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Original Research Article

Evaluation of Susceptibility Pattern of Gram Negative Bacilli with Special Reference to Ciprofloxacin in a Tertiary Care Hospital

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ABSTRACT

Introduction: Bacterial resistance to antibiotics is a growing therapeutic problem, both in the community as well as in the hospitals. It involves most of the antibiotics including fluoroquinolones. **Aim:** Our study aims to assess the prevalence of ciprofloxacin resistance in Gram negative bacilli isolated from various clinical samples over a period of six-months. **Methods:** Ciprofloxacin was evaluated along with other commonly-used antibiotics against a total of 480 clinical isolates obtained from various clinical samples. Minimal inhibitory concentration of ciprofloxacin was measured for all resistant isolates. Isolates, resistant or with a decreased susceptibility to ciprofloxacin (≤20 mm) were then screened for their minimum inhibitory concentration (MIC) by using the E-test. **Results:** Out of 480 Gram negative bacilli, 131(27.29%) isolates were resistant to ciprofloxacin. The minimum inhibitory concentration of ciprofloxacin for these isolates ranged between 4 and >32 µg/ml. High resistance to ciprofloxacin was detected among Pseudomonas species, Acinetobacter, Proteus, Klebsiella followed by E.coli. **Conclusion:** This study indicates emerging ciprofloxacin resistance among most of the bacterial pathogens isolated from UTI. The considerably high MIC values for ciprofloxacin in this study reflect the limited treatment options which are available for these resistant isolates and a need for the continuous evaluation of the commonly used antibiotics.

Key words: Gram negative bacilli, Ciprofloxacin, Antibiotic resistance, Flouroquinolones

INTRODUCTION

Bacterial resistance is a growing therapeutic menace, both in the community and as well as in the hospitals, involving most of the antibiotics, which include fluoroquinolones. Ciprofloxacin is a broad-spectrum antibiotic which is active against variety of Gram-positive and Gram-negative

bacteria. It belongs to the fluoroquinolone class of antibiotics. [1]

Fluoroquinolones block bacterial DNA synthesis by inhibiting bacterial topoisomerase II (DNA gyrase) and topoisomerase IV. [2] Topoisomerase IV is the primary target for many Gram positive bacteria. In contrast, DNA gyrase is the primary quinolone target in many Gram negative bacteria. [3] Inhibition of DNA gyrase prevents the relaxation of positively supercoiled DNA that is required for normal transcription and replication, whereas inhibition of topoisomerase IV interferes with separation of replicated chromosomal DNA into the respective daughter cells during cell division. [4] A decreased susceptibility to fluoroquinolones arises mainly due to single-step mutations in the *gyrA* and the *parC* genes, which encode the topoisomerase enzymes, the target for fluoroquinolone group of antibiotics. [5]

More recently, two types of plasmid mediated resistance have been described. The first type utilizes Qnr proteins, which protect DNA gyrase from the flouroquinolones. The second type is a variant of an aminoglycoside acetyltransferase capable of modifying ciprofloxacin. Both mechanisms confer low level resistance that may facilitate the point mutations that confer high level resistance. [4]

This study was undertaken to evaluate the susceptibility pattern of Gram negative bacilli to various antibiotics and to know the prevalence rate of ciprofloxacin resistance in our hospital.

MATERIALS AND METHODS

A total of 480 Gram-negative bacilli isolated from various clinical samples: urine, pus, sputum, blood and other body fluids, which were received in the Microbiology Laboratory over a period of six months (April 2011- Sept 2011) were subjected to the study. The identification of the isolates was done based on their colony morphology on MacConkey's agar and blood agar and by the standard biochemical reactions. [6] Antibiotic susceptibility testing was done on Muller Hinton agar (MHA) after standardizing the suspension to 0.5 McFarland's standards. [7] The antibiotic panel consisted of amoxycillin (25 µg), co-trimoxazole (23.75/1.25 µg), cefotaxime(30µg), ceftazidime(30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), imipenem (10 µg) and nitrofurantoin 300 µg (for urinary isolates). The results were interpreted as recommended by the CLSI guidelines. [8] The isolates with resistance or decreased susceptibility to Ciprofloxacin (≤20mm) were confirmed by breakpoint minimum inhibitory concentration (MIC in µg/ml) by using E-test strips. The isolates with MIC value ≥ 4 µg/ml were defined as resistant isolates, as outlined by CLSI guidelines. [8]

RESULTS

Most of the isolates were cultured from urine (51.5%), wound swabs (26.01%), sputum (18.12%) or blood (4.37%). Escherichia coli (26.87%) was the predominant isolate which was found among the Gram negative bacilli, followed by Klebsiella sp.(25.20%), Pseudomonas aeruginosa (25%), Pseudomonas sp.(12.70%), Proteus sp.(6.45%) and Acinetobacter (3.75%) as shown in [Table 1].

