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Comparative *in vitro* potency of four fluoroquinolones on clinical isolates over a year period

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This study aimed at evaluating the efficacy of four common fluoroquinolone drugs over a year period on some clinical isolates. It also aimed at comparing statistically the average effects of each drug on the isolates. Five different clinical samples (urine, sputum, wound, blood and high vaginal swab [HVS]) from patients attending a university medical centre (between June 2011 and May 2012) were analysed for the purpose of bacteria isolation. The isolates were tested with commonly used flouroquinolones: pefloxacin (30 µg), ofloxacin (30 µg), sparfloxacin (10 µg), and ciprofloxacin (10 µg). Each sensitivity test was done in duplicate and a mean average of zone of inhibition was recorded. One hundred and eighty eight bacteria were isolated: *Staphylococcus aureus* (44.7%), *Streptococcus pyogenes* (6.4%), *Escherichia coli* (28.2%), *Pseudomonas aeruginosa* (8.5%), *Klebsiella pneumonia* (8.0%), and *Proteus mirabilis* (4.3%). All drugs were equally potent against the isolates, but a higher potency was seen in ofloxacin against *P. mirabilis*. The fluoroquinolones are a group of broad spectrum drugs effective in clinical cases. Their efficacy should be preserved by ensuring strict compliance to local drug policies.

Key words: Clinical, fluoroquinolones, efficacy.

INTRODUCTION

The health and well-being of human populations relies, in large, on the control of communicable diseases, as well as the availability of efficient and potent drugs for treatments of such diseases. Infectious diseases continue to take a toll, especially in most developing countries, accounting for nearly 50% of all deaths. The introduction of antimicrobial agents in the early 20th century brought a great relief in medicine; however, this relief was not to endure a while, especially due to the indiscriminate and uncontrolled use of these agents. Resistance, an unfriendly term in medicine, became a global problem when frequently used antimicrobials in human and veterinary medicine were observed to be impotent against known bacterial infections (Smith, 1999). Serious infections, notably in hospitals and other health care facilities are associated with the emergence of antibiotic-resistant organisms, and these organisms appear to be biologically competent to cause serious threat to life (Schwartz et al., 1997; Spellberg et al., 2008; Mulvey and Simor, 2009; Sibi et al., 2011; Taddele et al., 2012). The principal area of concern to this has been the increasing emergence of resistant phenotypes in both clinically relevant strains and normal commensal microbiota (Chikwendu et al., 2008).

Fluoroquinolones, as a class of drugs, have gained some importance during the last two decades because of their potent antibacterial activity against wide varieties of Gram positive and Gram negative pathogenic bacteria

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Table 1. Isolates per clinical sample.

Isolate	Urine	Sputum	Wound	Blood	HVS	Occurrence (%, n = 188)
E. coli	36	-	08	-	09	28.2
S. aureus	52	04	-	08	20	44.7
P. aeruginosa	04	04	08	-	-	8.5
K. pneumoniae	08	-	07	-	-	8.0
P. mirabilis	04	-	-	-	04	4.3
S. pyogenes	04	-	04	-	04	6.4

