RESISTANCE PATTERN OF URINARY TRACT INFECTION BACTERIAL ISOLATES TO SELECTED QUINOLONES

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ABSTRACT

The Quinolones inhibit bacteria by interacting with DNA topoisomerases (gyrases) of which four subunits (two A and B monomers) have been identified thus, inhibiting bacterial DNA gyrase. High level resistance to quinolones can be produced by serial exposure of bacteria to subinhibitory concentration. A Total of 408 suspected UTI and high vagina swab (HVS) samples were examined for bacteria and the isolates obtained tested against the newer guinolones. Prevalence of Bacterial isolates revealed Escherichia coli 110(92%) as the most isolated organism from urine, while Staphylococcus aureus 31(32%) was the most isolated species from HVS samples. Bacterial species such as coliforms 55(70%) and Klebsiella spp 42(84%), equally had high prevalence rate in urine samples. Pseudomonas aeroginosa 19(66%) was next to Staphylococcus aureus in terms of prevalence of isolated strains from HVS samples. The resistance pattern observed for these isolates, showed that the strains were least resistant to Ciprofloxacin, followed by Ofloxacin and Perfloxacin, while they were most resistant to Nalidixic acid. There was statistical significance however no (P<0.001) between the use of Ofloxacin and Perfloxacin, however. ANOVA showed a significant difference (P<0.05) between the pattern of Klebsiella spp resistance against Perfloxacin when compared to Proteus vulgaris.

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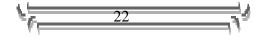
Keywords: Bacterial resistance, Quinolones.

INTRODUCTION

The introduction of Nalixidic acid in 1962, began the history of the newer 4quinolone antibacterial agents. The clinical significance of these drugs is based on their broad antibacterial spectrum, unique mechanism of action, good absorption from the gastrointestinal tract, excellent tissue distribution as well as low incidence of adverse reaction¹.

Recent studies indicate that the mechanism of action of these drugs is the inhibition of DNA topoisomerases (gyrases), thus inhibiting the bacterial synthesis^{2,3}. The drugs DNA are bacteriocidal. with а single most bacteriocidal concentration and in greater or lesser concentrations, kill few bacteria.⁴. This Paradoxical effect of decreased killing at higher concentrations is most likely the result of dosedependent inhibition of DNA synthesis⁵.

Antimicrobial Activity: Nalixidic acid has greater antibacterial activity against Gram negative rods than Gram positive bacteria. It is active against most strains of *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* spp and other Coliforms at concentrations easily achieved in the



urine i.e. 16pg/ml or lower ⁶ Gram positive organisms like *Staphylococcus aureus*, *Streptococcus pneumonia* and *Streptococcus faecalis* are resistant to Nalixidic acid.⁷

Norfloxacin which is 100 times more active compared to Nalixidic acid, has a spectrum that includes entrococci. Staphylococci and Pseudomonas species.⁸ It is active against most Gram positive and Gram negative bacteria implicated in UTI at concentrations easily attained in the urine. Norfloxacin is active against Haemophilus influenza, Neisseria gonorrhoea, regardless of beta-lactamase activity. However, it is less active against methicillinresistant strains of Staphylococcus aureus. 9,10

Ciprofloxacin is said to be more potent than Norfloxacin, and is also active against most Gram positive and Gram negative infectious bacteria at concentrations easily attained in most tissues and body fluids.¹¹. Ciprofloxacin has excellent activity against Chlamydia trachomatis and genital Mycoplasma, inhibiting 90% of isolates at 1ug/ml 13,14 12, concentrations. However, reduced susceptibility of these Ciprofloxacin organisms to occurs following exposure the serial of organisms subinhibitory to drua concentrations, this invariably may lead to cross resistance to other guinolones by the organisms¹⁴.