Table 1: Total number of Gram-negative Bacilli isolated from various clinical samples (n=480)

Sl.No	Organism	Total number isolated	Percentage
1.	Escherichia coli	129	26.87 %
2.	Klebsiella species	121	25.20 %
3.	Pseudomonas aeruginosa	120	25 %
4.	Pseudomonas sp.	61	12.70 %
5.	Proteus species	31	6.45 %
6.	Acinetobacter	18	3.75 %

Out of 480 Gram-negative bacilli, 131(27.29%) isolates were resistant to ciprofloxacin. High rates of resistance were observed for amoxycillin, followed by co-trimoxazole, nalidixic acid and cefotaxime, while low levels of resistance were observed for ceftazidime, amikacin, nitrofurantoin and imipenem as shown in [Table 2]. The minimum inhibitory concentration of ciprofloxacin for these isolates ranged between 4 and >32 µg/ml [Table 3].

Table 2: Percentage of resistance to the selected antimicrobial agent (n=480)

SL.No.	Antibiotics	Total no. of sensitive isolates (%)	Total no. of resistant isolates (%)
1.	Amoxycillin	130 (27%)	350 (73 %)
2.	Co-trimoxazole	164 (34%)	316 (66 %)
3.	Nalidixic acid	231 (48%)	249 (52 %)
4.	Cefotaxime	283 (59%)	197 (41 %)
5.	Ciprofloxacin	349 (72.71)	131 (27.29 %)
6.	Ceftazidime	365 (76%)	115 (24%)
7.	Amikacin	394 (82%)	86 (18 %)
8.	Nitrofurantoin (for urinary isolates)	394 (82%)	86 (18 %)
9.	Imipenem	427 (89%)	53 (11 %)

Table 3: MIC values of the resistant isolates to Ciprofloxacin (n=131)

Ciprofloxacin MIC Values	4µg/ml	8µg/ml	16µg/ml	32µg/ml	>32µg/ml
Total No. of isolates	24 (18.32%)	20 (15.26%)	21 (16.03%)	24 (18.3%)	42 (32.06%)

DISCUSSION

Ciprofloxacin is effective in a broad range of infections. Because of wide-spectrum bactericidal activity, oral efficacy and good tolerability, it is being extensively employed for empirical therapy of many infections. [1]

The prevalence of ciprofloxacin resistance in our study is 27.29 %. Most of the ciprofloxacin resistant isolates were obtained from urine samples. This may be because, fluoroquinolones are more preferred in the empirical treatment for UTI because of their excellent activity against the pathogens which are commonly encountered in UTI. [9] This emphasizes the importance of the reassessment of the antibiotics which are used in the empirical treatment.

In our study, majority of the isolates from UTIs are susceptible to nitrofurantoin, amikacin and imipenem. This suggests that nitrofurantoin can still be successfully used in the treatment of UTI.

The MIC of ciprofloxacin against Gram negative bacilli is usually <0.2 μg/ml. [1] The minimum inhibitory concentration of ciprofloxacin for the resistant isolates in our study ranged between 4 and >32 μg/ml.

Resistance to quinolones develops during therapy via mutations in the bacterial chromosomal genes encoding DNA gyrase or topoisomerase IV or by active transport of the drug out of the bacteria. Resistance has increased after the introduction of fluoroquinolones especially in Pseudomonas and staphylococci. [3]

In our study, the isolated bacteria showed wide differences in their susceptibility to ciprofloxacin. High rate of resistance to ciprofloxacin was observed among Pseudomonas sp., Acinetobacter, Proteus sp., Klebsiella followed by E.coli. The high resistance pattern which was seen in our study was probably due to the inappropriate prescription of antibiotics and the poor infection control strategies.

The drugs which showed maximum activity against most of the isolates were imipenem, nitrofurantoin and amikacin. Though carbapenems remain the final options for treating these infections, there is a possibility that the increasing use of carbapenems may lead to a rapid emergence of carbapenem resistance.

CONCLUSION

The present study indicates emerging ciprofloxacin resistance among most of the bacterial pathogens isolated from UTI. The situation in this region, in terms of antimicrobial drug usage, is not so different from that of many developing countries, where people usually take antimicrobial drugs without prescription or without performing the necessary culture testing. The widespread use, and more often the misuse of antimicrobial drugs in this region, has led to a general rise in the emergence of resistant bacteria, particularly to ciprofloxacin.

The considerably high MIC values for ciprofloxacin in this study reflect the limited treatment options which are available for these resistant isolates and a need for the continuous evaluation of the commonly used antibiotics. Increasing resistance against ciprofloxacin demands co-ordinated monitoring of its activity and rational use of the antibiotic. Repeated surveillance, formulation of an antibiotic policy and prudent prescription of antibiotics can be used to curb the rapid emergence and the spread of these resistant isolates.

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