From the antibiotic susceptibility profile, it can be clearly inferred along with colistin and tigecycline, CSE-1034 and amikacin can be a drug of choice for patients infected with carbapenem resistant strains. Moreover, considering the value of carbapenems as one of the last option for various MDR bacterial infections, irrational consumption of carbapenems should be stopped.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1 Xu Yanling, Gu Bing, Huang Mao, Liu Haiyan, Xu Ting, Xia Wenying, et al. Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia. *J Thorac Dis* 2015;7(3):376–85. Doi: 10.3978/j.issn.2072-1439.2014.12.33.
- 2 Perez F, Van Duin D. Carbapenem-resistant Enterobacteriaceae: A menace to our most vulnerable patients. *Cleve Clin J Med* 2013;80(4):225–33. Doi: 10.3949/ccjm.80a.12182.
- 3 Biedenbach D, Bouchillon S, Hackel M, Hoban D, Kazmierczak K, Hawser S, et al. Dissemination of NDM metallo- β -lactamase genes among clinical isolates of Enterobacteriaceae collected during the SMART global surveillance study from 2008 to 2012. *Antimicrob Agents Chemother* 2015;59(2):826–30. Doi: 10.1128/AAC.03938-14.
- 4 Mathers Amy. Mobilization of Carbapenemase-Mediated Resistance in Enterobacteriaceae. *Microbiol Spectr* 2016;4(3). Doi: 10.1128/microbiolspec.EI10-0010-2015.
- 5 Logan Latania K, Weinstein Robert A. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. *J Infect Dis* 2017;215(suppl_1):S28–36. Doi: 10.1093/infdis/jiw282.
- 6 van Duin David, Doi Yohei. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 2017;8(4):460–9. Doi: 10.1080/21505594.2016.1222343.

- 277 7 Walsh Timothy R. Emerging carbapenemases: a global perspective. *Int J Antimicrob Agents* 2010;**36**
278 **Suppl 3**:S8-14. Doi: 10.1016/S0924-8579(10)70004-2.
- 279
- 280 8 Teo Jocelyn Qi Min, Cai Yiyang, Lim Tze-Peng, Tan Thuan Tong, Kwa Andrea Lay-Hoon.
281 Carbapenem Resistance in Gram-Negative Bacteria: The Not-So-Little Problem in the Little Red Dot.
282 *Microorganisms* 2016;**4**(1). Doi: 10.3390/microorganisms4010013.
- 283
- 284 9 Martínez-Martínez Luis, González-López Juan José. Carbapenemases in Enterobacteriaceae: types
285 and molecular epidemiology. *Enferm Infecc Microbiol Clin* 2014;**32 Suppl 4**:4–9. Doi:
286 10.1016/S0213-005X(14)70168-5.
- 287
- 288 10 JMID-00660.pdf n.d.
- 289
- 290 11 Wattal Chand, Goel Neeraj, Oberoi JK, Raveendran Reena, Datta S, Prasad KJ. Surveillance of
291 multidrug resistant organisms in tertiary care hospital in Delhi, India. *J Assoc Physicians India*
292 2010;**58 Suppl**:32–6.
- 293
- 294 12 Gupta Ekta, Mohanty Srujana, Sood Seema, Dhawan Benu, Das Bimal K, Kapil Arti. Emerging
295 resistance to carbapenems in a tertiary care hospital in north India. *Indian J Med Res* 2006;**124**(1):95–
296 8.
- 297
- 298 13 Datta Priya, Gupta Varsha, Garg Shivani, Chander Jagdish. Phenotypic method for
299 differentiation of carbapenemases in Enterobacteriaceae: study from north India. *Indian J Pathol*
300 *Microbiol* 2012;**55**(3):357–60. Doi: 10.4103/0377-4929.101744.
- 301
- 302 14 Kazmierczak Krystyna M, Biedenbach Douglas J, Hackel Meredith, Rabine Sharon, de Jonge
303 Boudewijn LM, Bouchillon Samuel K, et al. Global Dissemination of blaKPC into Bacterial Species
304 beyond *Klebsiella pneumoniae* and In Vitro Susceptibility to Ceftazidime-Avibactam and Aztreonam-
305 Avibactam. *Antimicrob Agents Chemother* 2016;**60**(8):4490–500. Doi: 10.1128/AAC.00107-16.
- 306
- 307 15 Mouloudi Eleni, Protonotariou Euthymia, Zagorianou Alexia, Iosifidis Elias, Karapanagiotou
308 Areti, Giasnetsova Tatiana, et al. Bloodstream Infections Caused by Metallo-
309 *β*-Lactamase-*Klebsiella pneumoniae*
310 Carbapenemase-Producing *K. pneumoniae* among Intensive Care Unit
311 Patients in Greece: Risk Factors for Infection and Impact of Type of Resistance on Outcomes. *Infect*
312 *Control Amp Hosp Epidemiol* 2010;**31**(12):1250–6. Doi: 10.1086/657135.
- 313
- 314 16 Oberoi Loveena, Singh Nachhatarjit, Sharma Poonam, Aggarwal Aruna. ESBL, MBL and
315 Ampc *β* Lactamases Producing Superbugs – Havoc in the Intensive Care Units of Punjab India. *J Clin*
316 *Diagn Res JCDR* 2013;**7**(1):70–3. Doi: 10.7860/JCDR/2012/5016.2673.
- 317
- 318 17 Sahu Manisa, Sanjith S, Bhalekar Pallavi, Keny Dipti. Waging War Against Extended Spectrum
319 Beta Lactamase and Metallobetalactamase Producing Pathogens- Novel Adjuvant Antimicrobial
320 Agent Cse1034- An Extended Hope. *J Clin Diagn Res JCDR* 2014;**8**(6):DC20-DC23. Doi:
321 10.7860/JCDR/2014/8802.4504.
- 322

