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Original Article

A Prospective Comparative Study to Evaluate the Susceptibility of Bacterial Strains Isolated from Patients with Respiratory Tract Infection to Cefpodoxime Plus Clavulanic Acid and to Amoxicillin Plus Clavulanic Acid

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Abstract

Respiratory tract infections (RTIs) are one of the major public health problems in India. Susceptibility tests conducted to ascertain the susceptibility of the isolated bacteria to various antibiotics and to identify emerging trends of antibiotic resistance are helpful for antibiotic selection for initiating empirical treatment while awaiting results of the culture and sensitivity. This study was conducted to evaluate the susceptibility of bacterial strains isolated from patients with RTIs, determined by *in-vitro* strip method, to amoxicillin plus clavulanic acid in comparison to cefpodoxime plus clavulanic acid in terms of the Minimal Inhibitory Concentration (MIC) values a hundred subjects of either gender more than 3 months age, with clinical diagnosis of RTI and having culture positive samples with either *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae,* and/or *Moraxella catarrhalis* were considered. Primary efficacy endpoint was to compare MIC_{90} of the two antibiotics (cefpodoxime plus clavulanic acid and amoxicillin plus clavulanic acid) against these bacteria. Secondary efficacy endpoints were MIC_{50} of the two antibiotics and the geometric mean MIC of the two antibiotics. The MIC_{90} and MIC_{50} values of cefpodoxime plus clavulanic acid are found to be lower as compared to that of amoxicillin plus clavulanic acid for all four bacterial strains, that is, *S. aureus*, *S. pneumoniae*, *S. pyogenes* and *M. catarrhalis*. Further, the mean MIC values of cefpodoxime plus clavulanic acid are significantly lower in comparison with that of amoxicillin plus clavulanic acid for *S. aureus*. Pain and discomfort during collection of throat swab were adverse events recorded in the study.

Key words: Amoxicillin, Antibiotic resistance, Cefpodoxime, India, Respiratory tract infections

INTRODUCTION

Respiratory tract infections (RTIs) are one of the major public health problems and one of the leading causes of morbidity and mortality in both developed and developing countries. RTIs account for over 50 million deaths each year globally.[1] In fact, RTIs are one of the commonest types of infections affecting the Indian population, with prevalence rates ranging around 52%. Most of the RTIs are limited to the upper respiratory tract and only 5% involve the lower respiratory tract. As per the main locus of the infections, RTIs are categorized as – upper RTI (URTIs) and lower RTI (LRTIs). URTIs involve the nasal passages, pharynx, tonsils, and epiglottis, whereas LRTIs involve the bronchi and alveoli and include two serious conditions – acute bronchitis and pneumonia.[1,2] Some of these respiratory infections such as common cold, pharyngitis, and otitis media are more common among children and peak from infancy to 5 years.[3]

Paediatric RTIs are one of the most common reasons for physician visits and hospitalization. These infections present one of the major complaints in children and adolescents.[4] On an average, children below 5 years of

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age suffer about 5 episodes of acute respiratory infection per child per year, thus accounting for about 238 million attacks and about 13 million deaths every year in the world.[5] The child with recurrent respiratory infections presents a difficult diagnostic challenge. The recurrent respiratory infections in infants and children are among the most common causes of counseling and admission to the hospital. They are responsible for significant morbidity measured by school days lost.[6] Cough and cold lead to missed school days for children, this in turn leads to missed office days for working parents who need to stay at home to take care of their children.[7]

Physicians, in general, rely on clinical signs and symptoms to diagnose respiratory infections; the causative microbes are rarely identified. The uncomplicated URTIs are most likely caused by viruses. The viruses detected most frequently during acute RTI are human *rhinoviruses*, *paramyxoviruses*, *coronaviruses,* and *bocavirus*.[6] Bacterial URTIs are mainly caused by *Streptococcus*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Proteus*, *Enterobacter*, and *Haemophilus*. However, the responsible pathogens are usually un-identified in 50% of the patients despite of thorough diagnostic tests being carried out.[8] The choice of antibiotics in these cases is mostly empirical, usually based on the severity of illness, the known probabilities of the pathogens in specific geographical areas, resistance patterns of the most commonly implicated etiological agents, and associated co-morbidities.[8] The high disease and economic burden of RTIs call for evidence-based public health approaches, including a better understanding of causative microorganisms, for prevention and treatment of RTI.[9]

