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Determination of Minimum Inhibitory Concentration of Moxifloxacin Hydrochloride by Cup Plate Method Using *Staphylococcus aureus* and *Escherichia coli*

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Original Research Article

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Abstract: In this present study minimum inhibitory concentration of Moxifloxacin hydrochloride is determined by using cup plate method. Moxifloxacin Hydrochloride belongs to fourth generation fluoroquinolone antibiotic which is effective against both gram positive and gram negative microorganisms. Since moxifloxacin hydrochloride is a broad spectrum antibiotic Staphylococcus aureus and Escherichia coli is selected as test organisms. Serial dilution of Moxifloxacin Hydrochloride were made in the concentration ranging from 5000 µg/ml to 2.43µg/ml. Minimum inhibitory concentration of Moxifloxacin Hydrochloride was found out at 312.5µg/ml when seeded with S aureus and 19.5µg/ml when seeded with E coli.

Keywords: Minimum inhibitory concentration, Cup Plate method, Moxifloxacin hydrochloride.

INTRODUCTION

Antimicrobial agents or antibacterial agents are the chemical substances which may be bacteriostatic or bactericidal agents, which are used to kill or inhibit the growth of microorganisms. Antibiotic property had been assayed for two different bacteria (one gram-positive and one gram negative) by agar diffusion method. Usually, the antibiotics property is represented in terms of its capacity to inhibit the growth of bacteria in nutrient agar or broth. The inhibition by bacteria can be analysed by two different methods 1. Serial dilution method 2. Diffusion method

The serial dilution method is much useful for analysing the antibiotics propert. But it is not the preferred method qualitative detection of the concentration and evaluation of large number of compounds is not possible by this method. The agar diffusion technique consists of:

Cup-plate method:- In this technique with the use of a sterile borer, disc or cup is made in
the nutrient agar. The standard drug solution or test solution is added in this cup around that we see the zone of inhibition after incubation.

Filter paper strip method:- In this method the test solution which has to be analysed is adsorbed onto filter paper which is later placed over sterile plate containing organism and zone of inhibition is observed after incubation.

In this current investigation cup-plate method is used. In this method disc or cups of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums ie Staphylococcus aureus which belongs to gram positive bacteria and Escherichia coli which belong to gram negative bacteria [1].

As mentioned in the abstract moxifloxacin hydrochloride was selected for the current work. Moxifloxacin hydrochloride belongs to 4th generation fluoroquinolone antibiotic. Moxifloxacin hydrochloride is used for treatment of sinusitis and lung infections like

pneumonia and chronic Bronchitis, treatment of bacterial eye infections.

The Mechanism of action of moxifloxacin is due to inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV [2].

MATERIALS AND METHODS

MATERIALS

Moxifloxacin hydrochloride was procured as gift sample from micro labs Bangalore. The various other ingredients were of analytical grade.

APPARATUS AND MEDIA

- Sterile petriplates (12 Numbers)
- Live E Coli and S aureus
- Sterile measuring cylinder (25 ml capacity)
- Sterile Borer
- Sterile Nutrient agar media (20 ml for each plate)
- Incubator
- Permanent marker
- Cotton swab
- Micro pipette [3].

PREPARATION OF NUTRIENT AGAR MEDIA [4]

300 ml of agar was prepared (8.4 grams of pre-digested agar in 300 ml of distilled water) and sterilized by autoclaving at 121 degree C for 20 minutes at 15 PSI. (150 ml for E coli and 150 ml for S aureus).