- 18 Chaudhary Manu, Payasi Anurag. Rising Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolated from Clinical Specimens in India. *J Proteomics Bioinform* 2013;**6**(1). Doi: 10.4172/jpb.1000253.
- 19 Shanmugam Priyadarshini, Meenakshisundaram Jeya, Jayaraman Perumal. blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae in a Tertiary Care Hospital. *J Clin Diagn Res JCDR* 2013;**7**(12):2736–8. Doi: 10.7860/JCDR/2013/7759.3747.
- 20 Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the emergence of carbapenem resistance in *Pseudomonas aeruginosa*. *Hippokratia* 2012;**16**(4):303–7.
- 21 Mosca Adriana, Miragliotta Luisa, Del Prete Raffaele, Tzakis Gerasimos, Dalfino Lidia, Bruno Francesco, et al. Rapid and sensitive detection of bla KPC gene in clinical isolates of *Klebsiella pneumoniae* by a molecular real-time assay. *SpringerPlus* 2013;**2**(1):31. Doi: 10.1186/2193-1801-2-31.
- 22 Rodríguez-Martínez José-Manuel, Poirel Laurent, Nordmann Patrice. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2009;**53**(11):4783–8. Doi: 10.1128/AAC.00574-09.
- 23 Campana Eloiza Helena, Xavier Danilo Elias, Petrolini Fernanda Villas-Boas, Cordeiro-Moura Jhonatha Rodrigo, Araujo Maria Rita Elmor de, Gales Ana Cristina. Carbapenem-resistant and cephalosporin-susceptible: a worrisome phenotype among *Pseudomonas aeruginosa* clinical isolates in Brazil. *Braz J Infect Dis Off Publ Braz Soc Infect Dis* 2017;**21**(1):57–62. Doi: 10.1016/j.bjid.2016.10.008.
- 24 Horii Toshinobu, Muramatsu Hideaki, Morita Motoki, Maekawa Masato. Characterization of *Pseudomonas aeruginosa* isolates from patients with urinary tract infections during antibiotic therapy. *Microb Drug Resist Larchmt N* 2003;**9**(2):223–9. Doi: 10.1089/107662903765826831.
- 25 Farra Anna, Islam Sohidel, Strålfors Annelie, Sörberg Mikael, Wretling Bengt. Role of outer membrane protein OprD and penicillin-binding proteins in resistance of *Pseudomonas aeruginosa* to imipenem and meropenem. *Int J Antimicrob Agents* 2008;**31**(5):427–33. Doi: 10.1016/j.ijantimicag.2007.12.016.
- 26 Bonomo Robert A, Szabo Dora. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2006;**43** Suppl 2:S49-56. Doi: 10.1086/504477.
- 27 Han Lei, Lei Jine, Xu Jiru, Han Shaoshan. blaOXA-23-like and blaTEM rather than blaOXA-51-like contributed to a high level of carbapenem resistance in *Acinetobacter baumannii* strains from a teaching hospital in Xi'an, China. *Medicine (Baltimore)* 2017;**96**(48). Doi: 10.1097/MD.00000000000008965.
- 28 Walther-Rasmussen Jan, Høiby Niels. OXA-type carbapenemases. *J Antimicrob Chemother* 2006;**57**(3):373–83. Doi: 10.1093/jac/dki482.