Though, exact mechanism of bacterial resistance to the quinolones is unknown, it may occur when serine in position 83 of subunit A is replaced by trypophan ¹⁵. Or when inhibition of topoisomerase 1V interfere with replicated chromosomal DNA ¹⁶. Finally, it could be due to a mutation in the gene coding for DNA gyrase or a mutation that alters the bacteria's outer-membrane porins ¹⁷.

In this work, we identified and assessed the prevalence of bacteria in UTI as well as determined the current trend in the resistance pattern of the bacteria isolates to the selected quinolones.

MATERIALS AND METHODS

Specimen:

A total of 408 clinical specimens comprising of 305(75.25%) urine samples and 103(25.75%) of high vaginal swab (HVS) samples. All specimen were transported to the laboratory and were processed within 2hours of collection.

Isolation and identification:

The specimens were innoculated onto Nutrient agar, Blood agar and MacConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37°C for 24 hours. After 24 hours of incubation, discrete colonies were picked up and Gram stained and further subculturing was done to obtain pure cultures and biochemical tests carried out.

Antimicrobial Susceptibility Testing

This was done by the disc diffusion method. All isolates were subjected to testing using Nalidixic acid, Perfloxacin, Ciprofloxacin and Ofloxacin.

Staphylococcus aureus, Oxford stain NCTC 6751 was used as control for Gram-positive organisms while *Escherichia coli* NCTC 10418 was used as control for Gram negative organisms.

The results of the susceptibility test were interpreted as sensitive, intermediate or resistant, using the criteria below:

A zone's radius equal to or not more than 3mm smaller than the control was reported as sensitive.



A zone's radius more than 3mm smaller than the control but not less than 3mm was reported as intermediate or moderate.

A zone's radius of 2mm or less (i.e., no zone of inhibition) was reported as resistant.

A total of 408 suspected UTI and vaginitis samples were analysed during this study. Of these samples 305(75.25%) were urine samples and 103(25.75%) were high vagina swab (HVS) samples. Males contributed 156(51.51%) of the urine samples while a total of 103(100 *L) HVS samples were collected from females 18years and above.

RESULTS

Table 1: Prevalence of Bacterial Strain	Obtained From Urine and HVS Samples
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Destarial Studius	No of Isolates obtained from			
Bacterial Strains	Urine Samples	HVS Samples		
Escherichia coli	110(92%)	9(8%)		
Staphylococcus aureus	67(68%)	31(32%)		
Coliforms	55(70%)	23(30%)		
Klebsiella spp	42(84%)	89(16%)		
Proteus mirabilis	18(60%)	21(40%)		
Pseudomonas aeroginosa	10(34%)	19(66%)		
Proteus vulgaris	3(100%)	0(0%)		
Total	305(75.25%)	103(25.75%)		

Table 1.shows the prevalence of bacterial strains obtained from both urine and HVS samples. *Escherichia coli* has the highest prevalence among the bacterial isolates, with 119 samples. Of this total, 110(92%) were isolated from urine and 9(8%) was isolated from

HVS samples. *Staphylococcus aureus* were isolated in 98 samples, 67(68%) from urine samples and 31(32%) from HVS samples. *Klebsiella* species were isolated were isolated from 50 samples 42 from urine sample (84%) and 8 from HVS samples (16%).



Bacterial Strains	No (%) Resistance to the Quinolone				
	n	PER	CRP	OFX	NA
Escherichia coli	119	30(252%)	37(31.1%)	28(23.5%)	45(37.8%)
Staphylococcus aureus	98	16(16.3%)	8(8.2%)	8(8.2%)	37(37.6%)
Coliforms	78	18(22.8%)	6(7.6%)	18(22.8%)	45(60.0%)
Klebsiella spp	50	10(20.0%)	10(20.0%)	8(16.0%)	22(44.0%)
Proteus mirabilis	30	5(16.7%)	2(6.7%)	7(23.3%)	5(16.7%)
Pseudomonas aeroginosa	29	6(20.7%)	0(0.0%)	9(31.0%)	9(31.0%)
Proteus vulgaris	3	2(66.6%)	0(0.0%)	0(0.0%)	1(33.3%)

Table 2: The Susceptibility Pattern of Bacterial Isolates and the Percentage (%) Resistance

 to the Selected Quinolones

Key:

n = number of strains tested

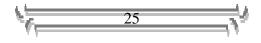
PEF	=	Perfloxacin
CRP	=	Ciprofloxacin
OFX	=	Ofloxacin
NA	=	Nalixidic acid.

