ORIGINAL ARTICLE

Evaluation of Cefixime-Clavulanate Combination by Comparative Disk Diffusion Method in *Klebsiella pneumoniae* Clinical Isolates-An *In-Vitro* Study

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Abstract:

Background: Resistance to cephalosporins due to β-lactamases is a major concern worldwide. However recent trend is to use β-lactamase inhibitor combinations. Potential combination is cefixime-clavulanate. *Objective:* Present study aims at the comparative evaluation of Fixed-Dose Combination (FDC) of cefixime-clavulanate and cefixime-alone in *Klebsiella pneumoniae* clinical isolates. *Material and Methods:* Study included 200 clinical isolates of *K. pneumoniae*. The Comparative Antimicrobial Susceptibility Test (AST) of cefixime-clavulanate (5µg/10µg) combination and cefixime-alone(5µg) was done by measurement and comparison of zone of lysis produced by both. All values were expressed in mean ± SD. Paired 't' test was used to determine statistical difference between different groups under study. P values < 0.05 were considered statistically significant. Isolates were tested for Extended-Spectrum β-lactamase (ESBL), AmpC β-lactamase (AmpC) and metallo β-lactamase (MBL) production by Clinical Laboratory Standards Institute - Phenotypic Disk Confirmatory Test (CLSI-PDCT), AmpC β-lactamase sterile disk test and Imipenem-Ethylene Di-amine Tetracetic Acid – Double disk synergy test (Imipenem-EDTA DDST) respectively. *Results:* Comparative AST resulted in statistically significant (P < 0.001) increased zones in cefixime-clavulanate combination than cefixime-alone in all isolates studied. When zones were evaluated separately only in three β-lactamase producing isolates; cefixime-clavulanate combination showed much higher zones in ESBL-producers (*n*=30) (P < 0.001), but not in AmpC-producers (*n*=32) (P = 0.5559) and MBL-producers (*n*=06) (P = 0.7815). *Conclusion:* Present study demonstrates the best bactericidal killing effect of cefixime-clavulanate compared to cefixime-alone. It is also of therapeutic significance in the treatment of infections caused by *K. pneumoniae* producing ESBLs. We recommend comparative AST method when commercially available newer β-lactamase inhibitor combination, for which no CLSI interpretive guidelines are available; to be studied systematically, before implementing it in treatment regimen.

Keywords: Comparative AST, FDC, ESBL producing *K. pneumoniae,* Cefixime-clavulanate

Introduction:

In the recent years bacteria have acquired a variety of resistance mechanisms. Among the various mechanisms, the most important mechanism is the production of β-lactamases which destroy penicillins and cephalosporins by hydrolyzing their β-lactam nucleus.

Third-generation cephalosporins have a broad-spectrum of activity and have proved to be good

in clinical and therapeutic conditions mainly for the treatment of many severe infections despite an escalating level of extended-spectrum β-lactamases (ESBLs) which are reducing the clinical usefulness of this class of antibiotics [1]. Originally, the more active cephalosporins were available for parenteral administration only, however during the last decade, a number of orally active third generation cephalosporins have become available. Their activity is similar to available parenteral drugs, showing pharmacokinetic advantages and; some of them, have better resistance to hydrolysis mediated by ESBLs. They may be good alternatives against many infectious syndromes [2].

Cefixime, the first oral third generation cephalosporin was quickly established in the western countries as a potent broad spectrum antibiotic. It is active against the most common pathogens involved in the infections for which it is indicated. A multinational, worldwide study has confirmed its excellent efficacy in children and adults [3]. However, these days resistance to cefixime is common and recently it has been reported in diverse organisms by few authors [4 -7].

