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COMPARATIVE STUDIES OF MINIMUM INHIBITORY CONCENTRATION OF CONVENTIONAL AND MOXIFLOXACIN AGAINST COMMON PATHOGENIC STRAINS

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ABSTRACT
In present study minimum inhibitory concentration (MIC) of moxifloxacin and few conventional antibacterial agents were investigated against some common pathogenic bacteria. MICs of antimicrobial agents were determined by Hicomb™ MIC test strips. The values of MIC of moxifloxacin against *Staphylococcus aureus* (ATCC 29737), *Staphylococcus aureus* (ATCC 6538), *Streptococcus aureus* (ATCC 6538 P) and *E. coli* (ATCC 10536) were 0.005µ g.ml^{-1}. The values of MIC of moxifloxacin against *Staphylococcus epidermis* (ATCC 12228), *Streptococcus fecalis* (ATCC 10541), *Pseudomonas aeruginosa* (ATCC 25619) and *E. coli* (NCTC 9002) obtained in this study was 0.100 µ g.ml^{-1}. The value of MIC of moxifloxacin against *E. coli* M 200 (ATCC 14169) was 0.06µ g.ml^{-1}. Relatively low MICs against target microorganisms, makes it a potentially useful drug for the treatment of infections caused by various organisms in goats.

KEY WORDS: MIC**,** Moxifloxacin, goat, ATCC

INTRODUCTION

Fluoroquinolones developed over the past few years have greater potency, a broader spectrum of activity, greater *in vivo* efficacy against resistant organisms and possess a better safety profile than other antimicrobial agents. Moxifloxacin is a new enantiomerically pure, fourth generation fluoroquinolone that was first synthesized by Petersen *et al.* (1996) and that has potent antimicrobial activity against both Gram-negative and Gram-positive bacteria, anaerobes and atypical organisms like *Mycoplasma* spp and *Chlamydia* spp. Moxifloxacin is a recently developed fluoroquinolone antibacterial with a methoxy group in the C-8 position and a bulky C-7 side chain. It has excellent efficacy against pneumococcal strains resistant to β-lactam and macrolide antibiotics and good activity against atypical respiratory tract pathogens (Dalhoff *et al.*, 1996; Rouse *et al.*, 1996).

MATERIALS & METHODS

Minimum inhibitory concentration of following listed bacteria was determined by Hicomb™ MIC Test strips as method described by Reeves, *et al.* (1978). Antibiotics purchased from Himedia® Laboratories Pvt. Limited, Mumbai, India. The different antibiotic strips used were Amoxicillin and Clavulinic acid (Amoxyclav), Ciprifloxacin, Moxifloxacin, Norfloxacin, Streptomycin, Tetracyclin, Sulphafurazole, Gentamicin, Ampicillin new and Cefepime new.

Hicomb™ MIC Test strips:

Hicomb consists of a strip made of an inert material, with extensions that carry the dics of 4 mm, resembling the tooth of a comb towards the stem of strip. A defined concentration of antimicrobial drug is loaded on each of the disc so as to form a gradient when placed on agar plate. Hicomb based on the principle of dilution and diffusion consists of a gradient that covers a continuous range of 16 two fold dilutions on 2 different strips as per conventional MIC method. When these strips are applied to the agar surface, the antibiotic instantaneously diffuses into the surrounding medium in high to low amounts from one end of the strip to the other. The gradient remains stable after diffusion, and the zone of inhibition created takes the form of ellipse.

Preparation of agar plate and broth

Glassware's used during study were sterilized by dry heat in hot air oven at 160°C for one hour. The culture media mainly used was Muller Hinton agar. The medium was prepared by mixing 38g of powder with one liter of distilled water in a large glass conical flask and was shaken vigorously. After that the flask was plugged with non-absorbent cotton and was autoclaved at 15lbs pressure/ sq inch for 15 minutes. All the sterile glass petri dishes were placed inside the Laminar air flow. The molten agar medium was allowed to cool up to 45-50^{0}C. Approximately 50-60 ml of cooled media was then poured in each glass petri dish to form a uniform layer of 4 mm thickness and allowed to solidify. After solidification the plates were refrigerated till used. Muller Hinton broth was used in this study. 22g of dehydrated broth powder was dissolved in 1000 ml of distilled water. The flask was kept in boiling water bath until the medium was clear and in liquid state. 5-6 ml of broth was transferred to sterile test tube. The test tubes were plugged with non-absorbent cotton and were autoclaved at 15 lbs pressure/ sq inch for 15 minutes. After cooling, all the test tubes were refrigerated till used.

Preparation of inoculums

The test tubes of broth media were kept for overnight incubation at 37°C for sterility testing. After that, all the test tubes containing 5ml broth were placed inside the Laminar air flow. The slant containing the broth was touched with the inoculation loop containing pathogenic strains to transfer the pathogens. The inoculated test tubes were kept at 37°C for 2-8 h.

Preparation of standard solution

Standard solution was prepared by mixing 0.5 ml of 1.175 % barium chloride and 99.5 ml of 0.36 N sulphuric acid. Turbidity of broth of different bacterial cultures in broth was matched with this McFerlan constant. Optical density of different broth cultures were also measured by UV VIS Spectrophotometer (Elico- SL 159, Ahmedabad) at 640 nm. The optical densities of different broth were 0.117-0.213 used for study.