Guidelines on antibiotic choice for RTI are generally not consistent.[10] The main class of antibiotic prescribed are penicillins, cephalosporins, macrolides, and fluoroquinolones. In many countries, plain amoxicillin and other penicillins are recommended as first-line therapy for most children with acute infections; however, amoxicillin-clavulanate, which is a broad-spectrum antibiotic, is more popularly used. Broad-spectrum antibiotics for URTIs would be the ones with activity against clinically important colonizing flora beyond *Pneumococcus* (the primary target for the specified acute RTI) such as *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Staphylococcus aureus*. Apart from amoxicillin-clavulanate, the other commonly prescribed broad-spectrum antibiotics in RTI are the cephalosporins and macrolides.

Susceptibility tests determine a microbe's susceptibility/vulnerability to antimicrobial drugs by exposing a standardized concentration of organisms to specific concentrations of antimicrobial drugs. Susceptibility testing can be done for microbes. Antimicrobial Susceptibility Testing (AST) can be used for drug discovery, epidemiology, and prediction of therapeutic outcome.[11] First introduced in 1929, *in vitro* AST methods are considered to be the most valuable in determining the efficacy of antimicrobial compounds against various microorganisms.[12] In general, AST methods combine one or more antimicrobial agents with bacteria to assess bacterial growth. This testing is essential to determine the possible suitability of specific antibiotics on inhibiting the bacteria and/or to determine if the bacteria have developed resistance to certain antibiotics. The results of this test can be used to help select the particular antibiotics that can be expected to be most effective in treating an infection.

Accurate and appropriate susceptibility testing of microbes will guide the physician in choosing the antimicrobial agent for difficult-to-treat infections and ensure optimal effective patient-tailored therapy and avoid over-prescription. The results from susceptibility testing are reported as the MIC, which is defined by the lowest concentration of a drug in which no visible growth occurs. The MIC_{50} represents the concentration at which ≥50% of the isolates in a test population are inhibited; it is equivalent to the median MIC value. The tested microorganism is then classified as either clinically susceptible, intermediate, or resistant to the tested drug. The MIC_{90} represents the MIC value at which ≥90% of the strains within a test population are inhibited; the 90th percentile.

Bacteria have the capability to develop resistance to antibiotics at any time. This means that antibiotics which once could kill or inhibit their growth may no longer be effective. Numerous resistant strains of major pathogens are emerging and so susceptibility testing will surely help out to observe their pattern of growth. This also helps to keep under scrutiny the susceptibility pattern of the existing-prevalent microbial strains.

Combination of amoxicillin with clavulanic acid and cefpodoxime with clavulanic acid are two popular antibiotics that are used by the General Practitioners, ENT specialists, and pediatricians for the management of URTIs. Both are broad-spectrum antibiotics with demonstrated efficacy against various aerobic (Gram-positive and Gram-negative) bacteria and anaerobic bacteria. Table 1 lists the microbes that are shown susceptible to these antimicrobials.

The current study was planned to evaluate the susceptibility of bacterial strains isolated from patients with upper RTI to amoxicillin plus clavulanic acid as compared to cefpodoxime plus clavulanic acid with respect to MIC values, determined by *in-vitro* strip method.

