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Original Article

Microbiology Section

In Vitro Fractional Inhibitory Concentration (FIC) Study of Cefixime and Azithromycin Fixed Dose Combination (FDC) Against Respiratory Clinical Isolates

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ABSTRACT

Introduction: Acute respiratory infections (ARI) contribute to more than 75% of health care seeking in primary health care facilities in India. Respiratory tract infections (RTIs) are managed frequently by β-lactam, macrolide and fluroquinolone class of antibiotics. However, these recommended classes of antibiotic have shown resistance in community settings. Antibiotic combinations may provide broader spectrum not only in terms of coverage but also to overcome multiple resistance mechanisms overcoming individual class limitations.

Aim: The study aimed to determine In vitro interactions interpreted according to calculated fractional inhibitory concentration (FIC) index between cefixime and azithromycin against common respiratory clinical isolates.

Materials and Methods: Forty four bacterial respiratory clinical isolates from microbiology department of tertiary care hospital from Mumbai were used to determine the minimum inhibitory concentration (MIC) values of cefixime and azithromycin. Synergy testing of cefixime combination with azithromycin was performed by checkerboard method. Interaction was determined according to calculated FIC index.

Results: MIC values were ranging from 2–128 µg/ml and 0.24–128 µg/ml for cefixime and azithromycin respectively against *K.pneumoniae*, *M.catarrhalis*, *S.pneumoniae* and *H.influenzae* isolates. All the tested isolates were resistant to cefixime. Azithromycin resistance was noted in all the isolates except six *M. catarrhalis* isolates. FIC index showed synergy and additive effect in 66% (29/44) and 34% (15/44) all bacterial clinical isolates. Maximum synergy between cefixime and azithromycin was observed against *K. pneumoniae* in 91% isolates.

Conclusion: This is one of the first attempts to check the rationality of fixed dose antibiotic combination of cefixime and azithromycin in India market. Though results of this study cannot be generalized considering the limitations of low sample size and in vitro model, our data provides stepping stone for further validation of cefixime and azithromycin fixed dose combinations (FDCs) in clinical setting by conducting randomized controlled trials. We think that judicious and rational use of FDCs may help to reduce the risk of selection of further drug resistance along with better clinical outcome.

Keywords: Acute respiratory infections, Fixed dose combination, Fractional inhibitory concentration index, Minimum inhibitory concentration, Synergy

INTRODUCTION

Globally, community acquired RTIs account for a large proportion of antibiotic prescriptions and visit to family practitioners [1]. ARIs contribute to more than 75% of health care seeking in primary health care facilities [2]. Majority of ARIs are of viral aetiology, but information from India on various respiratory tract bacterial pathogens and resistance pattern in hospital settings is inadequate [3]. Because of the commonness of the problem, antimicrobial therapy for ARIs is a major predictor for the spread of resistant strains of microbes in the community [2]. In India empiric therapy is often practiced, the tests of antibiotic susceptibility may not be routinely performed in the real life setting [4]. Often, the clinicians resort to clinical pointers of poor response to antibiotics such as lack of effervescence of fever, lack of symptom relief as a guide to estimate the presence of antibiotic resistance.

Lower RTIs particularly community acquired pneumonia (CAP) are common and can be potentially serious. These are managed frequently by β-lactam, macrolide and fluroquinolone class of antibiotics. But resistance towards these class of antibiotics in community settings of India is on rise [5].

Clinicians are increasingly opting for two or more antibiotics as empiric choice to ensure complete clinical cure. Antibiotic combinations are sought to provide synergistic killing, but its impact on the evolution of resistance is unclear. Synergistic interactions are usually thought of as advantageous since, for a given amount of drug, they more effectively inhibit the growth of drug-sensitive pathogens [6].

Antibiotic combinations may provide broader spectrum not only in terms of coverage but also to overcome multiple resistance mechanisms overcoming individual class limitations [Table/Fig-1]. Better clinical outcome of respiratory infections (particularly pneumonia) with antibiotics combination therapy than monotherapy has been documented by several studies [7].

Parameters which have been used to show interactions during combination therapy are the FIC indices, derived from checkerboard titrations [8]. In this study, in vitro synergy between cefixime and azithromycin in Cefixime-azithromycin FDC was investigated by evaluating FIC indices for *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Moraxella*, *catarrhalis* and *Streptococcus pneumoniae* respiratory clinical isolates.

MATERIALS AND METHODS

Forty four bacterial respiratory clinical isolates were collected from microbiology department of a tertiary care hospital in Mumbai, These included 11 *Haemophilus influenzae*, 11 *Klebsiella pneumoniae*, 11 *Moraxella catarrhalis* and 11 *Streptococcus pneumoniae*. Study period was from June 2014 to August 2014.