High Comb MIC Test

Agar plate was prepared with Muller Hinton agar. The medium in the plate was having depth of about 4 mm. Sterile non-toxic cotton swab was dipped in standardized inoculums and this swab was streaked on agar plate three times turning the plate at 60° angle between each streaking. High comb MIC strip was applied to the agar surface with the MIC scale facing upwards. The plate was incubated at 35-37 °C and examined after 18 to 24 hours. The zone of inhibition appeared in the form of ellipse that intersects the concentration marking which is expressed in terms of MIC of a particular antibacterial agent.

RESULTS & DISCUSSION

The values of MIC ($\mu g.ml^{-1}$) of different antibiotics against common pathogenic agents were presented in Table 1. The values of MIC of moxifloxacin against Staphylococcus *aureus* ATCC 29737, *Staphylococcus aureus* ATCC 6538, *Streptococcus aureus* ATCC 6538 P and *E.coli* ATCC 10536 were 0.005 $\mu g.ml^{-1}$. The value of MIC of moxifloxacin against *Staphylococcus epidermis* ATCC 12228, *Streptococcus fecalis* ATCC 10541, *Pseudomonas aeruginosa* ATCC 25619 and *E.coli* NCTC 9002 obtained in this study was 0.100 $\mu g.ml^{-1}$. The value of MIC of moxifloxacin against *E.coli* M 200 ATCC 14169 was 0.06 $\mu g.ml^{-1}$. Range of MIC value of moxifloxacin obtained in present study was 0.001- 0.06 $\mu g.ml^{-1}$.

TABLE 1: List of pathogenic bacterial strains purchased from FDA Baroda

Sr. No.	Pathogenic strains	ATCC No. / NCTC No.
1.	*Staphylococcus aureus*	ATCC 29737
2.	*Staphylococcus epidermis*	ATCC 12228
3.	*Staphylococcus aureus*	ATCC 6538
4.	*Streptococcus fecalis*	ATCC 10541
5.	*Streptococcus aureus*	ATCC 6538P
6.	*Pseudomonas aeruginosa*	ATCC 25619
7.	*E.coli*	ATCC 10536
8.	*E.coli*	NCTC 9002
9.	*E.coli* M 200	ATCC 14169

TABLE 2: Value of MIC ($\mu g.ml^{-1}$) of moxifloxacin and other conventional antimicrobial drugs against some common pathogenic ATCC & NCTC strains

Pathogenic strain	Antimicrobial agents (MIC in $\mu g.ml^{-1}$)							
	M	C	N	T	G	Cef	Am	A -cl
Staphylococcus aureus (ATCC 29737)	0.005	0.010	0.05	0.25	0.06	0.51	0.51	0.512
Staphylococcus epidermis (ATCC 12228)	0.100	0.100	0.100	0.1	0.064	0.512	1.024	R
Staphylococcus aureus (ATCC 6538	0.005	0.008	0.010	0.01	0.064	0.016	0.032	0.100
Streptococcus fecalis (ATCC 10541)	0.100	0.520	0.100	0.100	0.064	0.512	1.024	0.100
*Streptococcus aureus (*ATCC 6538P)	0.005	0.010	0.100	2.5	0.256	1.024	1.024	0.100
Pseudomonas aeruginosa (ATCC 2561)	0.100	0.500	0.400	1.00	0.064	1.00	R	R
E.coli (ATCC 10536)	0.005	0.001	0.010	0.100	0.064	0.032	0.016	2
E.coli (NCTC 9002)	0.100	1.00	1.00	30.00	0.064	2.00	R	R
E.coli M 200 (ATCC 14169)	0.060	0.520	0.001	0.10	0.128	0.032	R	R

R: Resistant. **M** denotes Moxifloxacin, and likewise **C**- Ciprofloxacin, **N**- Norfloxacin, **T**- Tetracyclin, **G**- Gentamicin, **Cef** - Cefepime new, **Am**- Ampicillin new and **A-Cl** --Amoxicillin and Clavulinic acid (Amoxyclav).

MIC is important for susceptibility testing of antimicrobial drugs because it is directly comparable with concentrations achievable in *vivo* and the true MIC can assist therapeutic choices of such drugs. The MIC of moxifloxacin against 90% of organisms (MIC_{90}) is less than 0.25 $mg.l^{-1}$ for commonly isolated community-acquired respiratory tract pathogens including penicillin-susceptible and resistant *Streptococcus pneumoniae*, *Haemophilus* sp, and *Moraxella catarrhalis* and less than 1.0 $mg.l^{-1}$ for atypical pathogens such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila* (Special *et al*., 2002). MIC value obtained in present study is 0.001- 0.06 $\mu g.ml^{-1}$. This is lowest in comparison to other antibacterial agents tested. This is in agreement with that reported by Duggirala *et al*. (2007) Where MIC values of moxifloxacin was lowest against 123 isolates as 68 were Gram-positive (*Staphylococcus* spp, *Streptococcus* spp, *Corynebacterium* spp, *Bacillus* spp.) and 55 were Gram-negative (*Pseudomonas aeruginosa*). Approximately, similar value of MIC of moxifloxacin (0.094 $mg.ml^{-1}$) reported by Duggirala *et al*. (2007) for Gram positive bacteria. Relatively low MICs of moxifloxacin against target microorganisms, except makes

it a potentially useful drug for the treatment of infections caused by various organisms in goats.

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