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Comparative Study of Antimicrobial Agents against Pathogens Associated with Diarrheal Disorders

Ibrahim Khan and M. Musaddiq

Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola, India

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Comparative study of antimicrobial activity of eight antibiotics which are used conventionally for the treatment of diarrhea, i.e. Ampicillin, Ceftriaxone, Doxycycline, Levofloxacin, Norfloxacin, Nalidixic acid, Trimethoprim sulfamethoxazoleand Tetracycline was studied with the use of pathogensisolated and identified from the stool samples collected from various diarrheal patients admitted at various Government and Private hospitals of Akola district. The antimicrobial activity was determined by studying Minimum Inhibitory Concentration (MIC)using Clinical and Laboratory Standard Institute (CLSI) guidelines.The microdilution procedure using 96 wells microdilutionplates was employed. Results revealed Increase in microbial resistance by enteric pathogen against antimicrobials Ampicillin, Nalidixic acid, Tetracycline and Doxycycline with high MIC_{50} and MIC_{90} values, However Levofloxacin, Ceftriaxone and Norfloxacin revealed promising MIC activity.*E.coli* shows high resistance as compared to *Salmonella* and *Shigella species*.

Key words: Diarrhea, Ampicillin, Ceftriaxone, Norfloxacin, Nalidixic acid, Minimum Inhibitory Concentration (MIC).

Acute diarrhea is a common cause of morbidity and mortality throughout the world. Generally, the most severe, as well as the most frequently occurring, forms of this disease in developing countries are of bacterial etiology[3]. Antimicrobial therapy is indicated for moderate to severe disease to reduce the duration of illness[1,2,4]. Antibiotic shave been found to shorten the duration of some types of bacterial diarrhea; however, because of the emergence of antimicrobial agent resistance among enteric pathogens, selection of appropriate therapy is often difficult[5].

Infectious diarrhea is the second most common cause of morbidity and mortality worldwide. In the US, it has been estimated that more than 200 million episodes of diarrheal illness occur each year, resulting in 73million physician consultations, 1.8 million hospitalizations, and 3,100 deaths. Economic costs associated with diarrheal illnesses in the US, including medical care and lost productivity, have been estimated at upto US$ 23 billion per year[2]. The causes of infectious diarrhea are many, and in spite of the magnitude of the problem, there have, until recently, been few new advances in pharmacotherapy for infectious diarrhea over the past 30–40 years.

Infectious diarrhea may affect the small intestine and/or the colon. Infections of the small intestine lead to watery diarrhea with high volume of liquid stools. These can be caused by enterotoxigenic *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Campylobacter jejuni*, *Cryptosporidium* spp and enteric viruses[6].

Resistance to commonly used antimicrobial agents among enteric bacterial pathogens has been reported worldwide[7-12], although data for resistance among pathogens causing diarrhea are limited. The in vitro activities

* To whom all correspondence should be addressed.
E-mail: Kim_ikhan@hotmail.com

of currently available and new antimicrobial agents were evaluated against pathogens causing diarrhea.

MATERIALS AND METHODS

Stool samples

Between June 2009 and May 2010, stool samples were collected from patients with diarrhea admitted in Main Hospital, Akola. A detailed history of the patients is obtained, including information on the age, sex and clinical presentation. Stool samples were collected in sterile bottles containing Cary-Blair transport medium having phosphate buffer saline for transportation. In the same period, stool samples were collected from children having complaints of diarrhea attending Lady Hording, Akola and various private hospitals at Akola. As per data collected most of the patient's area is densely populated and has poor sanitary and hygiene conditions.

Isolation

Immediately after collection, Samples are cultured on MacConkey's Agar, Bismuth sulphite agar and salmonella shigella agar. After Incubation at 37°C for 24 hours typical colonies are subjected to apiweb rapid identification system, at the same time the colonies are transferred on Eosin Methylene Blue agar and Triple Sugar Iron agar.

Identification

A single isolated colony is transferred into 5 ml sterile 0.85 % sodium chloride (NaCl) and emulsify till homogenous bacterial suspension is obtained. With the help of micropipette bacterial suspension were distributed into the wells of API 20 E test kit and incubated at 37°C for 18-24 hours. After incubation the observation are compared with the reading table and the organism identified based on the database of software provided.

