ANTIMICROBIAL SUSCEPTIBILITY TESTING OF NEWER QUINOLONES AGAINST GRAM POSITIVE AND GRAM NEGATIVE CLINICAL ISOLATES

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ABSTRACT

Antibiotic resistance development is an ongoing process associated with irrational antibiotic use. WHO recommends regular surveillance programs for monitoring of antibiotic resistance. The present study is a step in this direction. A total of 124 clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were collected from different hospitals in Karachi. *In vitro* antimicrobial susceptibility studies were carried out by agar dilution method using newer quinolones that included Gatifloxacin and Levofloxacin.

It was observed that 50% (*n*=30) isolates of *Staphylococcus aureus* were resistant to gatifloxacin. Gatifloxacin was more active against *Pseudomonas aeruginosa* (*n*=23) and showing complete susceptibility with MIC 1mg/L except for three very resistant strains that shown resistance at even higher concentrations. *Escherichia coli* (*n*=45) has shown 15.5% and *Klebsiella pneumoniae* (*n*=26) 34.61% resistance to gatifloxacin.

Levofloxacin was more active against *Staphylococcus aureus* and *Escherichia coli* showing complete susceptibility at 0.5 mg /L concentration. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found to be resistant to Levofloxacin showing 36.36% and 23.08% resistance respectively.

The study strongly recommends the adherence to the antibiotic policy and regular susceptibility testing to overcome the problem associated with antimicrobial resistance.

Keywords: Agar dilution antimicrobial testing, Antibiotic resistance, Gatifloxacin, Levofloxacin, Resistance of clinical isolates

INTRODUCTION

Infectious diseases still account for 25% of all deaths and up to 45% of deaths in developing countries. Antimicrobial agents are available to combat the majority of these diseases; this window of opportunity is closing as a result of the emergence of antimicrobial resistance (Williams, 2001).

The discovery of the naphthyridine derivative nalidixic acid in the 1960's began the steady advancement of the development of Quinolones. Throughout their evolution, Quinolones have been modified to improve their pharmacokinetics, which led to less frequent dosing and higher bioavailability, and to widen their spectra of activity. The late 1990's saw development of third-generation agents, namely, sparfloxacin, levofloxacin, grepafloxacin, moxifloxacin, and gatifloxacin, which have increased gram-positive activity (Bearden and Danziger, 2001).

Fluoroquinolones antibiotics target DNA gyrase and topoisomerase IV while these enzymes are functionally attached to the DNA strand, resulting in a drug-enzyme-DNA complex in which DNA probably remains broken. Cell death apparently results from release of double-stranded DNA breaks from numerous drug-enzyme-DNA complexes throughout the chromosome (Fish, 2001).

Antibiotic resistance is a direct consequence of antibiotic use in humans. Quinolones are broad-spectrum, bactericidal antibiotics whose potent activity, including activity even against intracellular pathogens, and ease of administration (oral, parenteral), has firmly established them both in the hospital and the community. The emergence of resistance is the natural response of microbes to the presence of antimicrobials, and it is widely accepted that the greater the use of antimicrobials, the greater will be the emergence of resistance (Williams R, 2001).Gram-positive and Gram-negative bacteria have been reported to be resistant to Quinolones.

Three mechanisms of resistance predominate with Quinolones: alterations in target enzymes, bacterial cell permeability, and drug efflux mechanisms (Bearden and Danziger, 2001). Although plasmid-mediated resistance was reported, this appears to be very rare compared with

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more typical chromosomally mediated mechanisms of resistance (Fish, 2001).

MATERIAL AND METHOD

Bacterial cultures

Clinical isolates were collected from different hospital settings in Karachi, and after identification were inoculated on slants containing Muller Hinton agar and stored at temperature between 2-4°C. before testing, isolates were brought to room temperature. Culture media was used Mueller-Hinton medium (Difco, Detroit, MI, USA) and the isolates were identified and verified by conventional techniques (Forbes *et al*., 1998).

Agar dilution susceptibility test

To determine the MIC for one or more bacterial isolates, the basal media used for testing was determined on the basis of the organisms and in some cases the antibiotic to be tested (Steers *et al*., 1959).Antibiotic Concentration Range was selected according to National Committee for Clinical Laboratory Standards (NCCLS) for both the antibiotic and the organism being tested. The drug was incorporated into the media in a water bath between 45°C and 50°C, swirling the flask to mix the content thoroughly. A series of Petri plates were prepared with increasing concentration of the drug and with the aid of a multiple inoculum replicator as many as 11 different strains can be spot inoculated onto each plate. The MIC is read as the first antibiotic concentration that inhibits the growth of the organism completely.

