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CORRELATION OF MINIMUM INHIBITORY CONCENTRATION OF CIPROFLOXACIN TO THE THERAPEUTIC RESPONSE OF PATIENT WITH URINARY TRACT INFECTION CAUSED BY *ESCHERICHIA COLI*

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ABSTRACT: The choice of an antibiotic depends solely on the identification of the species by determination of the sensitivity characteristics of the microorganism. Along with the determination of sensitivity pattern, understanding the susceptibility pattern of particular strain isolated from patient is equally important. Variation in patient and microorganism is known to be key factor for predicting the outcome for individual patient and establishing targets for clinical susceptibility. Dosage adjustment in relation to minimum inhibitory concentration (MIC) of drug, taking into account underlying pathogen might affect the therapeutic response and hence improve clinical outcome of patient. Therefore, *E coli* positive urine cultures of patients who were prescribed Ciprofloxacin were collected and their MIC was determined by agar well diffusion method. The response of patient was obtained by direct interview with them after 3 days of Ciprofloxacin therapy. There is a direct correlation between MIC and therapeutic outcome of antibiotic therapy. The clinical success rate increases when MIC is <1mg/l whereas, patient becomes non respondent as MIC approaches to >4mg/l which can be categorized as susceptible and resistant respectively. Therefore, for best clinical outcome MIC lies in the range <1-4 mg/l which could be used to discriminate success and failure of Ciprofloxacin treatment. Breakpoint of Ciprofloxacin was also derived from the study which is 4mg/l. This study concludes that obtaining only sensitivity pattern of antibiotic is not sufficient for optimal antibiotic therapy because MIC of sensitive strains varies and so does the response in relation to the minimum inhibitory concentration.

INTRODUCTION: Successful antimicrobial therapy of an infection depends on concentration of antibiotic at the site of infection that is high enough to kill or inhibit the growth of microorganism.

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The choice of drug depends solely on the identification of the species by determination of the sensitivity characteristics of the microorganism. However, along with the determination of sensitivity pattern, the understanding the susceptibility pattern of the particular strain isolated from patient is equally important [1].

The clinician purpose in prescribing an antimicrobial drug is to produce at the site of infection a concentration high enough to kill or inhibit the growth of microorganism.

Different strains of pathogenic species are known to have sensitivity characters constant to enable the choice of drug to be made solely on the basis of identification of the species; therefore, it is necessary for susceptibility pattern of the particular strain isolated from patient to be determined by the sensitivity test in the laboratory [1]. The main therapeutic outcome predictor for antimicrobial efficacy is the determination of MIC [2].

The MIC of Ciprofloxacin also varies according to different pathogens from 0.444mg/l to 128mg/l [3]. Ciprofloxacin MICs were between 0.015mg/l and 0.03mg/l in *E. coli* standard of ATCC 25922; however, *E. coli* isolates categorized as resistant to Nalidixic acid and susceptible to Ciprofloxacin the corresponding figure was between 0.12 and 0.25mg/l [4]. Trends suggest the higher MIC of Nalidixic acid may be due to its resistance pattern as shown by some *Salmonella* species. This resistance leads to decrease in the patient response[5].

As seen in Nalidixic acid, the difference in susceptibility pattern, leads to diverse therapeutic response on the individual. Dosage adjustment in relation to MIC's of drug, taking into account underlying pathogen might affect the therapeutic response and hence improve the clinical outcome of patient. Also, we can roughly estimate the dose that should be given to inhibit the organism, if we determine the MIC of the drug.

Most patients suggestive of urinary tract symptoms are started with an empirical therapy of Ciprofloxacin at a relatively fixed dose of 500mg BD. However, the therapeutic response of the patient might be in relation to the MIC of the drug. There is little research and fewer data to relate the response of patient to the MIC of Ciprofloxacin in patient with UTI and *E. coli*.

The aim of the study is to correlate MIC of Ciprofloxacin to the therapeutic response of patient with UTI caused by *E. coli* and to determine the MIC of Ciprofloxacin as well as to study the relation between the MIC and the therapeutic response of the patient. The Specific objective is to determine the MIC of Ciprofloxacin, to study the sensitivity pattern of Ciprofloxacin against *E. coli*, to study the therapeutic outcome of the UTI patient who are prescribed Ciprofloxacin, to study the relation between the MIC and the therapeutic response of the patient

Methodology: The proposal was approved from Department of Pharmacy, Kathmandu University on 20th April, 2012 and Institutional Review Committee, Kathmandu University Teaching Hospital, Dhulikhel on 13th May, 2012.

