

Original Research Articles

A new antibiotic adjuvant entity (ceftriaxone + sulbactam + disodium edetate): an alternative to carbapenems for the management of intensive care unit infection

Abstract

Aims: Carbapenem resistant bacteria infections have limited treatment options and are associated with high mortality. Here we present a retrospective analysis of treatment and outcome for ICU patients suffering from moderate to severe urinary tract infection (UTI), lower respiratory tract infection (LRTI) and intra-abdominal infections (IAI) to assess the efficacy of novel antibiotic adjuvant entity (AAE); ceftriaxone + sulbactam + disodium edetate, as an effective alternative for carbapenems in critically ill patients.

Material and Methods: A retrospective study was conducted to evaluate efficacy of AAE in 84 patients (showing sensitivity to AAE) with UTI, LRTI and IAI treated at IVY hospital, Mohali, India between January 2013 to November 2014 . The antibiotic therapy was initiated empirically and continued based on the results of the microbiological susceptibility testing and clinical outcome.

Results: 64 (76.19 %), patients were diagnosed with single-organism infections, among which, 14 (16.16%) bacteria were resistant to meropenem and all the bacteria were susceptible to AAE. Empirical meropenem treatment was given to 25 patients, out of which 18 (72 %) patients achieved clinical success. 24 (75 %) patients out of 32 patients treated with AAE, achieved clinical success and the remaining 8 patients were cured when colistin was given along with AAE. 20 (23.80 %), patients were diagnosed with multiple bacterial infections. Among 20 multiple bacterial infection patients, bacterial samples of 12 patients showed sensitivity towards AAE and meropenem, where as the remaining 8 (40%) samples showed intermediate susceptibility towards both cabapenem and AAE. 9 (45 %) patients cured with AAE, while the remaining 11 patients cured with AAE and colistin combination therapy.

Conclusion: From the above study, it can be concluded that patients experience similar rates of

clinical response in carbapenem susceptible cases and in some cases where patients failed to respond carbapenem therapy responded to AAE treatment. Hence, AAE can be as an alternative in the treatment of moderate and severe infections caused by Gram negative organisms.

Keywords: Ceftriaxone/sulbactam-EDTA, Intra-abdominal infections, Lower respiratory tract infections, Retrospective study, Urinary tract infections.

1. Introduction

Management of critically ill patients due to increasing antibiotic resistance is a major health-care problem affecting morbidity and mortality in the intensive care unit [1]. Gram-negative bacteria are predominantly responsible for severe diseases including those of abdomen (intra-abdominal), lower respiratory tract (LRTI), bacteremia and urinary tract (UTI) infections [2]. Beta-lactams are one of the most frequently used classes of antimicrobials in hospital settings, crucial for the treatment of infections caused by Gram-negative bacteria [3]. However, on account of the increased resistance towards beta lactam antibiotics, mainly due to production of extended spectrum beta lactamases (ESBL), Carbapenems have become the drug of choice to treat such severe infections [4]. Emergence of novel beta-lactamases with direct carbapenem-hydrolyzing activity has contributed to an increased prevalence of carbapenem resistant Gram negative bacteria, especially *Enterobacteriaceae* (CRE).

CRE are particularly problematic given the frequency with which *Enterobacteriaceae* cause infections [5], the high mortality associated with infections caused by CRE [6-8], and the potential for widespread transmission of carbapenem resistance via mobile genetic elements [9,10]. Increase in carbapenem usage has a direct relationship to increase in carbapenem resistant Gram negative bacteria [11]. Antibacterial drug discovery and development have slowed considerably in recent years [1]. The number of new antibacterial medicines entering the clinic has been declining and, in view of this fact, few compounds for multi-drug resistant Gram-negative bacteria will be available for more than 10 years [12,13]. The problems associated with escalating resistance and decreased

development of antibiotics with novel mechanisms of action has required more research into existing antibiotics. Scarcity of newer antibiotics and a declining drying antibiotic pipeline forces us to look for an effective alternate empiric antibiotic therapy equivalent to carbapenems.

