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Original Article

In Vitro Assay to Determine the Minimal Inhibitory Concentration$_{90}$ of β-lactam and β-lactam – β-lactamase Inhibitor against Community Acquired Respiratory Pathogens

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Abstract

Background: Respiratory tract infections (RTIs) accounts for two-third of all community-acquired bacterial infection. Antibiotic resistance in the form of penicillin-resistant *Streptococcus pneumoniae* or β-lactamase producing *Hemophilus influenzae, Moraxella catarrhalis, S. pneumoniae* forms the basis for overwhelming preference of β-lactam or β-lactam – β-lactamase inhibitor. However, with the increasing use of these drugs as empirical therapy, the prevalence of antibiotic resistance is on rise.

Objective: To determine minimal inhibitory concentration$_{90}$ (MIC$_{90}$) of β-lactam and β-lactam – β-lactamase inhibitor against community-acquired respiratory pathogens.

Materials and Methods: *In vitro* study was conducted to determine MIC$_{90}$ value of three commonly used antibiotics against three community respiratory pathogen. Isolates from patient's sputum, nasal swab, and throat swab were collected from 12 community centers across India, and the MIC$_{90}$ value for each antibiotic was determined using Broth dilution method.

Results: Totally 106 isolates were collected from 12 community center's across India. For isolates tested for *H. influenzae*, the mean MIC$_{90}$ of cefpodoxime-clavulanic acid was. 0.26 µg/ml, cefpodoxime was 0.72 µg/ml and amoxicillin-clavulanic acid 0.84 µg/ml. For isolates tested for *S. pneumonia* MIC$_{90}$ for cefpodoxime-clavulanic acid was 0.09 µg/ml; cefpodoxime was 0.69 µg/ml, and amoxicillin-clavulanic acid was 0.27 µg/ml. For isolate tested for *M. catarrhalis*, MIC$_{90}$ for cefpodoxime-clavulanic acid was 0.18 µg/ml, cefpodoxime was 1.01 µg/ml, and amoxicillin-clavulanic acid was 0.62 µg/ml. For all the isolate tested cefpodoxime-clavulanic acid demonstrated lower MIC$_{90}$ than cefpodoxime and amoxicillin-clavulanic acid for all the three pathogens. Cefpodoxime when used in combination with clavulanic acid there was a significant decrease in the MIC$_{90}$ against all the three pathogen which shows the synergistic action of the combination.

Conclusion: The fixed dose combination of cefpodoxime-clavulanic acid showed lower MIC$_{90}$ for the community respiratory pathogen and hence can be considered as a preferred therapy of choice for the treatment of RTI.

Key words: Amoxicillin-clavulanic acid, Cefpodoxime, Cefpodoxime-clavulanic acid, Minimal inhibitory concentration$_{90}$, Respiratory infection

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INTRODUCTION

Acute respiratory tract infections (RTIs) are common and persistent causes of morbidity, disability, and mortality. RTIs represent 60% of all community-acquired bacterial infections[1,2] and account for two-thirds of all antibiotic prescriptions written for the treatment of community-

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acquired infections.[3] It is the third commonest cause of mortality and morbidity worldwide.[4] Although most RTIs are caused by viruses, various bacteria, particularly *Streptococcus pneumoniae*, *Hemophilus influenzae* and *Moraxella catarrhalis*, are common causes of community-acquired pneumonia, acute exacerbations of chronic bronchitis, otitis media and sinusitis.[4]

Epidemiologically *S. pneumonia* accounts for 42% of acute sinusitis, 15% of acute exacerbation of chronic bronchitis and 20-75% of community-acquired pneumonia; *H. influenzae* accounts for 29% of acute sinusitis, 32% of acute exacerbation of chronic bronchitis and 3-10% of community-acquired pneumonia whereas *M. catarrhalis* accounts for 22% of acute sinusitis, 13% of acute exacerbation of chronic bronchitis and very less for community-acquired pneumonia.[3]

Antimicrobial therapy forms a mainstay in the treatment of all infectious disease. Ideally before starting an antimicrobial therapy an infectious disease diagnosis is reached by determining the site of infection, defining the host (e.g., Immunocompramised, diabetic, of advanced age), and establishing, when possible, a microbiological diagnosis. To optimize an accurate microbiological diagnosis, clinicians should ensure that diagnostic specimens are properly obtained and promptly submitted to the microbiology laboratory, preferably before the institution of antimicrobial therapy. Because microbiological results do not become available for 24-72 h, initial therapy for infection is often empiric and guided by the clinical presentation.[5]