- **Study Site:** The sample and patient response was collected in Department of Microbiology, Kathmandu University Teaching Hospital, Dhulikhel. MIC determination was done in Kathmandu University, Department of pharmacy, Dhulikhel.
- **Study Design:** The study is a prospective observational study.
- **Sample Size:** The total sample size for the study was 70.

Inclusion Criteria:

- All patient suffering from UTI who are prescribed Ciprofloxacin
- *E. coli* sensitive and resistant to Ciprofloxacin
- *E. coli* sensitive and resistant to Nalidixic acid
- In Patient/Outpatient
- Adult population aged over 15 years

Exclusion Criteria:

- Patients with more than one prescribed antibiotic
- Pediatric patient

Study Method:

1. **Sample Collection:** Clinical samples were collected from Department of Microbiology, Kathmandu University Teaching Hospital. All patients with provisional diagnosis of UTI were taken and their urine samples were cultured to determine sensitivity to the antimicrobials.
2. **Preparation of Inoculums stock:** All samples *E. coli* sensitive as well as resistant to Ciprofloxacin and also samples which were resistant and sensitive to Nalidixic acid were taken. A loop of *E. coli* was taken and it was inoculated in BHI broth. It was then incubated at 37°C for 24 hours and stored at 2-8°C until

further use. Their MIC was determined by agar well diffusion method at Kathmandu University, Department of Pharmacy.

3. **Collecting patient information:** For therapeutic response, patients were directly interviewed for details

MIC determination by Agar well diffusion method [1]:

a) **Preparation of agar dilution plates:** 20ml of cooled molten MHA was added to the petri plate. The plates were set to dry. The prepared inoculums were then swabbed in to the dried petri plate.

b) **Inoculation:** Using an 8.86 mm bore, holes were punched into the agar plate. 50-60µl of different concentration of prepared antibiotic solution was delivered into the punched well. The plates were then left for diffusion for at least 1 hour and then was incubated at 35-37^{o}C for 24 hours.

c) **Quality control:** ATCC 25922 control strain for *E. coli* was used, which was supplied from Department of Microbiology, Patan Hospital.

d) **Reading the result:** The lowest antibiotic concentration with the highest zone of inhibition was considered to be the MIC of the sample. The zone of inhibition was measured by Vernier caliper. MIC is lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye, disregarding a single colony or a thin haze within the area of the inoculated spot.

e) **Interpreting MIC:** The MIC of Ciprofloxacin for *E coli* has been categorized into susceptible, intermediate and resistant [6, 7].

TABLE 1: INTERPRETATION OF MIC

MIC(mg/l)	Interpretation
≤1	Susceptible (S)
1-4	Intermediate (I)
≥4	Resistant (R)

Statistical analysis of data: The collected data were analyzed using SPSS v 15 and Microsoft Excel 2007. Comparison of data was done by Chi-square test and P value <0.05 was considered significant.

RESULTS:

Prevalence of causative organism: Out of 88 UTI cases observed from 30th May, 2012, to 2nd July, 2012, the main organism isolated was *E. coli* (84.33%), followed by Klebsiella species (6.02%). The result observed is summarized in **Table 2**.

TABLE 2: PREVALENCE OF CAUSATIVE ORGANISM

Organism Isolated	Percentage (%)
Escherichia coli	81.33
Klebsiella species	6.02
Enterococcus	4.24
Pseudomonas aeuroginosa	3.61
Proteus mirabilis	2.4
Acienebacter species	2.4

Prevalence of UTI according to Gender and age: The total number of the patient included in the study was 70. Among the 70 patients suffering from UTI, 41 patients were female (58.57%) and 29 were male (41.42%). Their ages ranged from 15-60 years with highest incidence observed in 15-30 years age group (57.14%) and lowest in 46-60 year age group (15.71%).