STOCK SOLUTION OF MOXIFLOXACIN HYDROCHLORIDE- PREPARATION

- 500 mg of moxifloxacin hydrochloride was dissolved in 100 ml of distilled water in a 100 ml volumetric flask to get 5000 µg/ml concentration.
- 5 ml of above solution was diluted to 10 ml with distilled water to get 2500 µg/ml concentration.
- 2.5 ml of above solution was diluted to 10 ml with distilled water to get 1250 µg/ml concentration.
- 1.25 ml of above solution was diluted to 10 ml with distilled water to get 625 µg/ml concentration.
- 0.625 ml of above solution was diluted to 10 ml with distilled water to get 312.5 µg/ml concentration.
- 0.312 ml of above solution was diluted to 10 ml with distilled water to get 156.25 µg/ml concentration.
- 0.156 ml of above solution was diluted to 10 ml with distilled water to get 78.0 µg/ml concentration.
- From step 7 take 5 ml and dilute to 10 ml with distilled water to get 39 µg/ml concentartion.
- From step 8 take 5 ml and dilute to 10 ml with distilled water to get 19.5 µg/ml concentration.
- From 9 take take 5ml and dilute to 10 ml with distilled water to get 9.75 µg/ml concentration.
- From 10 take 5 ml and dilute to 10 ml with distilled water to get 4.87 µg/ml concentration.
- From 11 take 5 ml and dilute to 10 ml with distilled water to get 2.43 µg/ml concentration.

CUP PLATE METHOD [5]

- In 150 ml of sterilized agar E coli was seeded (3 ml) at a temperature around 45 degree C and in other 150 ml of sterilized agar S aureus was seeded (3ml) around 45 degree C.
- 20 ml of seeded agar media containing S aureus was transferred to each petriplates (6 plates) and allowed for solidification.
- 20 ml of seeded agar media containing E coli was transferred to each petriplates (6 plates) and allowed for solidification.
- After solidification, using permanent marker label the petriplates.
- After solidification 2 cavities are made in each plates, 24 cavities for 12 different concentration for E coli and S aureus.
- 2 drops ie 0.1ml of various dilutions is added into the cavity and incubated the plates for 24 hours at 37 degree Celcius.
- Measure and record the zone of inhibition.

RESULTS AND DISCUSSION

The zones of inhibition using S aureus and E coli are mentioned in the following figures (Figure 1 to 8). The diameter of zones of inhibition is listed in (Table-1).