Recent effort to maximize antibiotic activity and overcome drug resistance has led to the search for an alternate solution such as antibiotic adjuvants. Antibiotic adjuvants are moieties, non-antibiotic in nature, which in combination with antibiotics, enhance the antimicrobial activity of the latter [14]. Fixed dose combination of Ceftriaxone + sulbactam + adjuvant disodium edetate is one such novel Antibiotic Adjuvant Entity (AAE) approved by the Drug Controller General of India (DCGI) and increasingly used in Indian hospitals. Various reports of the in-vitro susceptibility studies [14] hint of the possibility of this AAE to overcome the hurdles of both ESBL (sulbactam effect) as well as metallo beta lactamases (MBL) (disodium edetate) producers clinically. Two recently published retrospective studies support the use of the AAE in septicaemia and sepsis [15,16]. Thus the main goal of this study was to retrospectively analyze the clinical and microbiological efficacy of this AAE in patients with moderate to severe IAI, LRTI and UTI.

2. Materials and methods

2.1 Patients and antibiotic therapy

Study was conducted at 200 bed tertiary care IVY hospital Mohali, Chandigarh. We retrospectively reviewed the case reports of 108 ICU patients suffering from different infections (IAI, LRTI, UTI) and who were treated at IVY hospital between January 2013 to November 2014. Of 108 patients, 84 patients were included in the study of which 34 patients were diagnosed with LRTI and each of 25 patients was diagnosed with IAI and UTI. Among these 84 clinically cured patients having culture positive infections and showing sensitivity to AAE were considered in the study, while the remaining 24 patients were excluded from the study: (8 patients which were resistance to meropenem or AAE, 12 patients which were changed to other antibiotics on discretion of investigator before sensitivity results and 4 patients, who expired during the course of treatment).

77 The patients were administered either meropenem or AAE empirically. Meropenem was
78 administered at the dose of 1000 mg *t.i.d.* and AAE was given at the dose of 1500-3000 mg *b.i.d.*
79 depending on the severity of infections. 1500 mg of AAE contains 1000 mg of ceftriaxone, 500 mg
80 of sulbactam and 37 mg of EDTA and 3000 mg of AAE contains 2000 mg of ceftriaxone, 1000 mg
81 of sulbactam and 74 mg of EDTA. For colistin therapy, a loading dose of 9 MIU followed by
82 *b.i.d.* doses of 4.5 MIU were used.

83 Case records were analyzed for age, sex, APACHE II score, co-morbadities, duration of
84 antimicrobial treatment, total leukocyte count, neutrophil count, liver function tests, renal function
85 test, culture sensitivity, serum electrolytes, radiological imaging, reason for the change of
86 antibiotics, dose, duration and route of administration of AAE or Meropenem and final
87 microbiological and clinical outcome.

88 The antibiotic therapy with AAE or meropenem was initially started empirically based on the
89 clinical presentation and treating physician's decision and was continued or modified based on the
90 *in-vitro* microbiological susceptibility tests and clinical outcome.

91 On retrospective evaluation, patients were divided into two main groups. i.e. group A-Single
92 bacterial infection [64 (76.2 %) patients] and group B-multiple bacterial infections [20 (23.8 %)
93 patients]. AAE was administered empirically to 64 patients in group A, 14 (21.9%) patients (group
94 G-1) with infection caused by meropenem resistant and AAE susceptible bacteria. In the 50
95 (78.1%) patients (group G-2) with meropenem and AAE susceptible bacterial infections,
96 meropenem was administered to 50% cases (group G2A) and AAE was given to the remaining
97 50% (group G2B). On the third day of treatment, along with bacteriological evaluations, the
98 progress of the therapy (measured in terms of the improvement in the symptoms) was also recorded
99 and the patients showing improvement with respective empiric therapies were continued on the
100 same antibiotic. Patients who failed to respond to meropenem clinically despite microbial
101 sensitivity to meropenem were switched to AAE therapy while patients who failed to respond to

AAE mono-therapy were changed to combination therapy with AAE and colistin. In polymicrobial infections caused by pathogens sensitive to AAE and meropenem (group G3) empirical treatment was continued and those showing intermediate susceptibility towards AAE and meropenem (group G4), were treated with colistin along with the empiric AAE once the susceptibility report was received .

