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# *IN VITRO* ACTIVITY OF FLUOROQUINOLONES AMONG BACTERIAL ISOLATES OF CANCER PATIENTS

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## ABSTRACT

Bacteraemic infections have become the major cause of mortality and morbidity in cancer patients. Floroquinolones have broad spectrum of antimicrobial activity and have increasingly been used for prophylaxis against infection in cancer patients. The activity of these fluoroquinolones was evaluated in cancer patients undergoing treatment against 60 g +ve and -ve bacterial strains isolated from blood cultures. The susceptibility was determined by broth dilution method according to National Committee Clinical Laboratory Standards (NCCLS), USA guidelines. The overall respective MICs at which 50% and 90% of isolates were inhibited (MIC50s and MIC90s) were as follows ciprofloxacin, 4 and 8ug/mL; ofloxacin, 16 and 64ug/mL; pefloxacin, 16 and 128ug/mL. The percent resistance of gram negative bacterial isolates against ciprofloxacin, ofloxacin and norfloxacin was 80%, 95% and 100% respectively. In gram positive bacteria high resistance was observed against ciprofloxacin where only 15% strains were susceptible in methecillin susceptible *Styphlococcus aureus* and 12% in methecillin resistant *Staphylococcus aureus*. Where as 100% resistance were observed in case of pefloxacin, ofloxacin and norfloxacin against both methecillin resistant and methecillin sensitive *Staphylococcus aureus*. High resistance observed in this study against clinical isolates of cancer patients against potent antimicrobial agent warrants the need of monitoring susceptibility of bacterial isolates from cancer patients for better management and treatment of patients.

**Key words:** Fluoroquinolones, Resistance in *Pseudomonas areuginosa*, Resistance in *Staphylococcus aureus*.

## INTRODUCTION

Infections are a major cause of morbidity and mortality among immunocompromised cancer patients undergoing chemotherapy. For this reason these patients are at risk for a wide variety of bacterial, viral, and fungal infections throughout the phases of immune recovery. The careful evaluation of bactermic episodes that develop during the course of disease is essential for the successful anti-infective treatment (Cruciani, 2000; Madani, 2000). In the face of the increasing worldwide problem of antimicrobial resistance, many classes of antimicrobial agents have become less useful for therapy (Kunin, 1997). Recently addition of the flouride to the quinolone antibiotic compounds yielded a new class of drugs, the fluoroquinolones, which have a broader antimicrobial spectrum and improved pharmokinetic properties (Paton and Deeve, 1988; Siporin *et al.*, 1989; Wolfson and Hooper, 1989; Hooper and Wolfson, 1991;Gootz *et al.*, 1994) Most of these agents, including norfloxacin, ciprofloxacin, ofloxacin, pefloxacin, have broader antimicrobial spectra and improved bioavailabilities in comparison with those of naladixic acid. Fluoroquinolones are bactericidal antibiotics that act by specifically targeting DNA gyrase (Just, 1993). In contrast to amino glycosides and beta-lactams, some floroquinolones are active against dormant and replicating bacteria (Fitton, 1992).

Fluoroquinolones is being used for the treatment of various infections, caused by both Gram positive and Gram-negative bacteria (Gootz *et al.*, 1994; Oh *et al.*, 1996; Cormicon and Jones, 1997; Hannan and Woodnutt, 2000). Some of these have been used for the infection prevention and for the treatment of established infection in neutropenic cancer patient (Karp *et al.*, 1987; Chan *et al.*, 1989 ;). However, the widespread prophylactic and therapeutic usage of quinolones in cancer patients has raised concern about the emergence of resistance to them and a reduction in their overall impact as clinically useful antibiotics. In this study we examined the in vitro activity of ciprofloxacin, pefloxacin, ofloxacin, norfloxacin against bacterial isolates obtained from patients with cancer that are being treated at our institution.

## MATERIALS AND METHODS

The study was carried out at the clinical pathology labs., Institute of Nuclear Medicine and Oncology over a period between January 2004 to June 2004.

**Patients:**

All hospitalized cancer patients undergoing anticancer therapy with suspected blood stream infections were studied. [illegible]ination was made on the basis of age or gender. Patients already on antimicrobial therapy and

those having fever due to non-infectious causes, such as blood transfusion, drug infusion etc. were excluded from the study.