Table 1: List of microorganisms that are shown susceptible to amoxicillin plus clavulanic acid and cefpodoxime plus clavulanic acid

Commonly susceptible microorganisms	
Amoxicillin plus clavulanic acid	**Cefpodoxime plus clavulanic acid**
Aerobic Gram-positive micro-organisms	
Enterococcus faecalis	*Staphylococcus aureus* (including penicillinase-producing strains)
Gardnerella vaginalis	Note: Cefpodoxime is inactive against methicillin-resistant *staphylococci.*
Staphylococcus aureus (methicillin-susceptible)	*Staphylococcus saprophyticus*
Coagulase-negative *staphylococci* (methicillin-susceptible)	*Streptococcus pneumoniae* (excluding penicillin-resistant strains)
Streptococcus agalactiae	*Streptococcus pyogenes**
Streptococcus pneumoniae	*Streptococcus agalactiae**
Streptococcus pyogenes and other	*Streptococcus agalactiae**
beta-hemolytic *streptococci*	*Streptococcus* spp. (Groups C, F, G)
Streptococcus viridans group	Note: cefpodoxime is inactive against *enterococci*
Aerobic Gram-negative micro-organisms	
Capnocytophaga spp.	*Escherichia coli*
Eikenella corrodens	*Klebsiella pneumoniae*
Haemophilus influenzae	*Proteus mirabilis*
Moraxella catarrhalis	*Haemophilus influenzae* (including beta-lactamase-producing strains)
Pasteurella multocida	*Moraxella* (Branhamella) catarrhalis
	Neisseria gonorrhoeae (including penicillinase-producing strains)
	Citrobacter diversus
	Klebsiella oxytoca
	Proteus vulgaris
	Providencia rettgeri
	Haemophilus parainfluenzae
	Note: Cefpodoxime is inactive against most strains of *Pseudomonas* and *Enterobacter*
Anaerobic micro-organisms	
Bacteroides fragilis	*Peptostreptococcus magnus**
Fusobacterium nucleatum	
Prevotella spp.	

*Safety and efficacy of cefpodoxime in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials

MATERIALS AND METHODS

The objective of this study was to evaluate the susceptibility of bacterial strains isolated from patients with upper RTI to cefpodoxime plus clavulanic acid as compared to amoxicillin plus clavulanic acid by *in-vitro* strip method. The study was conducted in compliance with the "National Ethical Guidelines for Biomedical and Health Research Involving Human Participants" issued by Indian Council of Medical Research, 2017 and in accordance with the ethical considerations laid down in Declaration of Helsinki, Fortaleza, Brazil (October 2013). The study/trial was conducted as a prospective, active-controlled *in-vitro* study in samples obtained from treatment-naïve patients suffering from RTIs. This was a study to compare MIC of two different antibiotic combinations and did not involve any treatment provided to the enrolled patients. As this was a study conducted to generate real-world data and to assess the antibiotic sensitivity scenario; hence, no formal sample size calculation was done. The patients enrolled in the study were treated by the physician/investigator as per his/her standard of care (routine clinical practice) and patients were not given any specific treatment as a part of this study. The Study Protocol and Patient Informed Consent Document (ICD) (in English and other applicable vernacular languages) were submitted to the applicable locally registered Institutional Ethics Committee before initiating the study at the site. The approval was received before the start of the study itself. Written informed consent was obtained before initiation of any of the study/trial-related activities on the ICDs from all patients willing to take part in the study. The patients who had provided their consent to participate in the study were then evaluated as per the inclusion/exclusion criteria. The main inclusion criteria were: (a) Patients of either gender more than 3 months, (b) patients with clinical diagnosis of RTI, and (c) patients or patient's legally acceptable representative willing to sign the ICD. The exclusion criteria were: (a) Patients who had taken antibiotics, antiviral agents, or interferon therapy in the past 30 days before enrolment into this study, (b) patients who were on immunosuppressive therapy, (c) patients who had taken any vaccine in the past 30 days, (d) patient who has participated in any other clinical study in the past 30 days, and (e) patients not willing to give a sample for analysis.

The two antibiotic combinations for which MIC was evaluated were:

- Antibiotic 1: Cefpodoxime plus clavulanic acid: Cefpodoxime/clavulanic acid Ezy MIC™ Strip (CPD) manufactured by HIMEDIA (Catalog No. EM138), capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml
- Antibiotic 2: Amoxicillin plus clavulanic acid: Amoxyclav Ezy MIC™ Strip (AMC) manufactured by HIMEDIA (Catalog No. EM003), capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml.