2.2 In-vitro microbial antibiotic susceptibility testing

Antimicrobial susceptibility testing of the pathogens isolated from the patients was done by Kirby-Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute [17].

2.3 Clinical analysis of patients

Clinical signs and symptoms associated with infections were evaluated prospectively initially, after 3 days of empirical antibiotic therapy, and also at the end of therapy. Sputum, bronchoalveolar lavage (BAL), endotracheal (ET) secretions, urine, blood, pus or peritoneal fluids from the patients were cultured for causative pathogens. Clinical response to therapy evaluated at the end of the treatment was recorded and classified as cured (complete remission of local and systemic signs and symptoms), improved (improvement of local and systemic signs and symptoms but without complete resolution) or failure (no improvement or deterioration of signs and symptoms).

3. Results

3.1 Patients and demographic characteristics

84 clinically cured cases of the 108 patients treated in the study period were diagnosed with culture-positive infections with bacteria sensitive to AAE. All the patients had moderate to severe infections with mean APACHE II score of 17.84 ± 2.16 ; 26 (30.95 %) of 84 patients had bacteremia. Out of 84 evaluable patients, 52 patients were identified with co-morbidities, including hypertension (26.1 %), diabetes (19.0 %), chronic obstructive pulmonary disease (11.9 %) and coronary artery disease (CAD) / ischemic heart disease (IHD) (4.7 %). LRTI was the predominant infection diagnosed

(40.47 %), followed by intra-abdominal (29.76%) and urinary tract infections (29.76%). 64 of 84 patients were diagnosed with a monomicrobial infection, while 20 were diagnosed with polymicrobial infections. 44 of 64 monomicrobial infections, 44 were caused by pathogens of the *Enterobacteriaceae* family, with *E coli* accounting for 30 (68.18 %) cases and *Klebsiella sp.* for 14 (31.81 %) cases of infection. The remaining 20 (5 *Acinetobacter sp.* + 15 *Pseudomonas sp.*) isolates were of non-*Enterobacteriaceae* family. Different combinations of either *E coli*, *Klebsiella sp.*, *Acinetobacter sp.*, *Pseudomonas sp* and *Neisseria gonorrhoeae* were identified in mixed bacterial infections.

3.2 *In-vitro* microbial antibiotic susceptibility testing

The results of the *In-vitro* microbial antibiotic susceptibility testing carried out for isolated pathogens. G1 – Single pathogen susceptible to AAE and resistant to meropenem, G2 – single bacteria Susceptible to both AAE and meropenem G3 – multiple Pathogens showing sensitivity to both AAE and meropenem and G4 – multiple Pathogens showing intermediate resistance to both AAE and meropenem (Figure 1). Out of 64 bacteria isolated from patients with single strain bacterial infections, 50 (78.13 %) showed susceptibility towards carbapenem and the remaining 14 (21.87 %) were resistant to it. Where as all the 64 (100 %) isolates showed showed susceptibility towards AAE. However all the tested pathogens showed low non significant (< 10 %) susceptibilities towards the other tested antibiotics. The susceptibility test carried out for bacterial samples isolated from patients with multi bacterial infections, 12 samples showed sensitivity towards AAE and meropenem, where as the remaining 8 (40%) samples showed intermediate susceptibility towards both cabapenem and AAE. From *Enterobacteriaceae* family *E. coli*, and *K. pneumoniae* were isolated while *Neisseria gonorrhoea*, *S. Pneumoniae*, *Acinetobacter sp.* and *P. aeruginosa* were also amongst the most common non-enterobacterial pathogens isolated. The overall susceptibility of all the isolated pathogens were 83.01 % and 69.81 % for AAE and meropenem respectively (Table 1).