**Bacterial strains and culture conditions**

A total of 60 bacterial isolates of *Pseudomonas aeruginosa* and *Stypholococcus aureus* isolated from blood of patients treated between January and June 2004, were studied. Isolation was made by adding Five ml blood obtained from peripheral veins of the patients to brain heart infusion (BHI) broth (Oxoid, Hampshire, UK). The blood culture bottles were incubated at $37^0$ C and regular subcultures were done. Identification of the isolates was done by Gram staining and standard biochemical tests according to the manual of clinical microbiology (Cheesbrough, 1984). All clinical isolates were identified and stored as glycerol stocks at –70°C.

**Antimicrobial agents and MIC determination**

Ciprofloxacin, Pefloxacin, ofloxacin and norfloxacin was obtained from commercial suppliers. Antibiotic sensitivity against fluoroquinolones was determined in *P. areuginosa* among gram negative isolates and *S. aureus* among gram positive bacteria. Minimal Inhibitory Concentration was determined in Mueller-Hinton broth (Oxoid, UK) containing serial two-fold dilutions of each antibiotic with inoculated bacterial suspensions of $5X10^5$ CFU/ml as outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 1997). The results were recorded after overnight incubation at 37°C. The MIC was defined as the lowest antibiotic concentration with no visible growth. The MIC50 and MIC90 was defined as the minimum concentration of antimicrobial that inhibited 50% or 90% of the isolates, respectively. For ciprofloxacin, NCCLS breakpoints of ≤ 1 μg/mL (susceptible), and ≥ 4 μg/mL (resistant) were applied. For ofloxacin, NCCLS breakpoints of ≤ 2 μg/mL (susceptible), and ≥ 8μg/mL (resistance) were applied. For pefloxacin, NCCLS breakpoints of ≤ 4μg/mL (susceptible), and ≥ 16 μg/mL (resistant) were applied. For norfloxacin, NCCLS breakpoints of ≤ 4μg/mL (susceptible), and ≥ 16μg/mL (resistance) were applied (NCCLS 1997, 2002).

**RESULTS**

**Bacterial Strains:**

A total 60 bacterial strains of gram negative and gram positive were isolated from patients under study. Only one isolate per patient was used for testing in order to avoid duplication.

Table 1. *In vitro* activity of fuoroquinolones agents against *Pseudomonas areuginosa.*

| Antimicrobial agent | Range μg/mL | MIC50 μg/mL | MIC90 μg/mL | %age sensitivity | %age resistance |
|---|---|---|---|---|---|
| Ciprofloxacin | 0.125-64 | 4 | 8 | 20% | 80% |
| Ofloxacin | 0.5-256 | 16 | 64 | 5% | 95% |
| Pefloxacin | 0.5-256 | 16 | 128 | 0% | 100% |
| Norfloxacin | 0.5-256 | 32 | 128 | 0% | 100% |

Table 2. *In vitro* activity of fluoroquinolones agents against methecillin susceptible *Staphylococcus aureus.*

| Antimicrobial agent | Range μg/mL | MIC50 μg/mL | MIC90 μg/mL | %age sensitivity | %age resistance |
|---|---|---|---|---|---|
| Ciprofloxacin | 0.125-64 | 8 | 16 | 15% | 85% |
| Ofloxacin | 0.5-256 | 32 | 64 | 0% | 100% |
| Pefloxacin | 0.5-256 | 32 | 256 | 0% | 100% |
| Norfloxacin | 0.5-256 | 64 | 256 | 0% | 100% |

**MIC determination**

The over all susceptibility results are shown in Table (1, 2 &3). Ciprofloxacin showed high activity against *P. aeuginosa* among gram negative isolates The $MIC_{50}$ of ciprofloxacin against gram negative bacteria was 4ug/mL and inhibited 20% of isolates of *P. areuginosa* at concentration 1μg/mL (susceptibility break point). However it inhibited all the isolates of *P. areuginosa* at concentration of 32μg/mL. Ofloxacin have moderate activity against *Pseudomonas areuginosa* with MIC ranging from 0.5-256μg/mL with more than 90% of isolates being susceptible at a concentration of 64μg/mL but 95% were resistant according to NCCLS resistance break point. The activity of pefloxacin and norfloxacin was least active against *P. areuginosa* than that of ciprofloxacin and ofloxacin for isolates tested demonstrating $MIC_{90}$ of 128 ug/mL (Table 2).