Ezy MIC™ Strips is a quantitative technique for determining the antibiotic susceptibility of a wide range of aerobic and fastidious organisms. The system comprises a predefined antibiotic gradient which is coated on a paper strip used to determine the Minimum Inhibitory Concentration (MIC), in μg/ml, of different antibiotic agents against a variety of microorganisms when tested on appropriate agar media under specific incubation conditions. As with other dilution methods, Ezy MIC™ Strip directly quantifies antibiotic susceptibility in terms of discrete MIC values. However, using a predefined, stable, and continuous antibiotic concentration gradient, MIC values observed using Ezy MIC™ Strip are more precise and reproducible results are obtained as compared to those from conventional procedures based on discontinuous two-fold serial dilutions.

Based on the clinical signs and symptoms, either of the samples was taken from the eligible patients: (1) Throat swab, (2) sputum, or (3) pus. Only one sample was taken per patient. The sample was collected following all requisite aseptic precautions and stored in wide-mouthed sterile bottles. These were then shipped to the diagnostic laboratory within 4 h of collection and processed in laboratory; sample culture was performed and the organism was identified.

If any of the six target bacteria were isolated from the sample collected (*Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus influenzae,* and *M. catarrhalis*), antibiotic sensitivity testing was done using strip method and MIC was determined for two antibiotic combinations under study. The primary efficacy endpoint was MIC_{90} of the two antibiotics combination (cefpodoxime plus clavulanic acid and amoxicillin plus clavulanic acid) against the different bacteria isolated. The secondary efficacy endpoints were (1) MIC_{50} of the two antibiotics combination against the different bacteria isolated, (2) MIC of the two antibiotics combination against the different bacteria isolated, and (3) proportion of samples sensitive for the two antibiotics combination as per criteria laid down by Clinical and Laboratory Standards Institute (CLSI).

As no particular medication was provided to the patients enrolled in the study, safety evaluation of study medication stands not applicable. However, like any other clinical trial procedure, adverse events were anticipated at the time of sample collection. Adverse events if any occurring during the sample collection process were recorded. After receiving the case record forms (CRFs), the data were checked for inclusion and exclusion criteria and then evaluated for analysis. The patients were to be treated by the investigator as per the routine clinical practice.

The data collected from the CRFs were analyzed for demographics, efficacy, and safety outcome using GraphPad Prism Version 8.0.1(244). Data were presented as mean (95% confidence intervals)/geometric mean (95% confidence intervals) or number (percentage). Descriptive statistics were used for different variables at baseline. $P < 0.05$ was considered as statistically significant. Standard statistical tests (unpaired *t*-test for continuous variables/ log-transformed MIC data and Fischer's exact test for categorical data) were used to analyze the data obtained.

RESULTS

Clinical Phase

A total of 100 subjects having culture-positive samples with either of the six target bacteria (*S. pneumoniae*, *K. pneumoniae*, *S. aureus*, *S. pyogenes*, *H. influenzae,* and *M. catarrhalis*) were considered for analysis in the study. It was a single-center study and all the samples were collected by the investigator and his team from routine Outpatient Department/in-house subjects with a clinical diagnosis of upper RTI. No deviations to the study protocol were reported during the study.

The mean age of the subjects enrolled in the study was 34.5 (30.3–38.6) years. The youngest subject was 3 months old, while the eldest subject enrolled in the study was 79 years. Among these 100 subjects, 58 were male, while 42 were female. The number of subjects when grouped on the basis of age was as follows in Table 2.

Types of Samples and Distribution of Target Organisms

Among the 100 culture-positive samples subjected to MIC evaluation during the study, 41 were swab samples, 32 sputum samples, and 27 pus samples.