In the present situation, emergence of resistance in organisms has limited the usefulness of many penicillins, cephalosporins, and other antimicrobial classes; driving increased utilization of carbapenems, in consequence of which metallo β-lactamases (MBLs) and AmpC β-lactamases (AmpC) have emerged. At present, there is a paucity of development of broad-

spectrum agents which can simultaneously target these resistant bacteria. Moreover, streamlining empirical antimicrobial therapy has not been successful. Therefore, β-lactam/β-lactamase inhibitor combinations offer a potential alternative to newer cephalosporins. ESBLs are usually susceptible to available β-lactamase inhibitors; hence these combinations are often seen as the only reliable alternatives for the treatment of infections caused by ESBL producing organisms. Such combinations have been successfully used in the clinical practice to treat and manage infections, including that of resistant bacteria [1]. In the recent years many newer β-lactamase inhibitor combinations are commercially available for therapeutic use. They are successfully being implemented in therapeutic regimen. Although Clinical and Laboratory standards Institute (CLSI) has not yet declared interpretive criteria for *in-vitro* evaluation of these newer combinations, few authors have tried to evaluate their *in-vitro* efficacy by using different methods like comparative diffusion methods, Minimum Inhibitory Concentration (MIC) studies and time kill curve studies [1,8].

A comparative evaluation of commercially available β-lactamase inhibitor combinations is extremely difficult and must be done under standardized test conditions [9]. Since its formulation, cefixime-clavulanate combination is being used for the treatment of various infections, however; the efficacy and usefulness of cefixime-clavulanate combination for better therapeutic effect has not yet systematically been

evaluated in the current literature. Until now, perhaps a single study done by Rawat *et al*. is available which has reported *in-vitro* efficacy of cefixime-clavulanate combination [10]. This study has reported efficacy of cefixime-clavulanate combination in comparison with amoxycillin-clavulanate, however the authors have extrapolated interpretive criteria of this combination from CLSI criteria of ceftazidime/cefotaxime-clavulanate combination due to the lack of CLSI interpretive guidelines for cefixime-clavulanate [10].

Cefixime-clavulanate is a fixed dose combination (FDC) of cefixime and clavulanate (*i.e.* clavulanic acid), a potent β-lactamase inhibitor along with suitable agent, which is used to overcome common bacterial infections. In this study, attempts have been made to evaluate cefixime-clavulanate combination in comparison with cefixime-alone by comparative study in *K. pneumoniae* clinical isolates producing three different types of β-lactamases *i.e.* ESBL, AmpC, and MBL. Even in the situation of the lack of CLSI guidelines, it sounds worthwhile to make an attempt to evaluate its efficacy based on purely comparative zone studies. To the best of our knowledge, there is no comparative study made to understand the impact of cefixime-clavulanate on its antimicrobial efficacy, and ours is the first such comparative study to evaluate commercially available susceptibility disks of cefixime-clavulanate with cefixime-alone in three different β-lactamase producing *K. pneumoniae* isolates implicated in varied clinical syndromes in a developing country setting.

Material and Methods:

The present study was carried out in the Department of Microbiology, Shri BM Patil Medical College, BLDE University, Bijapur, from 16th Oct 2009 to 28th Feb 2012.

Bacterial strains:

This cross sectional study included total 200 clinical isolates of *Klebsiella pneumoniae* obtained from different clinical syndromes.

Antibiotics:

Cefixime (5μg) and cefixime-clavulanate (5μg/10μg) used in the study were procured from HiMedia Lab. Pvt. Ltd., India.

Medium:

Mueller-Hinton agar (HiMedia Lab. Pvt. Ltd., India) was used for antibiotic susceptibility test.

Antimicrobial Susceptibility Testing:

The Comparative Antibiotic Susceptibility Test (AST) of cefixime-alone and cefixime-clavulanate combination was determined by measurement of zone of lysis *i.e.* susceptibility disks were placed on Mueller-Hinton agar plates pre-inoculated with test organisms and incubated overnight at 37^0C, after which lytic zones were read with the help of antibiotic zone reader [1,8,11]. Zones of cefixime-alone and cefixime-clavulanate were noted separately.

