

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

(bacterial)

Aims: Carbapenem resistant bacterial 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 (eliminate parentheses) 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 polymicrobial infections. Among 20 polymicrobial 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 were cured with AAE, while the remaining 11 patients were 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 to carbapenem therapy but responded to AAE treatment. Hence, AAE can be used as an alternative to carbapenems 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 infected with antibiotic-resistant organisms 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 these infections 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 and are crucial for the treatment of infections caused by Gram-negative bacteria [3]. However, because of increasing beta lactam-resistance primarily 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 for the treatment of 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 an 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

antimicrobial development
52 development of antibiotics with novel mechanisms of action has required more research into
53 the use of available existing antibiotics. Scarcity of newer antibiotics and a declining drying antibiotic pipeline forces us
54 to look for an effective alternate empiric antibiotic therapy equivalent to carbapenems. of severe infections.

55 Recent effort to maximize antibiotic activity and overcome drug resistance has led to the search for
56 an alternate solution such as the use of antibiotic adjuvants. Antibiotic adjuvants are moieties, non-antibiotic
57 in nature, which in combination with antibiotics, enhance the antimicrobial activity of the latter
58 [14]. Fixed dose combination of Ceftriaxone + sulbactam + adjuvant disodium edetate is one such
59 novel Antibiotic Adjuvant Entity (AAE) approved by the Drug Controller General of India (DCGI)
60 and increasingly used in Indian hospitals. Various reports of the in-vitro susceptibility studies [14]
61 suggest hint of the possibility of this AAE to overcome the hurdles of both ESBL (sulbactam effect) as well
62 as metallo beta lactamases (MBL) (disodium edetate) producers clinically. Two recently published
63 retrospective studies support the use of the AAE in septicemia and sepsis [15,16]. Thus the main
64 goal of this study was to retrospectively analyze the clinical and microbiological efficacy of this
65 AAE in patients with moderate to severe IAI, LRTI and UTI.

66 2. Materials and methods

67 2.1 Patients and antibiotic therapy

68 This study was conducted at 200 bed tertiary care IVY hospital Mohali, Chandigarh. We retrospectively
69 reviewed the case reports of 108 ICU patients suffering from different infections (IAI, LRTI, UTI)
70 and who were treated at IVY hospital between January 2013 to November 2014. Of 108 patients, 84
71 patients were included in the study of which 34 patients were diagnosed with LRTI and each of 25
72 patients was diagnosed with IAI and UTI. Among these 84 clinically cured patients had both
73 positive infections and showing sensitivity to AAE were considered in the study, while the
74 remaining 24 patients were excluded from the study: 8 patients which were resistance to
75 meropenem or AAE, 12 patients which were changed to other antibiotics on the discretion of the
76 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 ^{upon} 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. ^{Patients receiving} ~~For colistin therapy,~~ ^(were given a) loading dose of 9 MIU followed by
82 *b.i.d.* doses of 4.5 MIU ~~were used.~~

What
is
MIU?

83 Case records were analyzed for age, sex, APACHE II score, co-morbidities, duration of
84 antimicrobial treatment, total leukocyte count, neutrophil count, liver function tests, renal function
85 test, ^{and} culture ^{of isolated organisms} 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 ^{It} with AAE or meropenem was initially ^{begun} ~~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-^{Monomicrobial} ~~Single~~
92 ~~bacterial~~ infection [64 (76.2 %) patients] and group B-^{polymicrobial infection} ~~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).** ^{together} ~~along~~ 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 these infections were evaluated prospectively upon initiation of therapy 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 had comorbid conditions 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 were classified into 3 groups: 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 monomicrobial 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 polymicrobial multi bacterial infections, 12 samples showed sensitivity to AAE and meropenem, while the remaining 8 (40%) samples showed intermediate susceptibility towards both cabapenem and AAE. From Enterobacteriaceae family *E. coli*, and *K. pneumoniae* were isolated most commonly, while *Neisseria gonorrhoeae*, *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).

151 3.3 Efficacy of antibiotic therapy

152 14 patients with carbapenem-resistant and AAE-susceptible bacterial infections were treated with
153 AAE. Bacteriological eradication with successful clinical response was observed in 12 patients. 2
154 ^{who} patients failed to ^{clinically} respond to AAE ^{clinically} were switched to AAE and colistin combination
155 therapy. The mean treatment duration ^{for these} for these 12 patients ^{who} were cured with AAE was 6.91 days
156 ± 1.08 (SD). 25 ^{out} of 50 patients with carbapenem and AAE-susceptible ^{organisms} were treated
157 with meropenem. ^{Of these patients} Of these patients, 18 ^{of these} patients showed clinical improvement (measured in terms of
158 disease symptoms) after ~~initial~~ 3 days of treatment and were continued ^{on} carbapenem therapy.
159 For the remaining 7 patients, ^{treatment was changed} treatment was changed to AAE. ^{Of these} Of these, 4 patients showed clinical
160 cure. The mean treatment duration for these 4 cured patients with AAE therapy was 5.75 days ± 0.5
161 (SD). For the remaining 3 patients, clinical success was achieved after the administration of colistin
162 with AAE. ^{Of} Among 25 ^{Similar} patients (having infections with carbapenem and AAE-susceptible
163 ^{organisms and} bacterial cultures) but treated with AAE, 20 ^{patients} showed satisfactory clinical cure. The mean
164 treatment duration for these 20 cured patients was 5.8 days ± 1.36 (SD). ^{In the} In the 5 patients who did
165 not show clinical improvement after ~~initial~~ 3 days of AAE therapy, colistin was added ^{to the} to the
166 treatment regimen. The administration of colistin resulted ⁱⁿ in clinical cure of all ^{the} patients. ^{Of 20} Of 20
167 patients with polymicrobial infection, 12 patients with infection due to AAE and meropenem
168 sensitive strains, were continued ^{on} on empirical AAE treatment. Of these 12 patients, ^{nine were cured} nine were cured.
169 In the remaining 3 patients, colistin ^{was added to} was added to the ongoing AAE therapy. The mean treatment
170 duration for these 9 patients cured with AAE was (9.88 days ± 1.69 (SD)). However, 8 patients with
171 cultures showing intermediate resistance towards both AAE and carbapenem, clinical success was
172 achieved ^{with the addition of colistin to ongoing AAE treatment regimen after 3 days of treatment}.
173 The mean treatment duration for the AAE and colistin therapy was (9.87 days ± 2.64 (SD)).
174 None of the 84 patients had any serious ^{treatment-associated} therapy-related side effects. ^{No untoward therapy-related} No untoward therapy-related ^{or}
175 ^{changes in lab parameters were observed} changes in lab parameters were observed. All the patients had a good clinical response and were

176 stable and discharged ~~with~~ at the time of hospital discharge.

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 ^{recent years} ~~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 ^{attributed to} ~~contributed by~~ various factors ^{including} ~~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 ^{other} ~~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 ^{of Gram-negative bacteria} ~~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

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. ~~and an antimicrobial agent~~

Handwritten notes:
- "provides" written above line 233.
- "The results of this retrospective study" written above line 234.
- "regimen" written to the right of line 234.
- "both" written above line 236.
- "therefore" written to the left of line 237.
- "to" written above line 235.
- "antimicrobial agent" written to the right of line 237.

238 Consent

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

240 Ethical Approval

241 Not applicable

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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 the 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, thereby providing 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

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