3.3 Efficacy of antibiotic therapy

14 patients with carbapenem-resistant and AAE-susceptible bacterial infections were treated with AAE. Bacteriological eradication with successful clinical response was observed in 12 patients. 2 patients failed to respond to AAE clinically were switched to AAE and colistin combination therapy. The mean treatment duration for these 12 patients who were cured with AAE was 6.91 days $\pm$ 1.08 (SD). 25 out of 50 patients with carbapenem and AAE susceptible organisms were treated with meropenem. Of these patients, 18 patients showed clinical improvement (measured in terms of disease symptoms) after initial 3 days of treatment and were continued on carbapenem therapy. For the remaining 7 patients, treatment was changed to AAE. Of these, 4 patients showed clinical cure. The mean treatment duration for these 4 cured patients with AAE therapy was 5.75 days $\pm$ 0.5 (SD). For the remaining 3 patients, clinical success was achieved after the administration of colistin with AAE. Among 25 Similar patients (having infections with carbapenem and AAE susceptible bacterial cultures) but treated with AAE, 20 patients showed satisfactory clinical cure. The mean treatment duration for these 20 cured patients was 5.8 days $\pm$ 1.36 (SD). In the 5 patients who did not show clinical improvement after initial 3 days of AAE therapy, colistin was added to the treatment regimen. The administration of colistin resulted in clinical cure of all the patients. Of 20 patients with polymicrobial infection, 12 patients with infection due to AAE and meropenem sensitive strains, were continued on empirical AAE treatment. Of these 12 patients, nine were cured. In the remaining 3 patients, colistin was added to the ongoing AAE therapy. The mean treatment duration for these 9 patients cured with AAE was (9.88 days $\pm$ 1.69 (SD)). However, 8 patients with cultures showing intermediate resistance towards both AAE and carbapenem, clinical success was achieved with the addition of colistin to ongoing AAE treatment regimen after 3 days of treatment. The mean treatment duration for the AAE and colistin therapy was (9.87 days $\pm$ 2.64 (SD)).

None of the 84 patients had any serious therapy related side effects. No untoward therapy-related changes in lab parameters were observed. All the patients had a good clinical response and were

176 stable and discharged.

177 4. Discussion

178 Carbapenems are broad-spectrum antibiotics which possess stability against hydrolysis by ESBL
179 and AmpC chromosomal β -lactamase enzymes and are often reserved to treat the most serious
180 infections [18-20]. The carbapenems have been effectively used to treat serious infections caused
181 by ESBL producing bacteria [21,22]. However in past few years, carbapenem resistance among
182 Gram negative bacteria has been reported increasingly throughout the world including India [21-
183 26]. This carbapenem resistance is contributed by various factors which include production of MBL
184 enzymes (carbapenamases), .increased production of AmpC chromosome-encoded
185 cephalosporinase, reduced outer membrane porin OprD expression, over expression of efflux
186 pumps and associated factors are known to contribute to carbapenem resistance [27-30]. Several
187 studies have demonstrated the role of EDTA in MBL inhibition and efflux pump down-regulation.
188 However, the role of EDTA in OprD and AmpC is not well reported (31-33).

189 In view of this increased resistance towards carbapenems, many researchers from the Indian sub-
190 continent have recommended the use of beta lactamase and beta lactamase inhibitor combinations
191 (BL + BLI) in place of carbapenems [27,34]. However, treatment with BL + BLI combinations may
192 not effectively eradicate both ESBL and MBL producers. Hence we have used an adjuvant
193 antimicrobial FDC, a novel combination of Ceftriaxone+sulbactam+disodium edetate to treat the
194 patients with infections caused by carbapenem resistant bacteria.