Antimicrobial activity of drugs is usually assessed by determination of the minimal inhibitory concentration $_{90}$ (MIC_{90}) and the minimal bactericidal concentration of the drug *in vitro* after overnight aerobic incubation in a protein-free liquid medium at pH 7.2. The MIC_{90} is defined as the minimal concentration of antibiotic that prevents the clear suspension of 105 CFU/mL from becoming turbid after overnight incubation; turbidity usually connotes at least a 10 fold increase in bacterial density. It is the lowest concentration of antimicrobial agent required to inhibit the microorganism. The MIC_{90} is a measure of the potency of an antimicrobial drug. Sensitive strains have relatively low MIC_{90}s, and resistant strains have relatively high MICs.[6] The MIC_{90} can guide the choice of antimicrobial used in treatment by predicting efficacy. If pharmacokinetic and pharmacodynamics principles are met by careful selection of a specific anti-MIC_{90} robial given at an appropriate dosage, it will lead to a clinical cure, eradication of carrier status of a specific organism, and prevention of selection of resistance.

The present study was conducted to determine the MIC_{90} of the three most commonly used antibiotic: Cefpodoxime, cefpodoxime-clavulanic acid and amoxicillin-clavulanic acid in the treatment of RTI against three most common organisms responsible for RTI: *S. pneumonia*, *H. influenzae* and *M. catarallis*.

MATERIALS AND METHODS

The present *in vitro* study was conducted for 4 months (June 2015 to September 2015) to determine MIC_{90} of three commonly used antibiotic, that is, cefpodoxime, cefpodoxime-clavulanic acid and amoxicillin-clavulanic acid against three community respiratory pathogen, that is, *S. pneumonia*, *H. influenzae* and *M. catarrhalis*. Isolates from patient's sputum, nasal swab and throat swab were collected from 12 different community centers across India, and the MIC_{90} for each antibiotic was determined using Broth dilution method. 10 isolates per center were subjected for evaluation. Susceptibility of antibiotics or combination was evaluated as per the Clinical and Laboratory *Standards* Institute (CLSI) breakpoint criteria.[7] The data obtained was kept confidential and was used for the purpose of study. Data were compiled, analyzed and expressed in terms of arithmetic mean and percentage. The difference was statistically determined using GraphPad Prism software version 7.

RESULTS

The present *in vitro* study was conducted to analyze the MIC_{90} of three commonly used antibiotics in the treatment of RTI against three common organisms. 106 isolated were collected of which, 36 were for *H. influenzae,* 42 for *S. pneumoniae* and 28 for *M. catarallis*. Samples from patient's sputum, nasal swab and throat swab were analyzed for MIC_{90} of three antibiotics (i.e., cefpodoxime-clavulanic acid; cefpodoxime and amoxicillin-clavulanic acid) against *H. influenzae*. The mean MIC_{90} of cefpodoxime-clavulanic acid was 0.26 ± 0.41 μg/ml (0.01-1) in comparison to cefpodoxime (0.72 ± 1.21 μg/ml) and amoxicillin-clavulanic acid (0.84 ± 0.79 μg/ml) (Figure 1). Statistically significant result (P = 0.0002) was seen between the MIC_{90} of cefpodoxime-clavulanic acid with cefpodoxime monotherapy (P = 0.03) (Table 1). Among the isolate tested 5 (13.8%), resistance strain was seen. Cefpodoxime-clavulanic acid retained sensitivity for 3 out of 5 resistant strains (60%), but the strains remained resistant to amoxicillin-clavulanic acid.

For all the isolates tested for *S. pneumonia*, cefpodoxime-clavulanic acid demonstrated lower MIC_{90} (0.09 ± 0.12 μg/ml) (0.01-0.1); than cefpodoxime (0.69 ± 0.35 μg/ml) and amoxicillin-clavulanic acid (0.27 ± 0.39 μg/ml) (Figure 2). Statistically significant result (P = 0.0054) was seen between

the MIC_{90} vales of cefpodoxime-clavulanic acid with cefpodoxime monotherapy ($P = 0.0001$) (Table 2). Among the isolate tested 2 (4.7%), resistant strain were seen. Cefpodoxime-clavulanic acid and amoxicillin-clavulanic acid retained sensitivity for all the resistant strains.