Doses: Out of 70 cases of UTI, 33 episodes were treated with Ciprofloxacin 500mg for 3 days, 22 episodes for 500mg for 5 days and 10 episodes for 7 days. 3 episodes of UTI were treated with Ciprofloxacin 750mg for 3 days and 2 episodes for 5 days.

Sensitivity pattern of Nalidixic Acid and Ciprofloxacin: According to the inclusion criteria, the antibiotic included in the study is Ciprofloxacin. Therefore, patients included were only those who were prescribed Ciprofloxacin. However, sensitivity test was done for Nalidixic acid as well. The total number of patient incorporated was 70. 30 out of 70 isolates were seen sensitive to Ciprofloxacin and 18 out of 70 were seen sensitive to Nalidixic acid. Whereas, resistance rate were higher in Nalidixic acid group where, 52 out of 70 were seen resistant, and 40 out of 70 were seen resistant to Ciprofloxacin. The sensitivity pattern of Nalidixic acid and Ciprofloxacin against *E. coli* is summarized in **Table 3**.

TABLE 3: SENSITIVITY PATTERN OF NALIDIXIC ACID AND CIPROFLOXACIN

			Ciprofloxacin	
			Sensitive	Resistant
Nalidixic acid	Sensitive	%	22.85	2.85
	Resistant	%	20	54.28

MIC obtained: MIC was determined for all 70 patients; the reference range of Ciprofloxacin taken was 0.03 – 128mg/l. The MIC was categorized on the basis of **Table 4**.

TABLE 4: CATEGORIZATION OF MIC AND ITS FREQUENCY

MIC (mg/l)	Frequency	Percent
<1 (susceptible)	19	27.1
1-4 (intermediate)	8	11.4
>4 (Resistant)	43	61.4

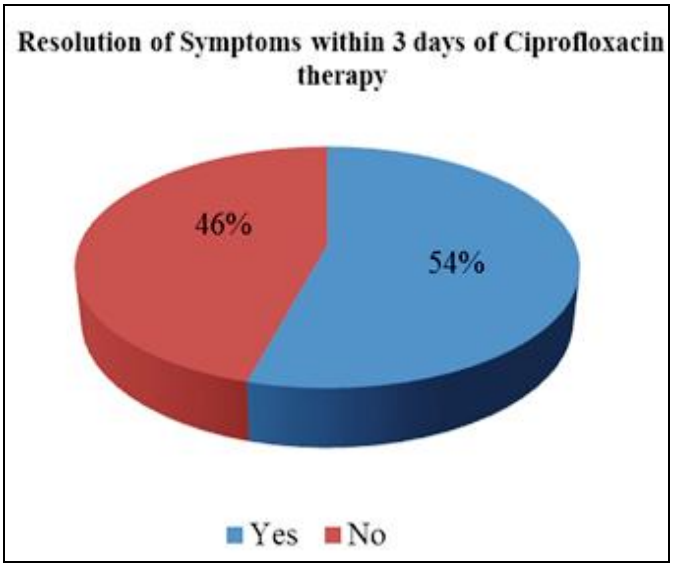
The percentage of the obtained MIC that are on the resistance range i.e. >4mg/l are 61.4% whereas 27.14% was grouped under susceptible i.e. <1mg/l.

Quality Control: A standard of E coli ATCC 25922 was used for quality control purpose and MIC of the strain was found to be 0.015mg/l.

The target MIC for the reference strain is 0.015mg/l for ATCC 25922 and NCTC 10418 [8].

Treatment Outcome: Based on the inclusion criteria, patients included in the study were those who were prescribed Ciprofloxacin only. Therapeutic response obtained was based on direct interview with the patient. Therapeutic success was defined by the resolution of symptoms such as fever, burning micturition for 24 hours of completion of 3 days Ciprofloxacin therapy and was not taking Paracetamol. Therapeutic failure was defined as persistent symptoms for more than 24 hours even after completion of 3 days of Ciprofloxacin therapy.

Patients treated within 3 days of Ciprofloxacin therapy were categorized as 'yes' and the patient who said their symptoms have not subsided or they were not treated were categorized as 'no'. Out of 70 patients who were on Ciprofloxacin therapy, 54.28% said they were treated within 3 days of therapy, and 45.71% said they were not treated within 3 days of therapy as shown in **Figure 1**.