370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385

386

387

388

389

390

391

392

393

394

395

396

397

398

399
400

29 Nordmann Patrice. [Gram-negative bacteriae with resistance to carbapenems]. *Med Sci MS* 2010;**26**(11):950–9. Doi: 10.1051/medsci/20102611950.

30 Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000;**44**(12):3322–7.

31 Srikumar R, Paul CJ, Poole K. Influence of mutations in the mexR repressor gene on expression of the MexA-MexB-oprM multidrug efflux system of *Pseudomonas aeruginosa*. *J Bacteriol* 2000;**182**(5):1410–4.

32 Chaudhary Manu, Kumar Shailesh, Payasi Anurag. A Novel Approach to Combat Acquired Multiple Resistance in *Escherichia coli* by using EDTA as Efflux Pump Inhibitor. *J Microb Biochem Technol* 2012;**4**(6). Doi: 10.4172/1948-5948.1000082.

401

402

403

404

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

407

Sr. no.	Name of clinical samples	Name of clinical samples	Number of carbapenem resistant pathogens isolated (%)
1	Urine	62	28 (45.2%)
2	Blood	19	8 (42.1%)
3	Wound	24	9 (37.5%)
4	Sputum	25	10 (40%)
5	Tracheal secretion	26	10 (38.5%)
6	Pus	25	9 (36)
	Total	181	74

408

409

410

411

412

413

414

415

416

417

Clinical samples	No. of isolates	<i>E. coli</i> (%)	<i>K. pneumoniae</i> (%)	<i>A. baumannii</i> (%)	<i>P. aeruginosa</i> & <i>P. luteola</i> (%)	*Others (%)
Urine	28	5 (45.45)	19 (54.28)	3 (23.07)	3 (25.0)	4 (66.66)
Blood	8	1 (9.09)	3 (8.57)	1 (7.69)	0	1 (33.33)
Wound	9	1 (9.09)	3 (8.57)	2 (15.38)	0	1 (33.33)

418

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

Formatted: Indent: Left: 0"

Formatted Table

426

427

428

429

430

Sputum	10	2 (18.18)	5 (14.28)	1 (7.69)	0	0
Tracheal secretion	10	1 (9.09)	3 (8.57)	5 (34.48)	1 (12.5)	0
Pus	9	1 (9.09)	2 (5.71)	1 (7.69)	5 (62.5)	0
Total	74	11 (14.86)	35 (47.30)	13 (17.57)	9 (10.81)	6 (4.05)

430

437

438

439

440

441

442

443

444 *Others incude *C. freundii*, *E. aerogenes* and *E. cloacae*

445

446

447

448

449

450

451

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

453

Clinical isolates		<i>E. coli</i> (n=11)	<i>K. pneumoniae</i> (n=35)	<i>A. baumannii</i> (n=13)	<i>P. aeruginosa</i> & <i>P. luteola</i> (n=9)	<i>Others</i> <i>C. freundii</i> (n=6)
Drug classes	Drugs					
AAE	CSE-1034	81.8	57.1	69.2	75	66.67
Sulphomides	Co-trimazole	54.5	14.29	7.69	0	33.33
BL	Ampicillin	0	0	0	0	0
BL-BLI	Amoxyclave	0	0	0	0	0
	Piperacillin-tazobactam	0	0	0	37.5	0
	Cefoperazone-sulbactam	0	0	61.5	12.5	0
Aminoglycoside	Gentamicin	36.36	8.57	30.76	37.5	16.6
	Amikacin	63.64	25.71	0	37.5	50
Cephalosporin	Ceftriaxone	0	0	0	0	0
	Cefuroxime	0	0	0	0	0
	Cefepime	0	0	0	25	0
Quinolone	Nalidixic acid	0	2.86	15.38	0	16.6
	Ciprofloxacin	0	2.86	15.38	25	16.6
	Levofloxacin	0	0	0	25	0
Peptide	Colistin	100	100	100	100	100
Glycylcycline	Tigecycline	90.91	60	84.62	0	100

*Others include *C. freundii*, *E. aerogenes* and *E. cloacae*