The MIC values of cefixime and azithromycin were determined for all 44 bacterial isolates. In order to determine the MICs, ultrapure water was used to dissolve the antibiotics so as to give stock concentrations of 5120 µg/ml. Subsequently, twofold serial dilutions of cefixime and azithromycin were made to give concentrations ranging from 1 to 512 µg/ml. An inoculum of 5×10^5 CFU/ml was

obtained by adding 500 µl of 10^6 CFU/ml bacterial suspension to the sterile capped test tubes. Another 500 µl of cefixime or cefixime and azithromycin combination were pipetted into the tubes. Control was prepared by adding the test bacteria to tube containing inert solvent to dissolve the antibiotics. After overnight incubation at 37°C, the tube containing lowest concentration of the antibiotic showing no visible growth was recorded for calculation of MIC. MIC values for isolates were interpreted according to CLSI criteria. Synergy testing of cefixime combination with azithromycin was performed by checkerboard method. Interaction was determined according to calculated FIC index.

RESULTS

MIC values were ranging from 2–128 µg/ml and 0.24–128 µg/ml for cefixime and azithromycin [Table/Fig-2-5] respectively against *K. pneumoniae*, *M. catarrhalis*, *S. pneumoniae* and *H. influenzae isolates*. All tested isolates were resistant to cefixime. Azithromycin resistance was noted in all isolates except six *M. catarrhalis* isolates [Table/Fig-2-5]. Comparison of mean MIC of each bacterial type with cefixime and azithromycin alone and cefixime in combination with azithromycin [Table/Fig-6] showed 4.4, 9.1, 12.8 and 5.8 fold reduction in cefixime MIC in combination against *K. pneumoniae*, *M. catarrhalis*, *S. pneumoniae* and *H. influenzae* respectively.

Similarly the mean MIC of azithromycin in combination with cefixime as compared to alone dropped by 5, 2.6, 3.9 and 4.5 fold for *K. pneumoniae*, *M. catarrhalis*, *S. pneumoniae* and *H. influenzae* respectively.

FIC index showed synergy in 66% (29/44) isolates, while additive effect in 34% (15/44) isolates. Maximum synergy of Cefixime-azithromycin FDC was observed against *K. pneumoniae* in 91% isolates.

Class	Limitation
β-lactam	No activity against atypical pathogen and development of *S. pneumoniae* resistant isolates > 50 %
Macrolides	30.9 % resistant *S. pneumoniae* isolates reported
Fluroquinolones	Increase potential for emergence of resistant strain of gram negative microorganism

[Table/Fig-1]: Individual antibiotic class limitation in RTI management

K. pneumoniae

Strains	MIC Azithromycin		MIC Cefixime		FIC index	Outcome
	Alone (µg/ml)	With Cefixime (µg/ml)	Alone (µg/ml)	With Azithromycin (µg/ml)		
A1	128	16	64	16	0.375	Synergy
A2	128	16	64	8	0.25	Synergy
A3	128	32	64	16	0.5	Synergy
A4	128	64	64	4	0.563	Additive
A5	128	16	128	32	0.375	Synergy
A6	128	32	128	32	0.5	Synergy
A7	64	16	64	16	0.5	Synergy
A8	64	16	128	32	0.5	Synergy
A9	128	16	32	8	0.5	Synergy
A10	256	64	128	32	0.5	Synergy
A11	128	8	64	16	0.313	Synergy

[Table/Fig-2]: MIC values of azithromycin alone and in combination with cefixime for *K. pneumoniae*

M. catarrhalis

Strains	MIC Azithromycin		MIC Cefixime		FIC index	Outcome
	Alone (µg/ml)	With Cefixime (µg/ml)	Alone (µg/ml)	With Azithromycin (µg/ml)		
A1	0.48	0.24	8	1	0.375	Synergy
A2	0.24	0.24	8	1	0.375	Synergy
A3	0.48	0.06	8	2	0.375	Synergy
A4	0.24	0.12	16	0.5	0.531	Additive
A5	0.24	0.06	8	2	0.5	Synergy
A6	0.24	0.12	16	1	0.563	Additive
A7	0.48	0.12	4	1	0.5	Synergy
A8	0.96	0.48	8	0.5	0.563	Additive
A9	0.96	0.12	4	0.5	0.25	Synergy
A10	0.48	0.24	8	0.5	0.563	Additive
A11	0.24	0.12	8	0.5	0.563	Additive

[Table/Fig-3]: MIC values of azithromycin alone and in combination with cefixime for *M. Catarrhalis*

S. pneumoniae

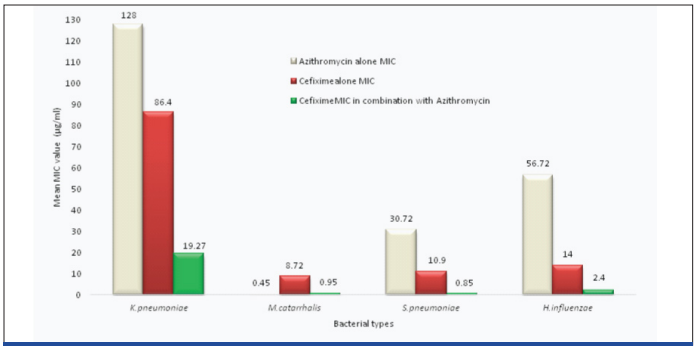
Strains	MIC Azithromycin		MIC Cefixime		FIC index	Outcome
	Alone (µg/ml)	With Cefixime (µg/ml)	Alone (µg/ml)	With Azithromycin (µg/ml)		
A1	16	4	4	0.5	0.375	Synergy
A2	16	4	8	2	0.5	Synergy
A3	16	8	16	2	0.563	Additive
A4	64	8	8	2	0.375	Synergy
A5	16	8	8	0.5	0.563	Additive
A6	64	32	8	0.5	0.563	Additive
A7	64	8	8	0.3	0.375	Synergy
A8	1	0.25	32	0.3	0.375	Synergy
A9	64	8	8	0.3	0.375	Synergy
A10	1	0.5	16	0.5	0.563	Additive
A11	16	4	4	0.5	0.5	Synergy