Correlation between MIC and Therapeutic response

TABLE 5: CORRELATION BETWEEN MIC AND THERAPEUTIC RESPONSE

MIC (mg/l)	Yes	No	P Value	χ^2 Value
<1 (Susceptible)	18	1	0.000	22.091
1-4 (Intermediate)	6	2		
>4 (Resistant)	14	29		

Chi square test was used to analyze the relation between MIC and therapeutic response and it was found that there is significant relation between MIC and treatment outcome of the patient (P=0.000, χ^2= 22.091).

Of the total infection, 94.7% (18 out of 19 episodes) due to isolates with Ciprofloxacin MIC <1mg/l responded to Ciprofloxacin treatment (**Table 5**). A response of 75% (6 out of 8 episodes) was observed when the infection were caused by isolates with MIC 1-4mg/l and a response of 32.6% (14 out of 43 episodes) was observed when the infection were caused by isolates with MIC ≥4mg/l.

Relation between MIC and Ciprofloxacin sensitive strains:

TABLE 6: CORRELATION BETWEEN MIC AND CIPROFLOXACIN SENSITIVE STRAINS

	<1 (Susceptible)	1-4 (Intermediate)	>4 (Resistant)	P Value	χ^2 Value
Yes	17	6	3	0.036	6.635
No	0	2	2		

Chi square test was used to analyze the relation between the MIC and the therapeutic response of the patient with Ciprofloxacin sensitive strains and it was found that there is significant relation between MIC and treatment outcome of the patient ($P = 0.036$, $\chi^2 = 6.635$).

All of the Ciprofloxacin sensitive strains i.e. 17 out of 17 strains, responded to the treatment with MIC <1mg/l (**Table 6**) giving the success response of 100%. A response of 75% (6 out of 8 Ciprofloxacin sensitive strains) was observed when the infection were caused by isolates with MIC 1-4mg/l and a response of 60% (3 out of 5 Ciprofloxacin sensitive strains) was observed when the infection were caused by isolates with MIC ≥4mg/l.

Patients those who did not respond to the Ciprofloxacin treatment, had MIC in the intermediate and resistant range even if the strain of *E. coli* was sensitive.

DISCUSSION: It has been advised that medical practitioners should be aware of increasing trends of resistance of urinary pathogens to commonly prescribed antibiotics as well as the profile of antibiotic resistance within their community. Therefore, periodic evaluation of sensitivity pattern is essential for rationale and appropriate use of antibiotics [9]. It is important to monitor the status of antimicrobial resistance among uropathogens in order to improve the treatment recommendation [10].

There has been rising resistance to Ciprofloxacin in recent years. In a study done by Kwan S.K et al, among 307 *E. coli* isolates, 30.3% were resistant to Ciprofloxacin [11]. Also, the study did in Netherland shows the increase in resistance to Ciprofloxacin among the urine isolates [12].

Similar results were seen in the study, where urinary isolates of *E. coli* were tested for the susceptibility testing for Ciprofloxacin and Nalidixic acid. 54.3% of the isolates were seen resistant to both Nalidixic acid and Ciprofloxacin, whereas, 22.9% were seen sensitive to both Nalidixic acid and Ciprofloxacin (Table 3).

However, to achieve an adequate response from an antimicrobial agent, it has to achieve a sufficient drug exposure in relation to MIC, at the site of infection, for optimal efficacy [13]. Determining MIC is considered to be the gold standard for the susceptibility of antibiotic to microorganism and is therefore used to judge the performance of all other methods of susceptibility testing [8]. Along with the sensitivity test of antibiotics, the bactericidal activity of antibiotics can be described by the investigation of bactericidal kinetics or by the determination of the minimal bactericidal concentration against a particular bacterial strain [14].

For Ciprofloxacin, the MIC is categorized into 3 different categories i.e. Susceptible, Intermediate and Resistant. The categorization of MIC is summarized in Table 1 [6, 7]. Similar results were obtained in our study, where we were able to categorize the MIC of Ciprofloxacin into 3 categories. It was found that, among 70 isolates of *E. coli* 62% were found to have MIC > 4mg/l i.e. it was grouped under resistant category. 27% were found to have MIC <1 mg/l and was grouped under susceptible category and 11% was grouped under intermediate category which had MIC between 1-4 mg/l (Table 4).