Preparation of antimicrobial plates

A stock solution of 10,000 µ g/mL or 1,000µ g/mL for each antibiotic was prepared in the sterile doubled distilled water or other appropriate diluents being tested. One liter of Mueller-Hinton agar was prepared for each concentration of antibiotic being tested. The media was cooled in a water bath to between 45°C and 50° antibiotic was added to the liquid agar media the content was mixed thoroughly. Sufficient volumes were prepared to fill each 9cm plate with 9 ml of agar. The diluted antimicrobial solutions were added to the melted and cooled medium in a ratio of one part of antimicrobial agent to 9 part of medium for each Petri plate. Petri plates were labeled for each concentration of antibiotic being tested. The plates were allowed to solidify at room temperature. The plates were stored at 2°C-8°C. In addition to preparing antibiotic dilution plates, control plates were also prepared. These plates consisted of only the agar-based media with no antibiotic added and labeled as control.

Preparing the Inoculum

Mueller-Hinton broth tubes containing 1 mL media were inoculated with a portion of three to five distinctive, clear isolated colonies of the organism to be tested. Tubes were incubated at 35°C for 2-6 hours. The growth should reach a turbidity that is equivalent to or greater than a 0.5 McFarland standard.

Inoculation and incubation of test plates

The agar test plates were inoculated with 1-2 µl of inoculum creating a 5-8-mm spot. This gives a final inoculum amount on the agar surface of 104 CFU/mL. Using the replicator containing 11 wire loops, one for the standard and 10 for the clinical isolates, inoculate the plates.

The plates were kept at room temperature until the inoculum spots have dried; the plates were inverted and incubated at 37°C for 16-24 hours. The MIC is read as the first antibiotic concentration that inhibits the growth of the organism completely. A faint haze caused by the inoculum or a single colony should not be read as growth (Hanlon *et al*., 2007). Acquisition of resistance was defined as increase in MIC of at least 4-fold. (Hamzehpour *et al*., 1995)

RESULTS

The present work consists on a total of 124 clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* recovered from different sources (table 1). The resistance pattern of these isolates was tested by agar dilution method (Hanlon A *et al*., 2007) against two quinolone antibiotics, gatifloxacin and levofloxacin.

The present study showed that overall; Gatifloxacin was more active against most of the clinical isolates as compared to Levofloxacin. *Escherichia coli* (*n*=45) mostly recovered from UTIs has shown a total percent resistance of 5.5 % this indicates that the activity of gatifloxacin is still promising against this strain. The MIC range for *E.coli* was determined 0.25 mg/L. *Pseudomonas aeruginosa* (*n*=23) mostly recovered from blood samples

Table 1: Summary of clinical isolates included in the study

Organisms	Source of isolate	Number of isolates *n*
E.coli	Urinary tract infections UTIs	45
Pseudomonas aeruginosa	Blood samples of burn patients	23
Klebsiella Pneumoniae	Blood samples	26
Staphylococcus. aureus	Wound Pus	30
	Total isolates	124

of burn patients has acquired almost 16 % resistance against Gatifloxacin with MIC observed 4 mg/L. *Klebsiella pneumoniae* (*n*=26) from blood samples has shown almost 34.61% resistance with MIC 0.5 mg/L. In the present study, *Staphylococcus aureus* (n=30) was observed to be most resistant strain with 15 % resistance and MIC of 0.5 mg/L. Methicillin resistance was not established for *Staphylococcus aureus* (table 2).

Levofloxacin antimicrobial has shown good activity against strains of *Escherichia coli* showing resistance under 5%. The present study shows 20.08 % resistance of strains of *Pseudomonas aeruginosa* to levofloxacin. Among the strains of *Klebsiella pneumoniae*, present study shows 23.08% resistance to levofloxacin with MIC 0.5 mg/L. Almost 100% strains of *Staphylococcus aureus* were susceptible to Levofloxacin with MIC 0.5 mg/L (table 2).

DISUSSION

Microbial resistance has always been a great matter of concern for the health care system and it has been associated with inappropriate and unmonitored use of antibiotics (Jarvis, 1996; Marr *et al*., 1988). One major factor that contributes in microbial resistance has been observed to be sub optimal and irrational and extensive use of antibiotics (Knox *et al*., 2003). Quinolones are now widely prescribed antimicrobials that may result in increased bacterial resistance. There are several reports that alerts on the heavy dispensing of Fluoroquinolones over the counter that could eventually lead to increased resistance of the pathogenic bacteria to these drugs (Al-Ghamdi MS, 2001; Naqai *et al*., 2001). In the present study, *Escherichia coli*, which were recovered from urinary tract infections, have shown susceptibility to gatifloxacin. (Shailaja *et al*., 2004) reported gatifloxacin and ciprofloxacin to be equally effective against

Table 2: MIC and percent resistance of Gatifloxacin and Levofloxacin against clinical isolates in Karachi