195 *In-vitro* microbial antibiotic susceptibility testing of the bacteria isolated from the 84 patients
196 yielded 4 groups of bacteria; G1 - with carbapenem resistant and AAE susceptible, G2 - both
197 carbapenem and AAE susceptible G3 – multiple pathogens showing susceptibility to both
198 meropenem and AAE and G4 – multiple Pathogens showing intermediate sensitivity to AAE and
199 meropenem. 12 of 14 group G1 patients treated with AAE achieved clinical success. This
200 established correlation of *in-vivo* results for the AAE treatment and justifies the selection of AAE to

201 treat infections caused by carbapenem resistant bacteria. Of the 25 group G2A patients receiving
202 carbapenem treatment 18 (72 %) achieved success. The results of the present study are in
203 accordance with the previous study by Chytra et al., who reported similar clinical cure rates
204 (74.3%) of critically ill patients using carbapenem (meropenem) therapy [1]. These clinical failure
205 rates may be attributed to the rise of carbapenem resistant bacteria, resulting from various
206 resistance mechanisms together with carbapenamase (MBL) production. One such resistance
207 mechanism is over-expression of efflux pumps like MexAB-OprM efflux system in *Pseudomonas*
208 *sp.* (specific to meropenem resistance) [35], AcrB efflux pumps in *E coli* [36], AcrAB efflux
209 pumps in *Klebsiella sp.*[37] and AdeABC type efflux pumps identified in *Acinetobacter sp.*[38]. Of
210 the remaining 7 patients from group two, 4 patients (57.14 %) achieved clinical success with AAE
211 treatment suggesting the treatment success with AAE over carbapenem. Similar trends were
212 observed in group G2B with 20 of 25 patients treated with AAE achieving clinical success. The
213 enhanced efficacy of AAE over meropenem may be attributed to the different ways in which AAE
214 target various resistance mechanisms in bacteria. The mechanisms include inhibition of conjugal
215 spreading of resistant gene from one bacteria to another. AAE does this by chelating Mg^{2+} ions
216 required for the activity of relaxases and thereby inhibiting conjugation process [39]. It has also
217 been reported that the AAE down-regulates the expression of MexAB-OprM and AcrAB-tolC
218 efflux pumps [40]. Sulbactam prevents inactivation of beta-lactam antibiotics by binding to the
219 beta-lactamases. EDTA, the adjuvant in AAE, chelates the divalent ions (Zn^{2+}) required the activity
220 of MBLs and thus deactivates the MBLs activity which in turn increases activity of the β -lactam
221 towards microorganisms [41]. Further, AAE is believed to disorganize the EPS and make the cell
222 wall more porous, thus enhancing its entry into the bacterial cells . It has also been found to inhibit
223 curli formation and bacterial adhesion [42]. The remaining patients were successfully treated when
224 colistin was administered with AAE, pressing us with a new therapeutic option in the patient who
225 failed AAE treatment. In 20 patients from the group G3 and group G4 clinical success was achieved

226 in 9 (45 %) patients with AAE treatment. In the remaining 11 patients, AAE and colistin therapy
227 was required to achieve clinical success. This antibiotic combination therapy provides us with an
228 efficient option to treat the single-drug resistant polymicrobial infections. The efficacy of this
229 combination might be due to the proven effect of AAE as an efflux pump inhibitor [40] which may
230 be helping colistin reach its site of action.

231 5. Conclusion

232 Increase in the carbapenem clinical failure rates in severe infections, and the efficacy of AAE
233 providing us with a carbapenem alternative in the treatment of moderate and severe infections
234 caused by resistant Gram negative bacteria. This study also provide us with an alternative to the use
235 of AAE with colistin to successfully treat patients with infections caused by organisms with
236 intermediate resistance to AAE and carbapenems. This is an encouraging result because of this, we
237 recommended the use AAE as a carbapenem sparing drug.