Similarly, while analyzing the relation between therapeutic response and MIC, it was found that there is a direct relation between these two parameters. More than 94% of the isolates responded to the Ciprofloxacin treatment when the isolate had Ciprofloxacin MIC <1mg/l. For these isolates with Ciprofloxacin MIC of 4mg/l the response was 32%. Therefore, an MIC of >1mg/l to 4mg/l could be used to discriminate the success and failure of Ciprofloxacin treatment. MIC target of Ciprofloxacin to achieve the clinical success is <1mg/l [7].

The lowest MIC breakpoint given is <1mg/l and the highest is >4mg/l [1, 7]. The clinical success rate in the study decreased as the MIC of Ciprofloxacin was greater than the breakpoint concentration i.e. 4mg/l (Table 5).

Correspondingly, while analyzing the relation between the response and MIC of Ciprofloxacin sensitive strains only, we found that, the response was 100% in susceptible category (Table 4). Even if the strains were classified as sensitive, the patient who did not respond to the treatment was 50%. The MIC of such strains was found to be >4mg/l. Consequently, we can say that, to obtain the clinical success with Ciprofloxacin, knowing only the sensitivity pattern is not sufficient. Determination of MIC is equally effective, as the clinical response depends upon the concentration of antibiotic at the site of infection, and the concentration can be best predicted by MIC. Also, while analyzing the pharmacokinetic parameter of ciprofloxacin it was found that effective killing concentration were achieved in pathogens with MIC less than 0.25 [15].

Although, MIC is important, it is not a surefire indication of clinical success or failure. Determination of MIC only cannot be used to access the therapeutic effectiveness of the drug. MIC together with the known pharmacokinetic properties of the substance can be used to assess the presumed degree of therapeutic effectiveness of the drug. In individual cases, additional special characteristics of the patient can be taken into account [14].

The interpatient variability in population pharmacokinetic parameter estimates has only been recognized as a key factor in predicting the outcome of individual patient and establishing the breakpoint and target for clinical susceptibility [2]. To best predict the efficacy of the antibiotic therapy, determination of pharmacodynamic parameter, i.e. AUC and the relation with maximum plasma concentration and dose i.e. Cmax/MIC, AUC/MIC dose/MIC relations are considered necessary. Various studies suggest that AUC/MIC ratio of 125 is required for optimal clinical effects for treatment of serious infection [15].

Some studies show the relevance of dose selection in optimizing target attainment, with important differences among pathogens, even those with MICs within the susceptible range [16]. The risk of Ciprofloxacin treatment failure was 27.8 times greater in those not achieving an AUC/MIC≥250 [16]. Also, the dose/MIC value can be considered as the threshold for the prediction of clinical cure or failure and increasing the exposure above this threshold can further increase the probability of cure [2]. It has been recommended to use higher dose of ciprofloxacin i.e. 1200mg/day to ensure optimal antimicrobial activity in cases of bacteria with higher MIC [15, 17].

However, in this study, AUC and the doses could not be adequately correlated due to lower sample size of dose 750mg and lack of variation in the dosage regimen used.

Therefore, we can say that prescribing practice of clinician mostly depends upon on the result of the sensitivity test. However, knowing only sensitivity pattern is not effective to actually predict therapeutic efficacy of antibiotic. Along with sensitivity test of antibiotics, bactericidal activity of antibiotics can be obtained by determination of the minimal bactericidal concentration against a particular bacterial strain which could influence the response shown by patient.

From this study, it was also found that, MIC was variable in case of sensitive drug too. This could affect the response shown by the patient since MIC and clinical success are interrelated.

CONCLUSION: This study gave way to optimize the treatment regimen for UTI, it aimed to obtain the relation between minimum inhibitory concentration and clinical success of the drug. It was found that, therapeutic response of Ciprofloxacin seen was maximum when MIC was in susceptible range i.e. <1mg/l. Therefore, an MIC of <1mg/l to 4mg/l could be used to discriminate success and failure of Ciprofloxacin treatment. Along with MIC, if we could obtain the equivalent AUC, it could confirm the breakpoint of the therapy as well as optimize the effective dosage regimen with 100% clinical cure.

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