Antimicrobial	Bacterial Organism (*n*)	MIC Conc.(mg/L)		MIC Conc. (mg/L) observed	% Resistance
		S	R		
Gatifloxacin	*E.coli* (*45*)	≤2	≥8	0.25	5.5 %
	Pseudomonas aeruginosa.(*23*)	≤2	≥8	1.0	15.5%
	Klebsiella Pneumoniae(*26*)	≤2	≥8	0.25	34.62 %
	Staphylococcus. aureus(*30*)	≤0.5	≥2	1.0	15 %
Levofloxacin	*E.coli* (*45*)	≤2	≥8	0.12	4.44%
	Pseudomonas aeruginosa.(*23*)	≤2	≥8	4.0	20 %
	Klebsiella Pneumoniae(*26*)	≤2	≥8	0.5	23.08 %
	Staphylococcus. aureus(*30*)	≤1	≥4	0.5	23.08 %

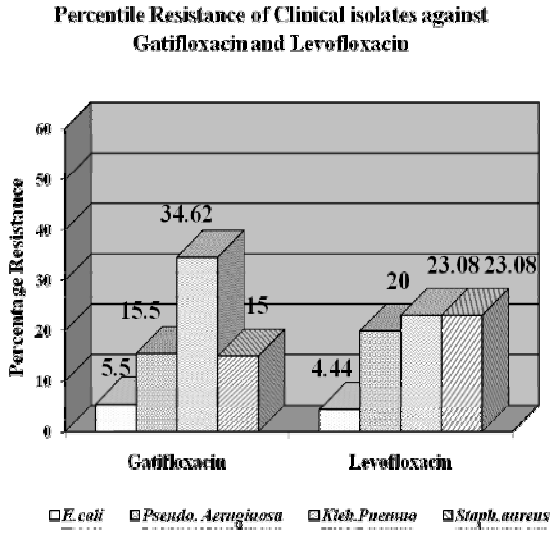

Fig. 1: Percentile Resistance of Clinical isolates against Gatifloxacin and Levofloxacin

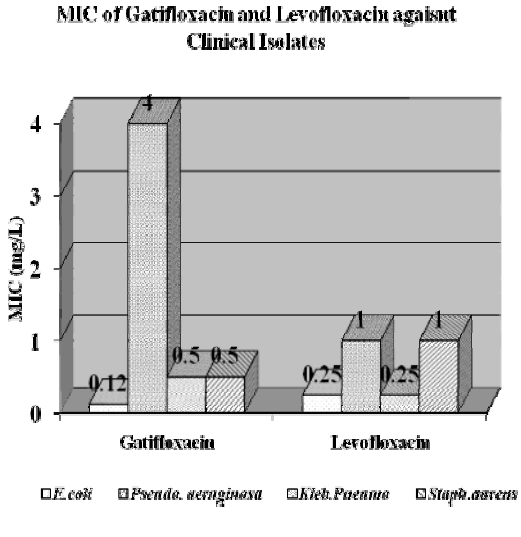

Fig. 2: MIC of Gatifloxacin and Levofloxacin against Clinical isolates

Pseudomonas aeruginosa and the present study was also supported by the work of Kowalski (Kowalski *et al*. 2003).

For *Escherichia coli*, levofloxacin has been a good antibiotic which was observed in the present study and was in strong agreement with the published reports (Drago *et al*., 2001; Weber *et al*., 2005; Matsuzaki *et al*., 1999). Good activity of Fluoroquinolones against many Gram-negative microorganisms, including *Pseudomonas aeruginosa* was reported (Algun *et al*., 2004) however; resistance to these antibiotics had been also reported in recent years as well. (Polk *et al*., 2004) reported that rates of Fluoroquinolone-resistant *Pseudomonas aeruginosa* increased from 29% in 1999 to 36% in 2001. Both community and hospital Fluoroquinolone use were predictive of rates of Fluoroquinolone-resistant *Pseudomonas aeruginosa*. Studies have shown good activity of Levofloxacin and ofloxacin against staphylococcal strains (Mascellino *et al*., 1998) compared with the majority of other antibiotics. Kowalski *et al*., (2003) studied that the fourth-generation Fluoroquinolones had increased susceptibility for *Staphylococcus aureus* isolates. Rolston *et al*., (2004) have reported a good activity of gatifloxacin on isolates obtained from cancer patients while the same antibiotic has shown moderate activity against staphylococcus aurueus, in the present study staphs has been observed to be moderately resistant with 15 % resistance at 1 mg /L MIC and *Klebsiella pneumoniae* has acquired moderate resistance against both the antibiotics. One such study have shown good activity of gatifloxacin and moderate activity of levofloxacin against gram positive MRSA *staphylococcus aureus* and gram negative *Pseudomonas aeruginosa* specie (Blondeau *et al*., 2000). These results suggested that the newer quinolones were promising antimicrobial agents against various strains of gram positive and gram negative bacteria, however, rational, appropriate and optimal use and monitoring programs for its sensitivity should be conducted regularly in order to control over the emergence of its resistance.

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