MIC determination

The method describes the intentional inoculation of specified microorganism to establish survival of inoculated test microorganisms against antibiotic under study.

Reagents

Sterile normal saline (0.9 % w/v), Antibiotic assay medium 3 (AM3), Muller Hilton Broth (MHB) Difco Laboratories, Soyabean casein digest agar (SCDA) HiMedia.

Procedure

Test organism

E.coli, Salmonella species and *Shigella* species isolated from diarrheal patients. *E.coli* ATCC 25922 as a control strain.

Preparation of inoculum

24 hr old culture of *E.coli* ATCC 25922 and *E. coli, Salmonellae* species and *Shigella* species isolates from diarrheal patients are grown on SCDA slants. Stock culture of these organisms is prepared by scraping the growth from the slants in sterile normal saline. .

Using sterile normal saline (0.9 % w/v), the above suspension is diluted suitably to bring the count to about 1 x 10^6 to 5 x 10^6cfu per ml by comparison with a 0.5 McFarland standard. 1:100 dilution of the above suspension was carried out using AM3 medium to obtain density of 1 x 10^4 to 5 x 10^4cfu per ml this suspension is used for the test procedure.

Preparation of antibiotic dilution range

Antibiotic ranges was prepared one step higher than the final range required i.e. if a final dilution of 0.5 mg/ml was required then 1 mg/ml was prepared to compensate for the addition of an equal volume of inoculum. Usual dilution range used was 4 mcg/ml to 2048 mcg/ml and 0.0195 mcg/ml to 10 mcg/ml, diluent used was suitable solvent specific to antibiotic and AM3 medium. The final concentration used was between 1 x 10^5 and 1 x 10^6 microorganisms per ml.

All MIC determinations were performed in volumes of 0.1 ml contained in 96 wells microdilution plates. First 0.1ml inoculum was dispensed with the help of multipipette than 0.1 of ranges of antibiotic concentrations in Freshly made or thawed plates were inoculated with a multiple-inoculum replicator so that the final inoculum was 1 x 10^5 to 5 x 10^5 colony-forming units per ml. Inoculated plates were incubated in incubator maintained at 37°C for 18 to 24hours and read with the aid of a magnifying mirror. The MIC was the lowest concentration of antimicrobial agent which inhibited visible growth.

RESULTS AND DISCUSSION

The MIC of antimicrobial tested for. *E.coli, Salmonellae* species, *Shigella* species isolated

from diarrheal patients are shown in Table 1. Minimum inhibitory concentration with the no. of isolates for Ampicillin (AMP), Ceftriaxone (CFO), Doxycycline (DOX), Levofloxacin (LEV), Nalidixic acid(NAL), Norfloxacin (NOR), Tetracycline (TET) and Trimethoprim and Sulfamethoxazole (TRI/SXT) is shown in Fig. 1-8. Ampicillin shows high MIC in the range of 64 – 1024 against E.coliwith MIC_{50} and MIC_{90}256 and 1024 mcg/ml respectively. Against SalmonellaMIC_{50} and MIC_{90} is 16 and 64 mcg/ml which is slightly low as compared to Jeannette Ouyang-Latimer et al, (2010). While NAL MIC_{50} and MIC_{90} against *E.coli* is 16 and 128 mcg/ml,whereas for *Salmonella Sp* MIC_{50} and $MIC_{90}$is 8 and 16 mcg/ml whichshowed a pattern of activity similar to that of the Harumi Gomi *et al*, 2001[11] LEV and DOX shows 10 times high MIC rate ranges from 0.156 – 10 mcg/ml and 8 – 512 mcg/ml respectively for enteropathogen *E.coli*. Traditional antibiotics, AMP, TET, DOX, and T/S, all showed poor in vitro activity in several studies worldwide. Resistance to SXT among enteric bacterial pathogens has increased dramatically over the last 14 years[5]. Therefore, it is essential of the selective administration to decrease the resistance development of these agent.