28 isolates from patient's sputum, nasal swab and throat swab were analyzed for *M. catarrhalis*. For all the isolates tested cefpodoxime-clavulanic acid demonstrated lower MIC_{90} 0.18 ± 0.31 μg/ml (0.01-0.1) than cefpodoxime (1.01 ± 1.16 μg/ml) and amoxicillin-clavulanic acid (0.62 ± 0.62 μg/ml) (Figure 3). Statistically significant result ($P = 0.0014$) was seen between the MIC_{90} vales of cefpodoxime-clavulanic acid cefpodoxime monotherapy ($P = 0.0001$) (Table 3). Among the isolate tested 2 (7.1%), resistant strain were seen. Cefpodoxime-clavulanic acid and amoxicillin-clavulanic acid retained sensitivity for all the resistant strains.

DISCUSSION

The increasing prevalence of antibiotic-resistant bacterial pathogen commonly associated with RTI and the variation in the rate of resistance to a range of antibiotic is now acknowledged to be a global problem. The antibiotic susceptibility (or resistance) of a strain cannot be measured directly but must be deduced from the *in vitro* activity of the antibiotic. Among the various methods available, MIC_{90} determination is the most widely used to assess *in vitro* activity for clinical categorization of clinical isolates.[8]

Table 1: Comparison of MIC_{90} of combination versus monotherapy for *H. influenzae*

Anti- microbial agents	*P* value
Amoxicillin-clavulanic acid versus cefpodoxime	0.61
Cefpodoxime-clavulanic acid versus cefpodoxime	0.03

MIC: Minimal inhibitory concentration, *H. influenza: Hemophilus influenza*

Table 2: Comparison of MIC_{90} of combination versus monotherapy for *S. pneumoniae*

Anti- microbial agents	*P* value
Amoxicillin-clavulanic acid versus cefpodoxime	0.001
Cefpodoxime-clavulanic acid versus cefpodoxime	0.0001

MIC: Minimal inhibitory concentration, *S. pneumonia: Streptococcus pneumoniae*

Table 3: Comparison of MIC_{90} of combination versus monotherapy for *M. catarrhalis*

Anti- microbial agents	*P* value
Amoxicillin-clavulanic acid versus cefpodoxime	0.12
Cefpodoxime-clavulanic acid versus cefpodoxime	0.0006

MIC: Minimal inhibitory concentration, *M. catarrhalis: Moraxella catarrhalis*

The present study was conducted to determine the MIC_{90} of the three common antibiotic used in the treatment of RTI against common community respiratory pathogen, that is, *S. pneumonia*, *H. influenzae* and *M. catarrhalis*. For all the isolate tested cefpodoxime-clavulanic acid demonstrated lower MIC_{90} than cefpodoxime and amoxicillin-clavulanic acid for all the three pathogens. A significant difference was seen in the MIC_{90} value of cefpodoxime-clavulanic acid and cefpodoxime for all pathogens.

The present study showed the MIC_{90} of amoxicillin-clavulanic acid to be 0.84 μg/ml for *H. influenzae* with 94.44% susceptibility, 0.27 μg/ml for *S. pneumonia* with 100% susceptibility and 0.62 μg/ml with 100%

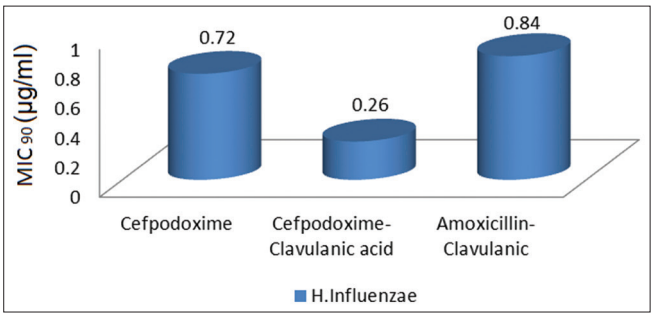

Figure 1: Comparison of minimal inhibitory concentration$_{90}$ values of three antibiotics against *Hemophilus influenzae*

