Quest Journals
Journal of Medical and Dental Science Research
Volume 4~ Issue 7 (2017) Aug. pp: 18-22
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

In Vitro Antimicrobial Susceptibility of Ceftriaxone Sulbactam EDTA (CSE 1034) And Other Β Lactam/ Β Lactamase Inhibitors And Carbapenems Against Enterobacteriaceae:A Comparative Study

Shashwati Nema[1], Prashant Nema[2]*, Khadija Mehdi[3], Kiran Tripathi[4], V. K.Ramnani[5]

[1] *Associate Professor, Dept.of Microbiology,AIIMS,Bhopal.*
[2]*Assistant Professor, Dept.of Surgery, People's College of Medical Sciences,Bhopal.*
[3]*Postgraduate Student, Dept.of Microbiology, LN Medical College & JK Hospital,Bhopal.*
[4]*Professor, Dept.of Microbiology, LN Medical College & J K Hospital,Bhopal.*
[5]*Professor & Head,Dept.of Microbiology, LN Medical College & J K Hospital,Bhopal.*
M.S.M.Ch.(Plastic & Reconstructive surgery)
Assistant Professor Dept.of Surgery, People's College of Medical Sciences,Bhopal
Corresponding Author: Dr.Prashant Nema

Received 14 July , 2017; **A**ccepted 09 September , 2017 © The author(s) 2017. **P**ublished with open access at **www.questjournals.org**

ABSTRACT: *Emergence of multidrug resistance among enterobacteriaceae has limited the treatment options. Therefore, it's high time to look ahead for newer possibilities. However, new antibiotic discovery and development has not kept pace with the existing demand of the same. An alternative aspect is use of potentiators of the already existing antibiotics the so called antibiotic adjuvants. The present study was designed to evaluate efficacy of new antibiotic adjuvant entity (AAE) - Ceftriaxone-Sulbactam-EDTA (CSE 1034) in comparison to other commonly used β lactam/ β lactamase inhibitors (BL+BLIs) and with carbapenems among enterobacteriaceae isolates. A total of 285 enterobacteriaceae obtained from different clinical specimens during study period of January 2015 to December 2015 were included. Antimicrobial susceptibility testing was performed for CSE 1034 and compared with susceptibility of amoxicillin-clavalunic acid, ampicilln-sulbactam, piparacillin-tazobactam, imipenem and meropenem. Escherichia coli (60.35%) was the predominant enterobacteriaceae obtained. Susceptibility of CSE 1034 was found to be significantly higher (p value <0.00001) than other antibiotics tested. It is recommended to include CSE 1034 in routine antibiotic sensitivity panel of enterobacteriaceae isolates. It may be considered as promising carbapenems sparer.*

Keywords: *β lactam/ β lactamase inhibitors, Carbapenems, CSE 1034, Enterobacteriaceae*

I. INTRODUCTION

Since the dawn of time, humankind has been afflicted with diseases that in some way or another have been associated with enterobacteriaceae as its causative organism. Members of enterobacteriaceae family are responsible for virtually any infectious disease and can be recovered from any specimen received in the laboratory. They produce infectious disease like diarrhea, dysentery, fever, septicemia, pneumonia. They have been identified as important nosocomial pathogens; infection can lead to severe morbidity and mortality, particularly in intensive care units (ICU), internal medicine and surgical units, and pediatric units [1]. Infections caused by enterobacteriaceae are treated with antibiotics, and the efficient agents are β-lactams and non β lactams group of antibiotics. β lactam antibiotics are the cornerstone of antibiotic for treatment which act effectively by inhibiting cell wall synthesis. A strong correlation between antibiotic use in the treatment of these diseases and antibiotic resistance development has been observed over the past half-century. Enterobacteriaceae have evolved sophisticated resistance mechanisms to combat the lethal effects of β lactams which involve an array of resistance determinants including altered expression of outer membrane proteins and efflux pumps along with an increasing arsenal of β lactamases[2]. β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics in gram negative bacteria[3]. ß-lactamases are the enzymes that target the ß-