The bactericidal activity of ceftriaxone results from inhibition of cell wall synthesis. Ceftriaxone has a highdegree of stability in the presence of beta-lactamases.Ceftriaxone has been shown to be active against most entero-pathogenstrains of microorganisms, both *in vitro* and in clinical infections. In our study Ceftriaxone shows high MIC_{50} and MIC_{90} values for *E.coli* 0.0391 and 0.3125 mcg/ml and lower values of MIC_{50} and MIC_{90} for *Salmonella* sp. i.e.0.0391 and 0.0781 mcg/ml. The MIC range of *Shigella* species also give quite promising results as compared. Since only three strains are tested more study is required to have proper idea of antimicrobial trend.

The mechanism of action oflevofloxacin and other fluoroquinolone antimicrobials involves inhibition of bacterial topoisomerase IV and DNA gyrase (both of which are type II topoisomerases), enzymes required for DNA replication, transcription, repair and recombination. Levofloxacin has in vitro activity against a wide range of gram-negative and gram-positive microorganisms. Levofloxacin is often bactericidal at concentrations equal to or slightly greater than inhibitory concentrations. MIC50 and MIC_{90} for *E.coli* is 0.625 and 10 mcg/ml and against

Table 1. MICs of 8 antimicrobials for 100 diarrheal pathogens

Antimicrobials	MIC mcg/ml					
	E.coli		*Salmonellae*		*Shigella*	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Ampicillin	256	1024	16	64	128	128
	Range 64 – 1024		Range 4 – 64		Range 128 – 128	
Ceftriaxone	0.0391	0.3125	0.0391	0.0781	0.0195	0.0195
	Range0.0195 – 0.3125		Range 0.0391 – 0.156		Range 0.0195 – 0.0195	
Doxycycline	16	128	64	512	3264	
	Range 8 – 512		Range 16 – 512		Range 32 – 64	
Levofloxacin	0.625	10	0.0391	0.0781	0.3125	0.625
	Range 0.156 – 10		Range 0.0195 – 0.0781		Range 0.3125 – 0.625	
Nalidixic acid	16	128	8	16	64	128
	Range 8 – 256		Range 4 – 32		Range 64 – 128	
Norfloxacin	0.0391	0.3125	0.0391	0.0781	0.0391	0.0781
	Range 0.0195 – 0.3125		Range 0.0195 - 0.0781		Range 0.0391 – 0.0781	
Tetracycline	128	512	128	128	128	128
	Range 64 – 1024		Range 64 – 512		Range 128 – 128	
Trimethoprim and Sulphamethoxazole	8	256	64	512	128	128
	Range 4 – 256		Range 8 – 512		Range 64 – 128	

50 and 90: MIC required to inhibit the growth of 50% and 90% of the strains tested respectively

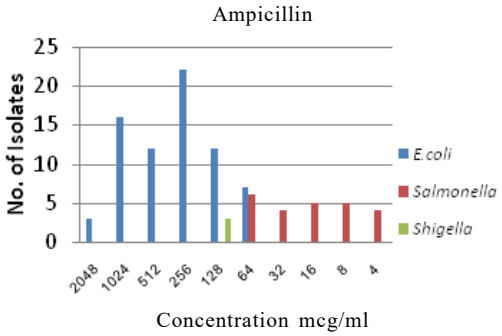
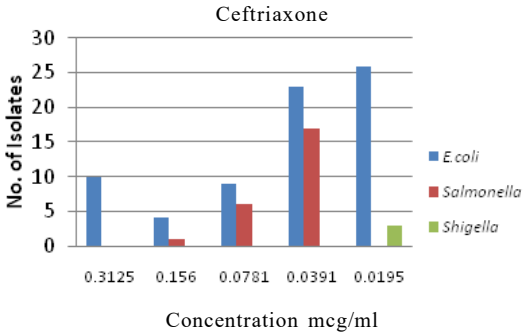


Fig. 1.