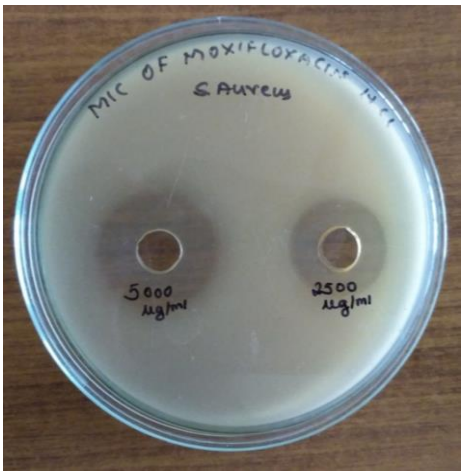

Fig-1: ZOI with 5000 µg/ml and 2500µg/ml with S aureus

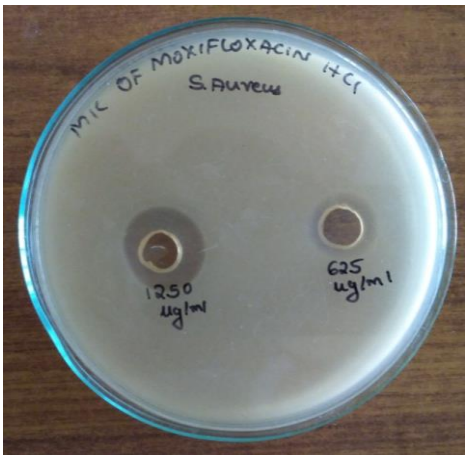

Fig-2: ZOI with 1250 µg/ml and 625µg/ml with S aureus

Fig-3: ZOI with 312.5 µg/ml and 156.25µg/ml with S aureus

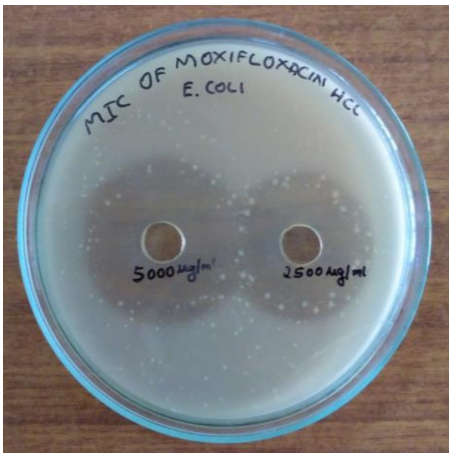

Fig-4: ZOI with 5000 µg/ml and 2500µg/ml with E coli

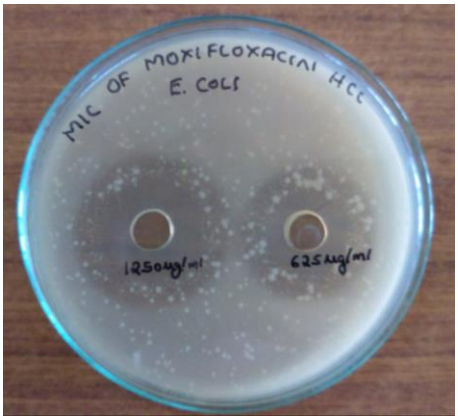

Fig-5: ZOI with 1250 µg/ml and 625µg/ml with E coli

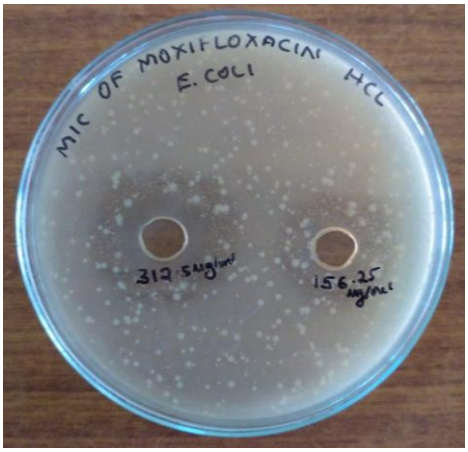

Fig-6: ZOI with 312.5 µg/ml and 156.25µg/ml with E coli

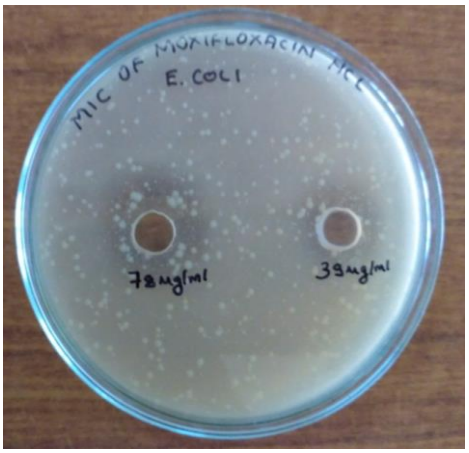

Fig-7: ZOI with 78.0 µg/ml and 39.0µg/ml with E coli

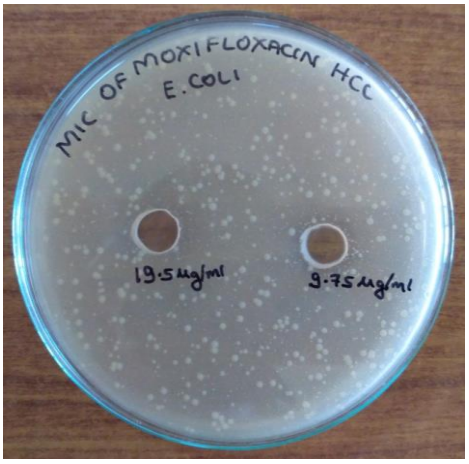

Fig-8: ZOI with 19.5 µg/ml and 9.75µg/ml with E coli (No inhibition)

Table-1: Diameter of zone of inhibition (mm)

Concentration (µg/ml)	Zone of inhibition (mm)	
	S aureus	E coli
5000	27	39
2500	21	36
1250	18	32
625	12	29
312.5	**10**	28
156.25	0	24
78.0	0	22
39.0	**0**	**18**
19.5	0	0
9.75	0	0
4.87	0	0
2.43	0	0

CONCLUSION

From this current work it can be observed that Moxifloxacin hydrochloride showed MIC at 312.5 µg/ml (10 mm) in presence of S aureus and 39.0 µg/ml (18 mm) in presence of E coli. Thus it can be concluded that Moxifloxacin hydrochloride showed zone of inhibition in both gram positive and gram negative microorganisms indicating it is a broad spectrum antibiotic.

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