with minimum toxic side effects and a different mechanism of action than other available antibacterial drugs (Talah and Gadad, 2006). To date, many fluoroquinolones have been introduced into clinical use with significant improvement in antibacterial spectrum and activity, thus forming an invaluable part of the present anti-infective armoury of the clinicians. This group of drugs are increasingly being used in both the hospital and community sectors to treat a broad range of infections (Bhanot et al., 2001). However, increased use has led to recent emergence of fluoroquinolone-resistant bacteria which has necessitated the search for newer drugs worldwide (Shindikar and Viswanthan, 2005; Foroumadi et al., 2006). Along with known mechanisms of resistance is the presence of fluoroquinolone resistant proteins (Qnr), codified by transmissible genes by means of plasmids, especially in *Enterobacter* species, *Escherichia coli,* and *Klebsiella pneumonia* (Luzzaro, 2008). Additionally, new specific resistance mechanisms have been described. AAC(6')-lb-cr represents the first enzyme able to inactivate, by acetylation, antimicrobials of two different classes, aminoglycosides and fluoro-quinolones; and an efflux-pump plasmid-mediated, codified by the QepA gene, acts as a selective mechanism (Luzzaro, 2008). In an over nine years of study, Adam et al. (2009) identified a significant strong relationship between increase in fluoroquinolone usage and rise in ciprofloxacin resistance in *Streptococcus pneumonia* from 0 to 4.5% in children (0 to 15 years), 0.2 to 5.4% in adults (16 to 64 years), and 1.4 to 11.6% in the elderly (≥65 years). In the last several years, resistance to fluoroquinolone has remained very high among methicillin-resistant *Staphylococcus aureus* (MRSA) strains in intensive care unit (ICU) patients, and it has increased among nosocomial isolates of *K. pneumonia, Serratia marcescens* and *Pseudomonas aeruginosa.* More worrisome are reports of an overall increase in resistance to fluoroquinolone among bacteria of community-acquired infections such as *E. coli, Salmonella* species, *Campylobacter* species and *Neisseria gonorrhoeae* (Acar and Goldstein, 1997). A research carried out in Imo State, Nigeria reports a high occurrence of resistance to ciprofloxacin in *S. aureus* isolated from medical samples (Ugbogu et al., 2007). In the same vein, Lamikanra et al. (2011) confirmed that the increase and uncontrolled use of fluoroquinolones paved way for resistance among *E. coli* in Nigeria. However, Olufunmilola et al. (2012) established the efficacy of fluoroquinolones in the treatment of typhoid fever in Ibadan despite evidence of emerging resistance. This study aimed at evaluating the efficacy of four common fluoroquinolone drugs over a year period on some clinical isolates.

METHODOLOGY

Five different clinical samples (urine, sputum, wound, blood and high vaginal swab [HVS]) from patients being attended to at a university medical centre (between June 2011 and May 2012) were analysed for the purpose of bacteria isolation on CLED, Mannitol salt, McConkey, EMB and Nutrient agars. A total of one hundred and twenty eight samples in all were analysed; thirty two samples per quarter. Isolates were characterised and identified in reference to Cowan and Stell (1993).

All isolates, suspended in normal saline at a density in comparison to 0.5 McFarland standard were subjected to antibiotic sensitivity test using disc diffusion method on Mueller Hinton agar (Oxoid, UK) (CLSI, 2006). The isolates were tested with commonly used fluoroquinolones; pefloxacin (30 µg), ofloxacin (30 µg), sparfloxacin (10 µg), and ciprofloxacin (10 µg). Each sensitivity test was done in duplicate and a mean average of zone of inhibition was recorded.

Statistical analysis

Analysis of variance (ANOVA) was applied to compare the average effect of the four fluoroquinolones on all isolates at 5% significant level.

RESULTS

From all the samples analysed, a total of one hundred and eighty-eight bacteria was isolated. Ninety six (96) were Gram positive isolates, while ninety two (92) were Gram negative isolates. Isolates were mostly of six species; *S. aureus* (44.7%), *Streptococcus pyogenes* (6.4%), *E. coli* (28.2%), *P. aeruginosa* (8.5%), *K. pneumonia* (8.0%), and *Proteus mirabilis* (4.3%). Urine samples had the highest number of isolates at 57.5%, while sputum and blood samples equally yielded the least number of isolates at 4.3% (Table 1 and Figure 1).

The antibiotic sensitivity test (AST) for the period of the study showed varying sizes of zone of inhibition in