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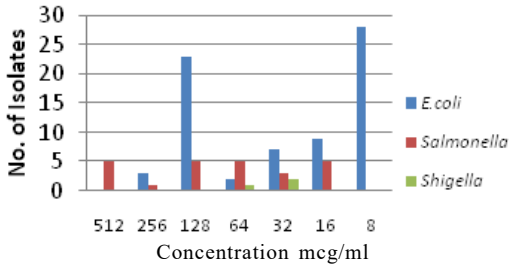

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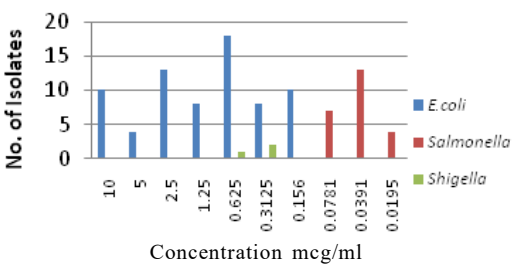

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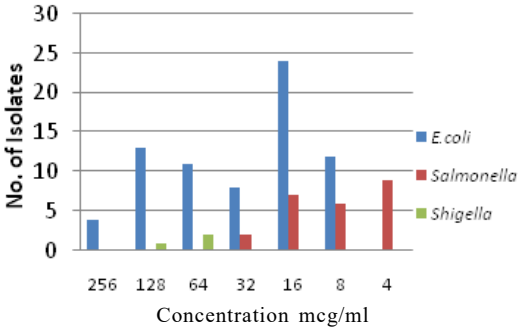

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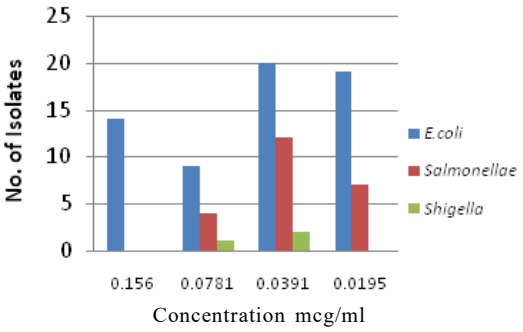

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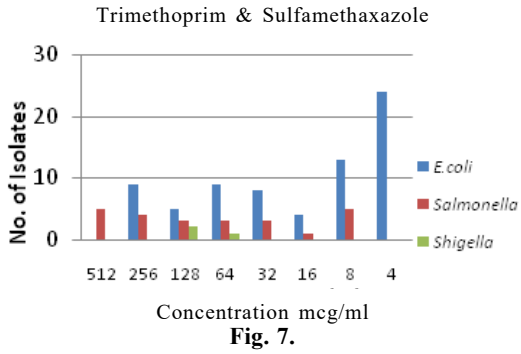

Fig. 7.

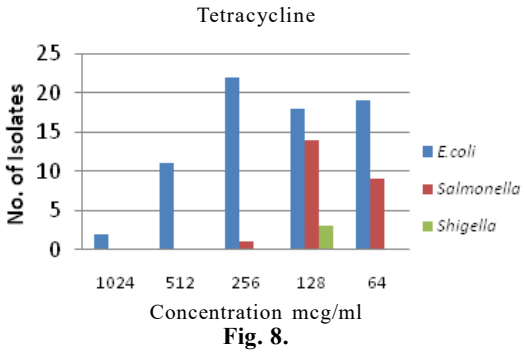

Fig. 8.

Fig. 1-8. Distribution of isolates by MIC value (in mcg/ml) for each antimicrobial

Salmonella Sp. MIC_{50} is 0.0391 and MIC_{90} is 0.0781 mcg/ml. Fluoroquinolones, including levofloxacin, differ in chemical structure and mode of action from aminoglycosides, macrolides and β-lactam antibiotics, including penicillins.

CONCLUSION

Continuous monitoring of the resistance patterns developed by antimicrobials is essential, and antimicrobial susceptibility testing should be carried out on clinical isolates, and empirical antimicrobial therapy need to be designed accordingly. Despite the limitation of our study, fluoroquinolones (NORand LVX) should still be considered the drugs of choice for treatment of diarrhea in adults in most regions of the world. At the same time oral rehydration therapy is also found to be more effective treatment for acute diarrheal dehydration. Followed by the use of various antibiotics, which help in decreasing the severity of diarrheal disease. A global and national multi-sectoral response is urgently needed to combat the growing threat of antimicrobial resistance.

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