Table 2: Age-distribution of the enrolled patients

Age (years)	No. of subjects
<12 years	21
Between 13 and 18 years	4
Between 19 and 65 years	70
Above 65 years	5

Among the 100 culture-positive samples subjected to MIC evaluation during the study, the distribution of the 4 target organisms isolated was 48 for *S. aureus*, 25 for *S. pneumoniae*, 21 for *S. pyogenes,* and 6 for *M. catarrhalis*.

Determining MIC_{50} and MIC_{90}

The MIC_{50} represents the MIC value at which ≥50% of the isolates in a test population are inhibited; it is equivalent to the median MIC value. Given n test strains and the values y1, y2……yn representing a graded series of MICs starting with the lowest value, the MIC_{50} was the value at position nx0.5, as long as n was an even number of test strains. If n was an odd number of test strains, the value at position (n+1) ×0.5 represented the MIC_{50} value.

The MIC_{90} represents the MIC value at which ≥90% of the strains within a test population are inhibited; the 90th percentile. The MIC_{90} was calculated accordingly, using nx0.9. If the resulting number is an integer, this number represented the MIC_{90}; if the resulting number was not an integer, the next integer following the respective value represented the MIC_{90}.

Both MIC_{50} and MIC_{90} values are presented as concentrations on the standard AST dilution series.

Efficacy Results

All patients with a positive culture and subjected to antibiotic sensitivity testing were considered for inclusion in the efficacy analysis. The MIC results obtained for the two antibiotics were evaluated as per the cut-offs mentioned in the CLSI guidelines. These were divided into susceptible or resistant as per the below table adopted from CLSI guidelines M100 (2018).

The primary efficacy endpoint was to compare MIC_{90} of the two antibiotics (cefpodoxime plus clavulanic acid and amoxicillin plus clavulanic acid) against the different bacteria isolated. The MIC_{90} values for the two study antibiotics are presented in Table 3.

There were two secondary endpoints, one of the secondary efficacy endpoint of the study was the MIC_{50} of the two antibiotics (cefpodoxime plus clavulanic acid and amoxicillin plus clavulanic acid) against the different bacteria isolated. The MIC_{50} values for the two study antibiotics are presented in Table 4.

The other secondary efficacy endpoint of the study was the geometric mean MIC of the two antibiotics (cefpodoxime plus clavulanic acid and amoxicillin plus clavulanic acid) against the different bacteria isolated. The geometric mean MIC values for the two study antibiotics are shown in Table 5.

The third secondary efficacy endpoint of the study was the proportion of samples sensitive for the two antibiotics as per CLSI criteria. The samples have been rated as sensitive and resistant based on the cut-offs given in CLSI guidelines and defied in the study protocol. The details are given in Tables 6 and 7.

Safety Results

All patients enrolled in the study and whose sample was collected were considered for inclusion in the safety analysis.

A total of 4 adverse events were reported in 4 study patients. The most common events recoded during sample

Table 3: MIC_{90} values for the two study antibiotics

Organism	Amoxicillin plus clavulanic acid		Cefpodoxime plus clavulanic acid	
	MIC_{90} (mcg/ml)	CLSI "Resistant" breakpoint(mcg/ml)	MIC_{90} (mcg/ml)	CLSI breakpoint (mcg/ml)
Staphylococcus aureus	4	8	2	8
Streptococcus pneumoniae	2	≤2	0.5	≤0.5
Streptococcus pyogenes	3	NAv	0.75	NAv
Moraxella catarrhalis	0.75	≤4	0.75	NAv

Values represent the MIC at which ≥90% of the strains within a test population are inhibited. NAv: Not available

Table 4: MIC_{50} values for the two study antibiotics

Organism	Amoxicillin plus clavulanic acid		Cefpodoxime plus clavulanic acid	
	MIC_{50} (mcg/ml)	CLSI 'Resistant' Breakpoint(mcg/ml)	MIC_{50} (mcg/ml)	CLSI Breakpoint(mcg/ml)
Staphylococcus aureus	1	8	0.5	8
Streptococcus pneumoniae	0.19	≤2	0.094	≤0.5
Streptococcus pyogenes	0.5	NAv	0.125	NAv
Moraxella catarrhalis	0.064	≤4	0.032	NAv