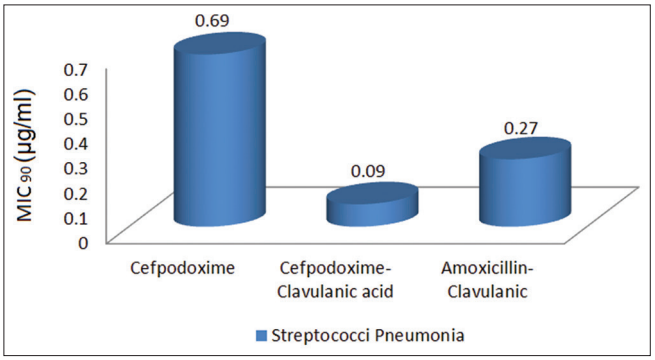

Figure 2: Comparison of minimal inhibitory concentration$_{90}$ values of three antibiotics against *Streptococcal pneumonia*

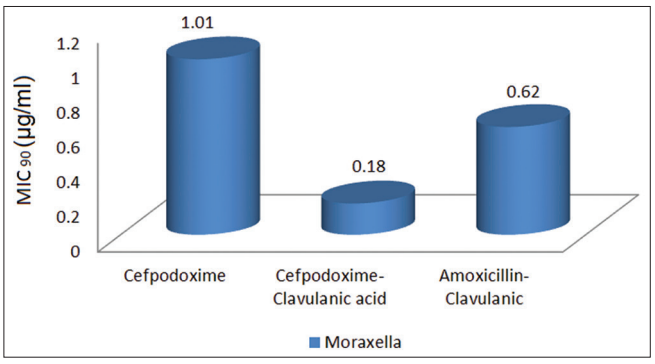

Figure 3: Comparison of minimal inhibitory concentration$_{90}$ values of three antibiotics against *Moraxella catarrhalis*

susceptibility for *M. catarrhalis*. The results of our study can be compared to MIC_{90} of amoxicillin-clavulanic acid conducted Koeth and team where the MIC_{90} was 1 μg/ml for *H. influenzae* with 98.7% susceptibility, 0.25 μg/ml with 100% susceptibility for *M. catarrhalis* and 1 μg/ml with 93.5% susceptibility for *S. pneumonia*.[9] Jacob and team in there susceptibility study for 10 oral antibiotics reported, MIC_{90} of amoxicillin-clavulanic acid to be 1 μg/ml for *H. influenzae* with 97.5% susceptibility, 2 μg/ml for *S. pneumonia* with 93.9% susceptibility.[10]

According to the CLSI susceptibility criteria,[7] for amoxicillin-clavulanic acid MIC_{90} of ≤2 μg/dl in a susceptible range and ≥8 μg/dl is in resistance range for *S. pneumonia*. For *H. influenzae* MIC_{90} of 2 μg/ml in a susceptible range and 16 μg/ml is in resistance range. For cefpodoxime MIC_{90} of 0.25 μg/ml is in a susceptible range and 1 μg/dl is in resistance range for *H. influenzae* whereas for *S. pneumonia* MIC_{90} of ≤0.5 μg/ml is in a susceptible range and for ≥2 μg/ml is in resistance range.

In vitro studies have shown the peak plasma concentration of cefpodoxime 200 mg single dose to be 2.18 mcg/ml[11] and that of clavulanic acid 125 mg single dose to be 2.2 mcg/ml.[12] microbiological studies have shown that antibiotic: Clavulanate ratio ranging from 1:1 to 16:1 improves sensitivity of antibiotic.[13] The peak plasma concentration of cefpodoxime-clavulanic acid lies within the ratio of 1:1 to 2:1.

The present study has also highlighted, when cefpodoxime was used in combination with clavulanic acid, there was a significant decrease in the MIC_{90} against all the three pathogen which shows the synergistic action of the combination. MIC_{90} of both the combination, that is, cefpodoxime-clavulanic acid and amoxicillin-clavulanic acid were within the susceptibility range according to the CLSI susceptibility criteria.

CONCLUSION

The recent increase in the resistance of the major respiratory pathogens to oral antimicrobial agents has produced a need to re-evaluate treatment options for RTIs. The fixed dose combination of cefpodoxime-clavulanic acid showed lowest MIC_{90} for the community respiratory pathogen and hence can be consider as a preferred therapy of choice for the treatment of RTI. The results of this study should be applied to clinical practice based on the clinical presentation of the patient so a randomized blinded clinical trial should be conducted to confirm the benefit obtained from our study.

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