lactam ring found in penicillins, cephalosporins, monobactams, and carbapenems. The selective pressures which are generated by the indiscriminate use of the ß -lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases such as the Extended Spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo-β-lactamases which have emerged as the most worrisome resistance mechanism which poses a therapeutic challenge to the health care settings [4]. β-lactam and β-lactamase inhibitor combinations (BL+BLIs) are used for ESBL producing isolates, but carbapenems seemed to be the best options to treat patients with ESBL. Unfortunately, emergence of metallo-beta-lactamases restricted the use of carbapenems [5,6]. Moreover, these isolates are resistant to other group of antibiotics as well. Looking beyond Carbapenems, options are very narrow, with colistin in focus and rather less hopeful results with tigecycline [7, 8]. Considering all these aspects, there is an urgent need to find out alternative antibiotic options. However, new antibiotic discovery and development has not kept pace with the existing demand of the same. An alternative aspect is use of potentiators of the already existing antibiotics the so called antibiotic adjuvants. These compounds can function either by reversing resistance mechanisms in naturally sensitive pathogens or by sensitizing intrinsic resistant strains [9]. Chaudhuri M et al [10], have put an effort to combat resistance among gram negative bacilli by evaluating novel adjuvant antimicrobial CSE 1034, a novel combination of Ceftriaxone+Sulbactam+EDTA . The addition of EDTA as a resistance breaker to ceftriaxone and sulbactam acts by interfering with the stability of outer membrane of microbes via chelating the cations and removing them from their binding sites thus elevating the permeability of the antibiotic [11]. EDTA has an ability to break biofilm[12] and down regulate over expression of efflux pump coding genes[13].

Keeping in view the above background, the present study was conducted to evaluate in vitro efficacy of new antibiotic adjuvant entity (AAE) - CSE 1034 in comparison to other commonly used BL+BLIs like amoxicillin-clavalunic acid, ampicillin-sulbactam and piparacillin-tazobactam as well as with carbapenems i.e.imipenem and meropenem among enterobacteriaceae isolates.

II. MATERIAL AND METHODS

2.1. Study design: Observational Cross-Sectional Study.
2.2. Study period: One year (January 2015-December 2015).
2.3. Study centre: Department of Microbiology L.N. Medical College and research centre, Bhopal, M.P.
2.4. Sample size: All the samples received in the microbiology laboratory during the study period.
2.5. Inclusion criteria

All biochemically confirmed, non-repetitive isolates of enterobacteriaceae with single type of growth.

2.6. Exclusion criteria
Enterobacteriaceae isolated from stool samples except *Salmonella spp, Shigella spp and Yersinia enterocolitica.*
2.7. Specimen collection
All types of clinical specimens such as urine, pus/wound swab, sputum, tracheal aspirate, blood, pharyngeal swabs, vaginal swabs, sterile body fluids etc. received in Microbiology laboratory during study period.
2.8. Processing of specimens
2.8.1. Microscopy

- Wet mount examination for urine specimens.
- Gram stain examination of heat fixed sample on a slide.

2.8.2. Culture
The samples were inoculated on Blood agar and MacConkey's agar and incubated for a period of 18- 24 hours at 37^0C.
2.8.3. Identification of the isolates
Bacterial growth on the medium was further identified using standard tests for enterobacteriaceae isolates [1].
2.8.4. Antimicrobial Susceptibility Testing
It was performed by Kirby Bauer Disc Diffusion method [14] as described by Clinical Laboratory Standard Institute (CLSI) guidelines[15].
The following antibiotics were tested

Antibiotics Group	Antibiotic
Novel AAE	Ceftriaxone – Sulbactam- EDTA
BL+BLIs	1. Amoxicillin-clavulinic acid 2. Ampicillin-sulbactam 3. Piperacillin-tazobactam
Carbapenem	1. Imipenem 2. Meropenem

The zone of inhibition was measured and interpreted as sensitive(S), intermediate (I), resistant (R) based on breakpoints.

2.8.5. Quality control

A quality control strain of E.coli ATCC 25922 was used throughout the study as per CLSI guidelines [15].
Quality control strain and antibiotic discs were procured from Himedia Mumbai.CSE 1034 disc was provided by Venus Medicine Research Centre,Baddi (H.P.), India.

2.9 Statistical Analysis

Chi Square test was applied to find out statistically significant difference between sensitivity of CSE 1034 and other antibiotics tested. p value was obtained.

III. RESULTS

A total of 285 Enterobacteriaceae isolates were obtained during study period.