Values represent the MIC at which ≥50% of the strains within a test population are inhibited. NAv – Not available

Table 5: Mean MIC of the two antibiotics against the different bacteria isolated

	Geometric mean MIC (mcg/ml)		
Organism	**Amoxicillin plus clavulanic acid**	**Cefpodoxime plus clavulanic acid**	***P*-value**
Staphylococcus aureus	0.73 (0.51–1.05)	0.41 (0.27–0.62)	0.04
Streptococcus pneumoniae	0.21 (0.11–0.42)	0.13 (0.08–0.20)	0.20
Streptococcus pyogenes	0.32 (0.15–0.70	0.14 (0.08–0.24)	0.07
Moraxella catarrhalis	0.10 (0.03–0.34)	0.05 (0.01–0.23)	0.44

Data presented as GMT (95% CI). *P*-values derived from unpaired 2 sample *t*-test with equal variance on log-transformed data

Table 6: Proportion of samples sensitive for the two antibiotics as per CLSI criteria

		Sensitivity as per CLSI criteria		
Organism	***n***	**Amoxicillin plus clavulanic acid (%)**	**Cefpodoxime plus clavulanic acid (%)**	***P*-value**
Staphylococcus aureus	48	42 (87.5)	44 (91.7)	0.74
Streptococcus pneumoniae	25	23 (92.0)	23 (92.0)	1.0

Data presented as n (%) *P*-values derived from Fisher's exact test

Table 7: Proportion of samples resistant for the two antibiotics as per CLSI criteria

		Resistance pattern as per CLSI criteria		
Organism	***n***	**Amoxicillin plus clavulanic acid (%)**	**Cefpodoxime plus clavulanic acid (%)**	***P*-value**
Staphylococcus aureus	48	6 (12.5)	4 (8.3)	0.74
Streptococcus pneumoniae	25	1 (4.0)	0 (0.0)	1.0

Data presented as n (%) *P*-values derived from Fisher's exact test

collection include pain and discomfort during the collection of a throat swab. No other adverse event was reported during the sample collection. All four events were judged as mild and resolved without any treatment.

DISCUSSION

Globally, respiratory infections are one of the major health problems and as per ones estimation, respiratory infections lead to over 50 million deaths per year.[8] As per a study conducted in 2019 to understand the rate and pattern of prescription of antibiotics in an out-patient setting, it was found that the majority (around 30%) of the antibiotic prescriptions were dispensed for acute upper respiratory infections, including cough and acute nasopharyngitis.[13] Further, the highest antibiotic prescription rates were observed in the children in the age group 0–4 years.[13]

RTI triggers inflammation and production of mucus, leading to nasal congestion, a runny nose, scratchy throat, and cough, which may last as long as up to 14 days. In pediatric patient practice, cough is seen to have continued for weeks after the upper respiratory infection has resolved.[14] Coughing in children can be distressing and can have a major impact on child's sleep, school performance, and ability to play.[7] Fever as high as 101–102° F is a very common feature in young children with acute respiratory infections. Other typical symptoms in children include decreased appetite, headache and body aches, lethargy, and a general feeling of illness (malaise), followed by either wheezing or stridor. Acute respiratory infections could further develop into otitis media or pneumonia. Further, in children predisposed to asthmatic conditions, RTI could often lead to an acute asthma attack.[14] This may be the reason that although the general clinical guidelines mention that the antibiotics should not be prescribed for the common cold and nonspecific URTIs,[15] general practitioners tend to take a cautious approach and prefer to initiate empirical treatment with a broad spectrum antibiotic rather than waiting for the results of culture and sensitivity.