Detection of β-lactamases:

All isolates were tested for ESBL, AmpC and MBL production. ESBL production was tested by CLSI proposed screening disk diffusion method

using five indicator drugs and confirmed by CLSI phenotypic disk confirmatory test (CLSI-PDCT) employing ceftazidime (30μg) and ceftazidime in combination with clavulanate (30μg/10μg) disks [12]. Isolates were further tested for AmpC β–lactamase production by cefoxitin (30μg) disk screen and confirmed by AmpC sterile disk method [13,14]. Testing for MBL production was done by imipenem (10μg), meropenem (10μg) and ceftazidime (30μg) disk screening and confirmation by Imipenem-Ethylene Di-amine Tetracetic Acid (0.5M EDTA) double disk synergy test (IE-DDST) [15-18]. Quality control was achieved by using *K. pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Statistical Analysis:

Results were analyzed statistically by comparing lytic zones obtained by cefixime-clavulanate combination with lytic zones obtained by cefixime-alone. All values were expressed in mean ± SD. Paired 't' test was used to determine statistical difference between different groups under study. P values < 0.05 were considered statistically significant.

Results:

Out of 200 isolates of *K. pneumoniae,* 30 isolates gave positive results for ESBL production, 32 were positive for AmpC and only six isolates produced MBL. Susceptibility zones of cefixime-alone in total 200 isolates and susceptibility

zones separately in three β-lactamase producing isolate only, indicated high level of resistance to cefixime (72% to 100%) (Table 1).

Table 1: Results of Cefixime Susceptibility as Per CLSI Guidelines

K. pneumoniae	Resistant (%)
Total study isolates (*n*=200)	170 (85)
ESBL-positive (*n*=30)	29 (97)
AmpC-positive (*n*=32)	23 (72)
MBL-positive (*n*=06)	06 (100)

Abbreviations: ESBL - Extended-spectrum β-lactamase, AmpC - AmpC β-lactamase, MBL - Metallo β-lactamase, CLSI – Clinical and Laboratory Standards Institute

Results of comparative AST revealed that the susceptibility zones of cefixime-clavulanate were more increased or enhanced in all 200 isolates studied. It resulted in statistically significant (*P* < 0.001) increased zone measurement in cefixime-clavulanate combination than cefixime-alone (Table 2). However, when susceptibility zones were evaluated separately in three β-lactamase producing isolates only; cefixime-clavulanate combination showed much higher zones (P < 0.001) in ESBL-producers but not in AmpC-producers (P = 0.5559) and MBL-producers (P = 0.7815) (Table 2).

Table 2: Results of Comparative AST of Cefixime-Clavulanate In Comparison With Cefixime-Alone

K. pneumoniae isolates	Zone diameter (mm) Mean±S.D.		Paired 't' test value	P value
	Cefixime	**Cefixime + Clavulanate**		
Total study isolates (*n*=200)	4.55 ± 8.518	6.25 ± 9.04	4.92	< 0.0001*
ESBL-positive (*n*=30)	2.63 ± 6.371	8.33 ± 8.18	4.76	< 0.0001*
AmpC-positive (*n*=32)	7.41 ± 10.18	7 ± 10.28	0.60	0.5559
MBL-positive (*n*=06)	2.67 ± 6.532	1.67 ± 4.08	0.29	0.7815

Abbreviations: ESBL - Extended-spectrum β-lactamase, AmpC - AmpC β-lactamase, MBL - Metallo β-lactamase

*(*extremely significant difference)*

Discussion:

Alarming antimicrobial resistance and worldwide rapid spread of MBL and AmpC β-lactamase producing organisms represents a global clinical and public health crisis in the recent years and it will undeniably increase in the near future, if appropriate and timely measures are not taken. Antibiotic pressure facilitates selection of mutants which produce β-lactamases at high levels and organisms develop cross-resistance to other β-lactam antibiotics during β-lactam antibiotic therapy. In ESBL producing bacteria, development of resistance to third generation cephalosporins has become a major concern across the globe.