Table 1: Distribution of Enterobacteriaceae isolates from clinical samples

Organism	**Number**	**Percentage**
Escherichia coli	172	60.35
Klebsiella pneumoniae	75	26.31
Enterobacter cloacae	11	3.85
Enterobacter aerogenes	06	2.1
Proteus mirabilis	08	2.8
Proteus vulgaris	05	1.75
Citrobacter fruendii	02	0.70
Citrobacter koseri	02	0.70
Providencia rettgeri	02	0.70
Morgenella morganii	01	0.35
Shigella flexneri	01	0.35
Total	285	100

Table 2: Comparison of antimicrobial susceptibility of BL+BLIs with CSE 1034

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	p value
Amoxycillin+clavalunic acid	47 (16.49)	10 (3.50)	228 (80)	< 0.00001
Ampicillin+sulbactam	95 (33.33)	13 (4.56)	177 (62.1)	
Piperacillin+ tazobactam	152 (53.3)	12 (4.21)	121 (42.45)	
CSE 1034	266 (93.3)	07 (2.45)	12 (4.21)	

Table 3: Comparison of antimicrobial susceptibility of Carbapenems with CSE 1034

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)	p value
Imipenem	211 (74.03)	18 (6.31)	56 (19.64)	< 0.00001
Meropenem	87 (30.5)	32 (11.2)	166 (58.24)	
CSE 1034	266 (93.3)	07 (2.45)	12 (4.21)	

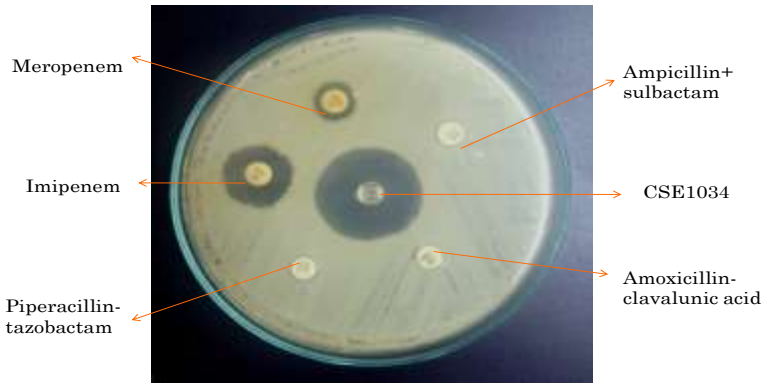

Image 1: Comparison of susceptibility of CSE1034 with BL+BLIs and Carbapenems

IV. DISCUSSION

Bacterial pathogens have developed greater ability to adapt and overcome the challenges of antibiotics in their environment that threaten to move us into the "post-antibiotic era" of infectious diseases. Indian hospitals have reported very high gram-negative resistance rates, with very high prevalence of ESBL (Extended Spectrum β lactamases) producers and also high carbapenem resistance rates [3, 4]. Among gram negative infections, enterobacteriaceae family members have been identified as important nosocomial pathogens; infection can lead to severe morbidity and mortality, particularly in intensive care units (ICU), internal medicine and surgical units, and pediatric units [1].

In the present study, 285 enterobacteriaceae isolates were obtained. Out of which *E. coli* was found to be the most prevalent pathogen followed by *K. pneumoniae* [Table 1].Earlier studies [16, 17] revealed that *E. coli* and *K. pneumoniae* have become the most common and opportunistic pathogens found in hospital-associated infections which supports current findings. The similar data was supported by Sachdeva et al [18] who also ascertained high prevalence (51.7%) of *E. coli* among all the clinical samples. Shilpa et al [19] determined the 23% prevalence of *K. pneumoniae* which is supported by Makkar et al [20] who also observed 22% *K. pneumoniae* among all clinical isolates. These findings are in accordance with our data.

Under a selective pressure induced by the extensive use of the cephalosporins, especially the third generation, ESBL producers appear and spread within the hospital. BL+BLIs are the drug of choice to treat such infections. In this study, susceptibility of commonly used BL+BLIs such as amoxicillin-clavalunic acid, ampicillin-sulbactam and piperacillin-tazobactam was observed. Amoxycillin-clavalunic acid and ampicillin-sulbactam showed high resistance whereas piperacillin-tazobactam combination was still found to be more effective than the other two [Table 2]. According to Peterson [21], piperacillin–tazobactam is clinically reliable for the treatment of serious infections caused by susceptible strains of ESBL-producing *E. coli* and *Klebsiella* spp. Al Zahrani et al [22] also reported similar findings. Susceptibility of existing BL+BLIs was compared with novel AAE, CSE1034. Susceptibility of CSE 1034 was found to be significantly higher than amoxycillin-clavalunic acid, ampicillin- sulbactam and piperacillin–tazobactam (p value < 0.00001) [Table 2]. In a similar study conducted by Jain S et al [23], sensitivity of CSE 1034 was reported to be better than piperacillin–tazobactam. Sahu M et al [6] also observed similar results.