238 Consent

239 Due to the retrospective study design, informed consent was not deemed necessary.

240 Ethical Approval

241 Not applicable

242 References

- 243 1. Chytra I, Stepan M, Benes J, Pelnar P, Zidkova A, Bergerova T, et al. Clinical and
244 microbiological efficacy of continuous versus intermittent application of meropenem in
245 critically ill patients: a randomized open-label controlled trial. Crit Care. 2012;16:R113.
- 246 2. Win WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, et al. G.
247 In: Williams L, Wilkins, editors. Colour atlas & text book of diagnostic microbiology. 6th
248 ed. Philadelphia: Pa USA; 2006. p. 211–302.
- 249 3. Khajuria A, Kumar A, Praharaj, Kumar M, Grover N. Carbapenem Resistance among

Enterobacter Species in a Tertiary Care Hospital in Central India. Chemother Res Pract 2014; Article ID 972646, 6 pages.

4. Paterson D, Ko W, Von Gottberg A. In vitro susceptibility and clinical outcomes of bacteremia due to extended spectrum β -lactamase (ESBL)-producing *Klebsiella pneumoniae*. Clin Infect Dis. 1998;27:956.
5. Hidron AI, Edwards JR, Patel J, Horon TC, Sievert DM, Pollock Da, et al. NHSN annual update: Antimicrobial resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol. 2008;29:996–1011.
6. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: A new threat to our antibiotic armamentarium. Arch Intern Med. 2005;165:1430–1435.
7. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol. 2008;29:1099–1106.
8. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother. 2008;52:1028–1033.
9. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 1991;35:147–151.
10. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2001;45:1151–1161.

11. Goel N, Wattal C, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Trend analysis of antimicrobial consumption and development of resistance in non-fermenters in a tertiary care hospital in Delhi, India. *J Antimicrob Chemother.* 2011;66:1625-1630.
12. Devasahayam G, Scheld W, Hoffman P. Newer antibacterial drugs for a new century. *Expert Opin Investig Drugs.* 2010;19:215-234.
13. Livermore D. Discovery research: the scientific challenge of finding new antibiotics. *J Antimicrob Chemother.* 2011;66:1941-1944.
14. Sahu M, Sanjith S, Bhalekar P, Dipti. Waging War Against Extended Spectrum Beta Lactamase and Metallo betalactamase Producing Pathogens- Novel Adjuvant Antimicrobial Agent Cse1034- An Extended Hope. *J Clin Diagn Res.* 2014;8:20-23.
15. Bhatia P. Alternative Empiric Therapy to Carbapenems in Management of Drug Resistant Gram Negative Pathogens: A New Way to Spare Carbapenems. *Res J Infect Dis.* 2015; 3:2.
16. Patil UN, Jambulingappa KL. A Combination Strategy Of Ceftriaxone, Sulbactam And Disodium Edetate For The Treatment Of Multi-Drug Resistant (mdr) Septicaemia: A Retrospective, Observational Study In Indian Tertiary Care Hospital. *J Clin Diagn Res.* 2015;9(11):FC29-32.
17. Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Vol 32. Clinical Laboratory Standard Institute. Wayne, Pennsylvania, USA; 2012.
18. Ayalew K, Nambiar S, Yasinskaya Y, Jantusch BA. Carbapenems in pediatrics. *Ther Drug Monit.* 2003;25:593–599.
19. Zhanel GG, Wiebe R, Dilay L, Thomson K, Rubinstein E, Hoban DJ, et al. Comparative review of the carbapenems. *Drugs.* 2007;67:1027–1052.
20. Brink AJ, Feldman C, Grolman DC, Muckart D, Pretorius J, Richards GA, et al. Appropriate use of the carbapenems. *S Afr Med J.* 2004;94:857–861.