Infections caused by such bacteria offering resistance to cephalosporins are being treated by a combination of β-lactam antibiotic with β-lactamase inhibitor. This kind of practice has become progressively widespread due to increased use of cephalosporins, particularly cefixime. ESBL producing members of family *Enterobacteriaceae* are often resistant to multiple classes of drugs, making therapy with oral antibiotics intricate [19].

Therefore, in order to overcome rising problem of resistance; combination of cefixime-clavulanate is recommended for the management of ESBL infections.

The comparative AST as described in this study is the method usually used for interpretation of lytic zone results of β-lactam/β-lactamase inhibitor combination in comparison with β-lactam agent-alone when CLSI interpretative guidelines are not available. With this novel method it is possible to plausibly evaluate efficacy of newer commercially available inhibitor combinations before implementing them in the treatment regimen. In this regard efforts have been made by notably very few authors [1, 8, 11]. Hence our efforts were to employ this method for evaluating the efficacy of new commercially available inhibitor combination cefixime-clavulanate in organism such as *K. pneumoniae* which is the most notorious multiple drug resistant (MDR) bug and a producer of all three β-lactamases *i.e.* ESBL, AmpC, MBL. The comparative disk

diffusion method as reported in our study has been also used by Chaudhary *et al*. [1] and Srivastava *et al*. [8,11], however the inhibitor combinations studied by them were ceftriaxone-sulbactam [1,11] and cefepime-sulbactam [8].

In the present study, susceptibility results for cefixime-alone showed high resistance (85%) in all study isolates (*n*=200) (Table 1). However, the comparative AST *i.e.* comparative lytic zone results of cefixime-clavulanate combination in all isolates under study have revealed statistically significant (P < 0.001) increased zone measurement in cefixime-clavulanate combination than cefixime-alone (Table 2). This clearly demonstrated that, a combination of cefixime-clavulanate has more bactericidal activity *in-vitro* than cefixime-alone in all isolates. At the same time, susceptibility results for cefixime-alone separately in three β-lactamase producing isolates only, showed resistance in all ESBL-producers (*n*=30) except one isolate (97%) (Table 1). Such a grave resistance to ESBL isolates makes it highly impossible for oral antibiotics to act. Jain *et al*. reported 91% (*n*=58) resistance to cefixime in ESBL producing isolates of *K. pneumoniae* [5]. The comparative AST *i.e.* comparative lytic zone results of cefixime-clavulanate combination in isolates producing only three β-lactamases showed that the zones produced by cefixime-clavulanate were statistically significantly higher (P < 0.001) in ESBL-producers only, but not in AmpC-producers (P = 0.5559) and MBL-producers (P = 0.7815). This indicates that, cefixime-clavulanate

combination is of therapeutic importance in the management of infections caused by ESBL producing organisms.

Efficacy of cefixime-clavulanate in comparison with amoxycillin-clavulanate has been evaluated by Rawat *et al*. in clinical isolates of *Gram-negative* bacteria including *K. pneumoniae* and their study also marked the superiority of cefixime-clavulanate over amoxycillin-clavulanate in the treatment of infections caused by ESBL producing isolates [10]. Campbell *et al*. also advocated the combination of clavulanate and cefixime as an interesting therapeutic alternative for urinary tract infection caused by *E. coli* producing ESBL, for which no or very few oral antimicrobials are available [19].

Conclusion:

The present study clearly demonstrates the best *in-vitro* efficacy *i.e.* bactericidal killing effect of cefixime-clavulanate in comparison with cefixime-alone. It could be of therapeutic importance in the treatment of community-acquired infections caused by organism under study. Addition of the β-lactamase inhibitor clavulanate to cefixime apparently adds upon antimicrobial activity of cefixime. Moreover this combination has proved its therapeutic implication in the treatment of infections caused by multiple drug resistant strains of *K. pneumoniae* producing ESBLs. We recommend comparative AST method when commercially available newer β-lactam/β-lactamase inhibitor combination, for which no CLSI interpretive guidelines are available; to be studied systematically, before implementing it in the treatment regimen.

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