[Table/Fig-4]: MIC values of azithromycin alone and in combination with cefixime for *S. pneumoniae*

H. influenzae

Strains	MIC Azithromycin		MIC Cefixime		FIC index	Outcome
	Alone (µg/ml)	With Cefixime (µg/ml)	Alone (µg/ml)	With Azithromycin (µg/ml)		
A1	64	4	2	1	0.563	Additive
A2	64	4	16	8	0.563	Additive
A3	16	8	8	0.5	0.563	Additive
A4	64	8	8	2	0.375	Synergy
A5	64	32	16	1	0.563	Additive
A6	64	4	16	0.5	0.188	Synergy
A7	16	4	16	1	0.313	Synergy
A8	16	4	32	8	0.5	Synergy
A9	64	32	16	1	0.563	Additive
A10	128	32	16	4	0.5	Synergy
A11	64	8	8	0.25	0.156	Synergy

[Table/Fig-5]: MIC values of azithromycin alone and in combination with cefixime for *H. influenzae*



[Table/Fig-6]: Comparison of cefixime mean MIC as alone and in combination with azithromycin against study bacterial types

DISCUSSION

Third generation cephalosporins and macrolides such as Azithromycin have been found to be effective drugs in the management of RTIs.

But rampant use of these antibiotics in the real life setting has led to the emergence of resistant strains of respiratory tract pathogens.

Cefixime is an orally active third-generation cephalosporin. It has broad spectrum of activity against various pathogens, including gram-negative organisms which are beta-lactamase producing strains [9]. Azithromycin is a macrolide with an expanded spectrum of activity and improved tissue pharmacokinetic. The drug is noted for its activity against some gram-negative organisms associated with RTIs, particularly. *H. influenzae* [10]. Azithromycin has similar properties to other macrolides against *S. pneumonia*e and (*M. catarrhalis*), and is active against atypical pathogens such as (*L. pneumophilae*), *C. pneumoniae* and *M. pneumoniae* [11].

The current study has demonstrated synergism of cefixime and azithromycin combination. There are few studies in literature documenting the in vitro effects of cefixime in combination with azithromycin. Furuya et al., demonstrated that cefixime can have synergistic effects in combination with azithromycin for Neisseria gonorrhoea [12].

The FIC of each agent was calculated as a ratio of the MIC when used in combination and the MIC when used alone. FIC index is the sum of the FIC of the two agents used in the combination [13]. FIC indexes were interpreted as previously defined synergy at a FIC index ≤0.5; additive at a FIC index >0.5 to 1; indifference at a FIC index >1–<2; and antagonism at a FIC index ≥2 [14]. Fall of MIC for both cefixime and azithromycin in combination along with combinational FIC index of less than one in all clinical isolates of has proved synergistic and additive effects of Cefixime-azithromycin.

Macrolide alone in *S.pneumoniae* has shown resistance upto 14% in Chawla et al., study. Prevalence of BLNAR positive *H.influenzae* is rising in South East Asian countries, showing upto 73% resistance towards conventional BL/BLIs. Incidence of atypical pathogens causing CAP in India is around 24% in Kashyap et al., study [15-18]. Macrolides once the cornerstone in the treatment of atypical pathogens, resistance is on the rise globally. Clinical studies by Waterer et al., Lodies et al., Rodrigo et al., Weiss et al., and Dudas et al., on the combination use of β-lactam and macrolide in CAP and pneumococcal bacteremia showed better results in terms of clinical outcome, length of stay and mortality [19-23].

Antibiotic combination therapy produces synergistic effects and reduces mortality at high risk for treatment failure, in comparison with monotherapy [24]. Various speciality societies like American Thoracic Society (ATS), British Thoracic Society (BTS), Infectious Disease Society of America (IDSA) and Canadian Infectious Disease Society (CIDS) recommended use of empiric combination therapy in management of RTI like CAP [5].

CONCLUSION

This is one of the first attempts to check the rationality of fixed dose antibiotic combination of cefixime and azithromycin in Indian market. Though results of this study cannot be generalized considering the limitations of low sample size and in vitro model, our data provides stepping stone for further validation of cefixime and azithromycin FDCs in clinical setting by conducting randomized controlled trials. We think that judicious and rational use of fixed dose antibiotic combinations may help to reduce the selection of further drug resistance along with proved clinical outcome.

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