In case of methecillin susceptible *S. aureus* $MIC_{50}$ against ciprofloxacin was 8ug/mL and ranged from 0.125-64 ug/mL. Only 15% were susceptible at NCCLS break point of 2ug/mL. In case of methecillin resistant *S. aureus* were 12%. In other fluoroquinolones (ofloxacin, pefloxacin, and norfloxacin) 100% resistance was observed against both methecillin susceptible and methecillin resistant *S. aureus* strains (Table 2 & 3).

Table 3. *In vitro* activity of fluoroquinolones against methecillin resistant *Staphylococcus aureus*.

| Antimicrobial agent | Range μg/mL | MIC50 μg/mL | MIC90 μg/mL | %age sensitivity | %age resistance |
|---|---|---|---|---|---|
| Ciprofloxacin | 0.125-64 | 8 | 16 | 12% | 88% |
| Ofloxacin | 0.5-256 | 32 | 64 | 0% | 100% |
| Pefloxacin | 0.5-256 | 32 | 256 | 0% | 100% |
| Norfloxacin | 0.5-256 | 64 | 256 | 0% | 100% |

## DISCUSSION

Our results indicated comparative activity of different quinolones against Gram-negative and gram positive microorganisms isolate from cancer patients. Ciprofloxacin was found active against gram negative and gram positive microorganism. It inhibited all the isolates of *P. areuginosa* at concentration of 32 ug/mL with only 20% being susceptible. The MIC of ciprofloxacin against members of the family *Pseudomonas* ranged from 0.125- 64 ug/mL, with more than 90% of isolates being susceptible at a concentration 8 ug/mL. The $MIC_{90}$ values for ofloxacin against isolates tested was 64 ug/mL which is eight fold higher than ciprofloxacin. In case of pefloxacin and norfloxacin 90% of isolates have MIC128 ug/mL. None of the isolate were found susceptible at NCCLS susceptible break point. In vitro activity against gram negative indicated that MIC values of ciprofloxacin are generally lower than those ofloxacin, pefloxacin and norfloxacin but higher than recommended NCCLS. Thus most of the strains are resistant.

Segatore *et al.* (1999) conducted a comparative survey on 334 clinical isolates of *P. areuginosa* and found higher number of ciprofloxacin resistant strains with 17.1% was susceptible to ciprofloxacin. A decreased susceptibility to fluoroquinolone was observed among gram negative isolates in Taiwan after wide use of these antimicrobial agents in different study periods reported (Sheng *et al.*, 2002). In Europe antimicrobial susceptibility of isolates from 3136 bacteraemic versus17261non-bacteraemic patients was reported in MYSTIC surveillance programme and found ciprofloxacin generally exhibited the lowest activities against the most commonly isolated organisms (Unal *et al.*, 2004).

Antimicrobial susceptibility of the pathogens of bacteraemia in the UK and Ireland between 2001-2002 was determined and a resistance rate of Pseudomonas aeruginosa to ciprofloxacin was found 7% (Reynolds *et al.*, 2004). Ciprofloxacin and other fluoroquinolones against *S. aureus* shown decreased susceptibility in our study. Ciprofloxacin resistance is considerably higher among methecillin resistance *S. aureus* than among methecillin sensitive *S. aureus* as shown in table 2 and 3. Goldstein and Acer in 1995 reported wide spread emergence of decreased susceptibility to ciprofloxacin and other floroquinolone against infection with *S. aureus*. Raviglione *et al.* (1990) studied ciprofloxacin resistance in methecillin resistant *Staphylococcus aureus* in an acute care hospital and 83% resistance was observed. Lei *et al.* (2001) reported 79.4% resistance to ciprofloxacin in methecillin resistant *S. aureus*. The activity of five different floroquinolones against *S. aureus* strain (13 methecillin resistant strains and nine methecillin susceptible strains and found ciprofloxacin and norfloxacin were the least active quinolones as compare to trvafloxacin, levofloxacin and sparfloxacin was studied (Sierra *et al.*, 2002).

This study provides important information on the current resistance pattern among bacterial pathogens of our patients against floroquinolones. It can also be argued that according to the accepted selective pressure theory hypothesis that a casual relationship exist between antimicrobials use and the development of resistance. An extensive use of floroquinolones in the wards either for therapy or antimicrobial prophylaxis might have contributed to such high resistance rate.

Antimicrobial resistance continues to increase and going surveillance of microbial pathogens is essential for better patient management. This study also warrants the need of rapid laboratory detection of resistance to prevent the spread of resistance among bacterial strains.

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