When precious lives are at stake, carbapenems are the last resort. However, resistances to carbapenems are upsurging especially among enterobacteriaceae members. The prevalence of the carbapenem resistant enterobacteriaceae varies from region to region. A review of the data from the US based National Healthcare Safety Network found that in 2009-2010, about 13% of the *Klebsiella* species which were reported from the Central Line-Associated Bloodstream Infections (CLABSIs) and the Catheter-Associated Urinary Tract Infections (CAUTIs) were carbapenem-nonsusceptible[24]. About 2% of *Escherichia coli* which were reported from the CLABSIs and the CAUTIs were carbapenem-nonsusceptible. In the present study, resistance to carbapenems was detected with 19.29% isolates resistant to imipenem and 58.24% isolates resistant to meropenem [Table 3]. Incidences of meropenem resistance higher than that of imipenem among nosocomial pathogens was also observed by Gupta *et al*.[25]. When sensitivity of carbapenems was compared with CSE 1034, statistically significant difference was observed. Susceptibility of CSE 1034 was found to be significantly higher than imipenem and meropenem (p value <0.00001) [Table 3]. Arora S et al [26] observed better sensitivity of CSE1034 than imipenem and meropenem for gram negative bacilli which coincide with the present findings. Sachdeva N et al [18] reported higher susceptibility rates of CSE1034 in comparison with meropenem but equivalent to imipenem.

V. CONCLUSIONS

The present study revealed increasing resistance to BL+BLIs and carbapenems which are used to treat enterobacteriaceae infections. An in vitro susceptibility result of CSE 1034 appears to be promising and better. Therefore, it is recommended to include CSE 1034 in routine antibiotic sensitivity panel of enterobacteriaceae isolates to generate the local data. It should be considered as an alternative option to spare carbapenems as reserve drug.

ACKNOWLEDGEMENT

We are thankful to Venus Medicine Research Centre, Baddi (H.P.), India for providing us CSE1034 antibiotic discs for our study.

REFERENCES

[1]. Win WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Woods G, editors. Color atlas and textbook of diagnostic microbiology. 6th edition Philadelphia: Lippincott William and Wilkins; Enterobacteriaceae 2006 Pg 211-302.

[2]. Win WC, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, Woods G, editors. Color atlas and textbook of diagnostic microbiology. 6th edition Philadelphia: Lippincott William and Wilkins; Antimicrobial Susceptibility Testing 2006 Pg 945-963.