21. Shah D, Narang M. Meropenem. Indian Pediatr. 2005;42:443–450.
22. Merrem I.V. (meropenem for injection) [package insert]. Wilmington, DE: AstraZeneca Pharmaceuticals. 2007.
23. Francis RO, Wu F, Della-Letta P, Shi J, Whittier S. Rapid detection of *Klebsiella pneumoniae* carbapenemase genes in *Enterobacteriaceae* directly from blood culture bottles by real-time PCR. Am J Clin Path. 2012;137:527-532.
24. Hu F, Chen S, Xu X, Guo Y, Liu Y, Zhu D, Zhang Y. Emergence of carbapenem-resistant clinical *Enterobacteriaceae* isolates from a teaching hospital in Shanghai. China J Med Microbiol. 2012;61:132-136.
25. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res. 2006;124:95-98.
26. Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, et al. Carbapenem-non-susceptible *Enterobacteriaceae* in Europe: conclusions from a meeting of national experts. Euro Surveill. 2010;15:46.
27. Varaiya A, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in diabetes and cancer patients. Indian J Pathol Microbiol. 2008;51:200-203.
28. Chaudhary M, Payasi A. Antimicrobial susceptibility patterns and molecular characterization of *Klebsiella pneumoniae* clinical isolates from north Indian patients. Int J Med Med Sci. 2013;46:1218-1224.
29. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hegde A, Soman R. New Delhi Metallo- β -lactamase (NDM-1) in *Enterobacteriaceae*: Treatment options with Carbapenems Compromised. J Assoc Physicians India. 2010;58:147-149.
30. Chakraborty D, Basu S, Das S. A study on infections caused by metallo-betalactamase producing Gram-negative bacteria in intensive care unit patients. Am J Infect Dis.

2010;6:34-39.

31. Chaudhary M, Payasi A. Inhibition of metallo- β -lactamases by Eloxal. J Antimicrob. 2013;128:177-182.

32. Chaudhary M, Kumar S, Payasi A. A novel approach to combat acquired multiple resistance in *Escherichia coli* by using EDTA as efflux pump inhibitor. J Microb Biochem Technol. 2012;6:126-130.

33. Chaudhary M, Payasi A. Ethylenediaminetetraacetic acid: A non antibiotic adjuvant enhancing *Pseudomonas aeruginosa* susceptibility. Afr J Microbiol Res. 2012; 6:6799-6804.

34. Livermore DM. Of *Pseudomonas*, porins, pumps and carbapenems. J Antimicrob Chemother. 2001;47:247-50.

35. Quale JS, Bratu J, Gupta, Landman D. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. Antimicrob Agents Chemother. 2006;50:1633-1641.

36. Ghafur A, Pushparaju R, Nalini S, Rajkumar K, Sureshkumar D. Sensitivity pattern of Gram negative bacteria to the new β -lactam/ β -lactamase inhibitor combination: Cefepime/tazobactam. J Microbiol Infect Dis. 2012;2:120-125.

37. Rodriguez-Martinez, Poirel L, Nordmann P. Molecular Epidemiology and Mechanisms of Carbapenem Resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2009;53:4783-534788.

38. Padilla E, Llobet E, Domenech-Sanchez A, Martinez-Martinez L, Bengoechea JA, Alberti S. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother. 2010;54:177-183.

39. Roca I, Espinal P, Marti S, Vila J. First identification and characterization of an AdeABC-like efflux pump in *Acinetobacter* genomospecies 13TU. Antimicrob Agents Chemother. 2011;55:1285-1286.

- 350 40. Chaudhary M, Payasi A. Sulbactamax prevents antimicrobial resistance development by
351 inhibition of conjugal transfer of F plasmids. Int J Drug Dev Res. 2012;4:337-345.
- 352 41. Chaudhary M, Payasi A. Rising Antimicrobial Resistance of *Pseudomonas aeruginosa*
353 Isolated from Clinical Specimens in India. J Proteomics Bioinform. 2013;6:5-9.
- 354 42. Chaudhary M, Kumar S, Payasi A. Role of CSE1034 in *Escherichia coli* Biofilm
355 Destruction. J Microb Biochem Technol. 2013;5:54-58.