A considerable percentage (>65%) of prescriptions is that of broad-spectrum antimicrobials such as amoxicillin-clavulanic acid, cephalosporins, macrolides, clindamycin, and piperacillin-tazobactam.[16] Both general physicians and pediatricians mainly prescribe cephalosporins, followed by fluoroquinolones, penicillins, and macrolides.[10,15]

Amoxicillin

Amoxicillin is semisynthetic penicillin that inhibits enzymes in the biosynthetic pathway of bacterial peptidoglycan, which in turn leads to weakening of the cell wall, followed by cell lysis and death. Amoxicillin is susceptible to degradation by beta-lactamases produced by resistant bacteria and therefore the spectrum of activity of amoxicillin alone does not include organisms which produce these enzymes. Combining with clavulanic acid

helps amoxicillin to exert a therapeutic effect against the bacteria resistant to amoxicillin alone.

Clavulanic Acid

Clavulanate potassium is structurally related to penicillin and possesses the ability to inactivate a wide variety of beta-lactamase enzymes, thereby preventing inactivation of the combining antibiotics, such as amoxicillin and cefpodoxime. Clavulanic acid alone does not exert any clinically effective antibacterial effect.

Cefpodoxime

Cefpodoxime paroxetil is a third-generation, orally-administered, broad-spectrum, and antibiotic of the cephalosporins class. While first- and second-generation cephalosporins could only be administered by intravenous or intramuscular injection, the development of third-generation cephalosporins that are broad-spectrum and can be administered orally has significantly increased their value in the management of RTIs. The presence of clavulanate potassium in cefpodoxime-clavulanic acid combination further extends the antibiotic spectrum of cefpodoxime to include many bacteria normally resistant to cefpodoxime alone.

The pathogens causing RTIs are susceptible to beta-lactam antibiotics that include penicillins and cephalosporins. The presence of bacteria producing beta-lactamase is a major cause of antibiotic resistance, and these can interfere with the action of beta-lactam antibiotics even when they are not the primary pathogens. The addition of clavulanic acid increases the spectrum and the susceptibility of amoxicillin and cefpodoxime. This is also observed in the present study where 44 (92%) of 48 samples of *S. aureus* were found to be sensitive to cefpodoxime and clavulanic acid combination and 42 (87%) of 48 samples of *S. aureus* were found to be sensitive to amoxicillin and clavulanic acid combination, whereas sensitivity of *S. pneumoniae* was found to be 92% for both antibiotic combinations. This high level of the sensitivity of two common bacteria which formed 73% of the samples collected for this *in vitro* study indicates a possible reason for the popular use of these antibiotics for empirical treatment of URTIs while awaiting the results of culture and sensitivity.

The widespread and repeated prescription of antibiotics, especially those with a broad-spectrum, is the single most important cause of the rise of drug resistance.[17] In prescribing the pragmatic antibiotic, clinicians should consider the following: (1) The site of infection and the organisms most likely to be colonizing that site, (2) knowledge/patient's medical history of any bacterial infections in recent past, and (3) the local geographical bacterial resistance patterns that are observed for important pathogens at most hospitals.[18]

When a pathogenic microorganism is identified in clinical cultures, AST is performed. AST measures the ability of a specific organism to grow in the presence of a particular drug *in vitro* and is performed using guidelines established by the CLSI[19] an organization that develops laboratory process standards through extensive testing and clinical correlation. 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.[20] The goal of AST is to predict the clinical success or failure of the antibiotic being tested against a particular organism. Data are reported in the form of MIC, which is the lowest concentration of an antibiotic that inhibits visible growth of a microorganism, and are interpreted by the laboratory as "susceptible," "resistant," or "intermediate," according to CLSI criteria. A report of "susceptible" indicates that the isolated microbe is likely to be inhibited by the usually achievable concentration of a particular antimicrobial agent when the recommended dosage is used for the particular site of infection.

Various testing methods are grouped based on their chronological development:[21] (1) Gold-standard clinical methods, (2) mechanical methods, (3) optical methods, (4) microfluidics and microdroplets methods, and (5) models of *in vivo* infection.

Gold standard methods are standardized by various organizations such as the Clinical and CLSI and the International Organization for Standardization (ISO). The interpretation of test results is, among others, standardized by the European Committee on AST (EUCAST).[22] A number of laboratory methods can be used to evaluate the *in vitro* antimicrobial activity.[11] The two basic principles on which these tests are established on are the disk-diffusion and broth or agar dilution methods.