Table 2 shows the selected quinolones used and the isolates susceptibility pattern. *Escherichia coli* had 30 strains resistant to perfloxacin, 37 were resistant to Ciprofloxacin, 28 and 45 isolates were resistant to Ofloxacin and Nalixidic acid. Nalixidic acid unarguably is the least sensitive of the selected quinolones, virtually all other bacterial isolates showed increased resistance to Nalidixic acid than the other selected quinolones.

Table 3: In-vitro Antimicrobial Activity of the Selected Test Quinolones against Isolated

 Bacterial Species.

Bacterial Strains -	Mic(µg/ml)			
	n	PER	CRP	
 Escherichia coli	0.039	0.250	0.125	5.000
Staphylococcus aureus	0.250	0.250	0.039	5.00
Coliforms	0.063	0.500	0.500	5.000
Klebsiella spp	0.250	0.500	0.250	10.000
Proteus mirabilis	0.250	0.500	0.250	10.000
Pseudomonas aeroginosa	0.500	5.000	5.000	10.000
Proteus vulgaris	0.063	0.250	0.250	0.500



In table 3, the minimum inhibitory concentration (MIC) in µg/ml of *Escherichia coli* to Nalixidic acid was observed to be 5.0µg/ ml, 0.059µg/ml to Perfloxacin, 0.250µg/ml and 0.125µg/ml to Ciprofloxacin and Ofloxacin respectively.

Ofloxacin was observed to be a more potent for *Staphylococcus aureus* with the organisms having a MIC of 0.039 to Ofloxacin, and 0.250µgml to both Perfloxacin and Ciprofloxacin respectively and 5.00µg/ml to Nalixidic acid.

DISCUSSION

The selected guinolones proved to be of chemotherapeutic value against the 408 samples analysed in the cause of this work. Some strains however were resistant. This work agrees with other similar works, especially with respect the incidence rates of Escherichia coli. Escherichia *coli* had earlier been reported as being the most prevalent implicated UTI^{18} . organism in Escherichia coli was observed in this work to have 29.17% prevalent rate of 24.02%. These prevalent rates makes Escherichia coli and Staphylococcus aureus the two most implicated organism in UTI, as well as the most prevalent Gram-negative and Grampositive organisms respectively.

Nalixidic acid was seen to be the least potent of the selected quinolones. However, there is no statistical significance (p< 0.001) between the use of Ofloxacin and Perfloxacin in the treatment of the isolated strains.

The pattern of *Klebsiella* spp resistance against Perfloxacin when compared to *Proteus vulgaris* was significant. In the same manner, the analysis of variance between the pattern of resistance against Perfloxacin shown by *Pseudomonas aeruginosa* compared to *Proteus vulgaris* was significant.

CONCLUSION

This study has been able to show that there is no real difference in therapeutic valve between the selected quinolones, safe for Nalixidic acid, which seen to have a wide range of resistance among the isolated organisms.

However, it is a first generation quinolone, compared to the other three which are second generation drugs.

The study has also shown that Gram-negative organisms, especially *Escherichia coli* are predominantly responsible for UTI, and *Staphylococcus aureus* is the most prevalent Grampositive organisms implicated in UTI, although other Gram-positive organisms like *Streptococcus* species could be implicated.

It is hoped that findings from this study would be of help in:

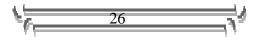
Monitoring antimicrobial susceptibility, to ensure informed usage of antibiotics and usefulness of these drugs for longer period of time.

Assist in effective management of UTI cases.

Give a guide-line in choice of drug when certain related organisms are implicated in a UTI.

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