[3]. Bradford PA. Extended-spectrum -lactamases in the 21st century: Characterization, epidemiology and detection of this important resistance threat. *Clin Microbiol Rev* 2001; 14(4):933-51.
[4]. Rice LB, Bonomo RA.-lactamases:Which ones are clinically important? *Drug resistance update* 2000;3:178-89.
[5]. Hu F, Chen S, Xu X, Guo Y, Liu Y, Zhu D, et al. Emergence of carbapenem-resistant clinical enterobacteriaceae isolates from a teaching hospital in Shanghai. *China. J Med Microbiol.* 2012; 61:132-36.
[6]. Sahu M, Sanjith S, Bhalekar P, Keny D.Waging War Against Extended Spectrum Beta Lactamase and Metallobetalactamase Producing Pathogens- Novel Adjuvant Antimicrobial Agent CSE1034- An Extended Hope. *Journal of Clinical and Diagnostic Research* 2014; 8(6):20-3.
[7]. Biswas, S, Brunel JM, Dubus, JC, Reynaud-Gaubert M, and Rolain, JM. Colistin: an update on the antibiotic of the 21st century. *Expert Rev. Anti Infect. Ther*. 2012; 10(8):917–34.
[8]. Prasad P, Sun J, Danner RL, Natanson C. Excess deaths associated with tigecycline after approval based on noninferiority trials. *Clin. Infect. Dis.* 2012. 54(12), 1699– 1709.
[9]. Bernal P, Molina-Santiago C, Daddaoua, A. and Llamas, MA. Antibiotic adjuvants: identification and clinical use. *Microbial Biotechnology* 2013; 6(5): 445–49.
[10]. Chaudhary M, Sudaroli M, Kumar S, Krishnaraju. V "Catering ESBL resistance challenge through strategic combination of Ceftriaxone, Sulbactam and Ethylenediaminetetra acetic Acid. *Int. J. Drug Dev. & Res.* 2012; 4(1): 72-81.
[11]. Sahalan AZ, Aziz AHA, Lian HH, Ghani MKA. Divalent Cations (Mg2+, Ca2+) Protect Bacterial Outer Membrane Damage by Polymyxin B. *Sains Malaysiana* 2013; 42(3): 301–06.
[12]. Manu C, Payasi A.(2012) Role of CSE1034 in bacterial lipids and polysaccharides involved in biofilm formation: a comparison with other drugs. *Afr J Microbiol Res* 6:6525-31.
[13]. Chaudhary M, Kumar S, Payasi A. A novel approach to combat acquired multiple resistances in *Escherichia coli* by using EDTA as efflux pump inhibitor. *J Microb Biochem Technol* 2012; 4(6); 126-130.
[14]. Bauer AW, Kirby WM, Sherris JC, Jurck M. Antibiotic susceptibility testing by a standardized single disc diffusion method. *Am J Clin Pathol* 1996; 45; 493-6.
[15]. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial susceptibility testing; 25th Informational supplement. CLSI document Jan 2015; M100-S25;Vol 35 No3.
[16]. Akter J, Chowdhury AMMA, Al Forkan M. Study on Prevalence and Antibiotic Resistance Pattern of Klebsiella Isolated from Clinical Samples in South East Region of Bangladesh. *American Journal of Drug Discovery and Development* 2014;4: 73-9.
[17]. Kumar M, Chaudhary S, Makkar DJ, Garg N, Chugh S. Comparative antimicrobial efficacy evaluation of a new product Elores against Meropenem of gram negative isolates. *Asian J Pharm Clin Res*. 2015; 8(4): 251-54.
[18]. Sachdeva N. Antibiotic sensitivity pattern of bacterial pathogens in Rajeev Gandhi Cancer Hospital, Delhi. *Int J Rec Sci Res.* 2016; 7(1):8480-85.
[19]. Shilpa K, Thomas R, Ramyashree A. Isolation and Antimicrobial sensitivity pattern of Klebsiella pneumoniae from sputum samples in a tertiary care hospital. *International Journal of Biomedical and Advance Research* 2016; 7(2): 53-7.
[20]. Makkar DK, Kumar M, Chaudhary S, Goyal S, Aggarwal P, Garg N. Prevelance and Susceptibility Analysis of Gram negative Pathogens. *Journal of Pharmacy and Biological Sciences* 2015; 10(3): 58-65.
[21]. Peterson LR. Antibiotic policy and prescribing strategies for therapy of extended spectrum – lactamase producing enterobacteriaceae: The role of piperacillin tazobactam. *Clin Microbiol Infect* 2008; 14:181-4.
[22]. Al-Zahrani AJ, Akhtar N. Susceptibility patterns of Extended spectrum – lactamase producing (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated in a teaching hospital.*Pakistan J Med Res* 2005;44:64-7.
[23]. Jain S,Gupta A,Khare V. In vitro antimicrobial susceptibility of Ceftriaxone/Sulbactam/Ethylene Diamine Tetra Acetic Acid and Comparison to other β lactam/ β lactamase inhibitors,Carbapenems and Colistin against Gram negative bacteria. *International Journal of Infectious Diseases* 2016;45(1):95.
[24]. Kallen A,Guh A.United States Centers for Disease Control and Prevention issue updated guidance for tackling carbapenem resistant enterobacteriaceae. *Euro Surveillance* 2012; 17(26).pii:20207.
[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; 124:95-8.
[26]. Arora S,Munshi N.Comparative assessment of antibiotic susceptibility pattern of gram negative pathogens isolated from intensive care unit patients in Pune. *British Microbiology Research Journal* 2015; 10(2):1-9.

* Dr. Prashant Nema "In Vitro Antimicrobial Susceptibility of Ceftriaxone Sulbactam EDTA (CSE 1034) And Other B Lactam/ B Lactamase Inhibitors And Carbapenems Against Enterobacteriaceae:A Comparative Study." Quest Journals Journal of Medical and Dental Science Research , vol. 04, no. 07, 2017, pp. 18–22.