In this study, the *in-vitro* strip method using the Ezy MIC™ Strip gradient technology, based on the combination of both the dilution and diffusion principles for AST, is utilized. This method involves unique MIC determination paper strip(s) which are coated with the two antibiotic combinations under study, namely, (1) cefpodoxime and clavulanic acid and (2) amoxicillin and clavulanic acid in a concentration gradient manner, capable of showing MIC's in the range of predefined gradient, when tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strips provides *in vitro* MIC values, which provide only a possible indication of pathogens potential in *in vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into

consideration several other factors and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible.

The widespread and repeated prescription of antibiotics, especially those with a broad-spectrum, can lead to antibiotic resistance and therefore, it has caused a lot of concern amongst regulators and clinicians. In India, key antibiotics are included in Schedule H1. Drugs included in Schedule H1 can only be sold with the prescription of a registered medical practitioner. Therefore, it is very important to regularly conduct such studies to continuously assess the susceptibility of various antibiotics and the MIC values to monitor emerging resistance patterns.

The results of this prospective, active-controlled *in-vitro* study of samples obtained from patients suffering from RTIs show that both the antibiotics, that is, cefpodoxime plus clavulanic acid and amoxicillin plus clavulanic acid are effective against the common respiratory pathogens, *S. aureus*, *S. pneumoniae*, *S. pyogenes*, and *M. catarrhalis* that were isolated from the isolated from the 100 patients with upper RTI, enrolled in this study, when respective MICs are compared against the CLSI 'resistant' breakpoint concentrations.

In this study, the MIC_{90} and MIC_{50} values of cefpodoxime plus clavulanic acid are found to be lower as compared to that of amoxicillin plus clavulanic acid for all the four bacterial strains, that is, *S. aureus*, *S. pneumoniae*, *S. pyogenes,* and *M. catarrhalis*. Further, the mean MIC values of cefpodoxime plus clavulanic acid are significantly lower as compared to that of amoxicillin plus clavulanic acid for *S. aureus*.

MIC values when compared with breakpoints provided in CLSI provide a fair estimate of the potential efficacy of these two antibiotics in URTIs caused by susceptible bacteria. However, there is a need to further substantiate these findings from this *in vitro* with clinical data. Further, such *in vitro* studies should be frequently planned in various geographies to assess the trend of microbial resistance against various antibiotics and antibiotic combinations. The results of these *in vitro* studies should be made available to the clinicians who can use the information on MIC to optimize the selection of the antibacterial and its dose considering the PK-PD data.

CONCLUSIONS

AST is done to measure the ability of a specific organism to grow in the presence of a particular drug *in vitro* and so, performed with a goal to predict the clinical success or failure of the antibiotic being prescribed against a particular organism. The results are reported in terms of Minimum Inhibitory Concentration(s), which is the lowest concentration of an antibiotic that inhibits visible growth of a microorganism, and are usually interpreted by the laboratory as "susceptible," "resistant," or "intermediate." In this study, the MIC_{90} and MIC_{50} values obtained for cefpodoxime plus clavulanic acid were found to be lower as compared to that of amoxicillin plus clavulanic acid for all the four bacterial, that is, *S. aureus*, *S. pneumoniae*, *S. pyogenes,* and *M. catarrhalis*. Further, the mean MIC values of cefpodoxime plus clavulanic acid are significantly lower as compared to that of amoxicillin plus clavulanic acid for *S. aureus*.

The susceptibility/sensitivity of a microbe toward a particular antimicrobial agent is better explained when MIC values obtained are assessed in conjunction with breakpoints provided in CLSI. This provides a fair estimate of the potential efficacy of the two antibiotics in URTIs caused by susceptible bacteria. Although there is a need to further corroborate these *in vitro* findings with real-world clinical data, the results from this study do indicate superiority or enhanced/better susceptibility of these bacterial strains to cefpodoxime plus clavulanic acid in comparison to amoxicillin plus clavulanic acid.

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