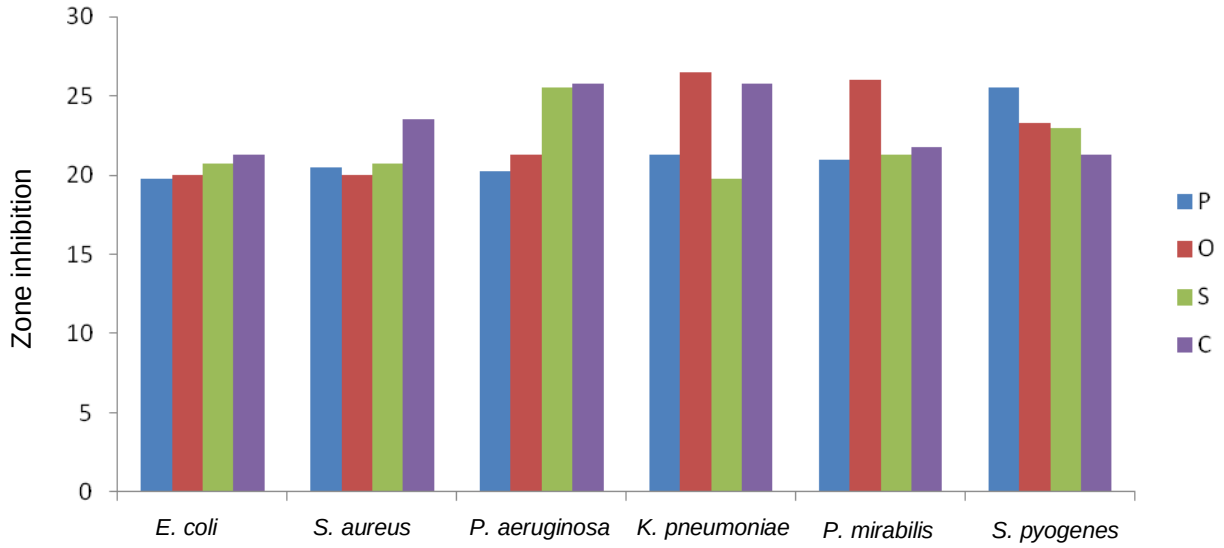

Figure 1. Overall average zone of inhibition. P: Pefloxacin; O: ofloxacin; S: sparfloxacin; C: ciprofloxacin.n

Table 2. The overall average zone of inhibition in millimetres (mm).

Isolate	P	O	S	C
E. coli	21.3	21.6	23.1	20.4
S. aureus	22.6	23.1	23.8	22.6
P. aeruginosa	21.9	22.0	22.2	23.6
K. pneumoniae	24.4	23.1	19.4	22.5
P. mirabilis	21.3	25.6	22.3	22.5
S. pyogenes	22.0	22.8	22.6	21.4

P: Pefloxacin; O: ofloxacin; S: sparfloxacin; C: ciprofloxacin.

millimetre (mm). The least zone of inhibition was 13 mm, recorded in the first quarter by sparfloxacin against *P. aeruginosa*, and in the fourth quarter by pefloxacin and sparfloxacin against *S. aureus* and *K. pneumonia*, respectively. The largest zone of inhibition recorded was at 35 mm in the first quarter by ciprofloxacin against *S. aureus* and in the second quarter by ciprofloxacin and pefloxacin against *K. pneumonia* and *E. coli*. The overall average zone of inhibition by each drug on each of the six isolates tested is as shown in Table 2 and Figure 2.

Statistical analysis showed that the average effect of all four drugs in comparison had no significant difference at 5% significant level against *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, and *K. pneumonia.* However, there was a significant difference in the average effects of the drugs on *P. mirabilis.* Multiple comparisons (using LSD test) showed that ofloxacin had a significant effect at 5% significant level on *P. mirabilis* unlike other drugs.

Of all the samples analysed, urine consistently had all test isolates analysed in this study. Independently of other sample isolates, statistical analysis of the average effect of the drugs on the urine isolates showed no significant difference at 5% significant level.

DISCUSSION

The six species isolated in this study are very common clinical isolates implicated in various clinical diagnoses. In virtually all these isolates, resistance to the first line drugs have been reported in different research works, which may have prompted the use of fluoroquinolones in empirical treatments. Many research works have been published in relations to resistance in these bacteria against the fluoroquinolones both from in-patients and out-patients. The most commonly prescribed of the fluoroquinolones is ciprofloxacin. Resistance to this drug was discovered in the mid-1990s, and it increased slowly from 1.2% in 1998 to 2.5% in 2001 (Kalowsky et al., 2002). The North American Urinary Tract Infection Collaborative Alliance (NAUTICA) study revealed that ciprofloxacin resistance increased to 5.5% in 2004 (Zhanel et al., 2006). Uropathogens studied between the years 1996 and 2009 in the province of British Columbia demonstrated an increase in fluoroquinolone resistance. The resistance rates in *E. coli* and *K. pneumoniae* increased from <2% in 1996 to ≥20% in 2009; the resistance rates of fluoroquinolones for *P. mirabilis*

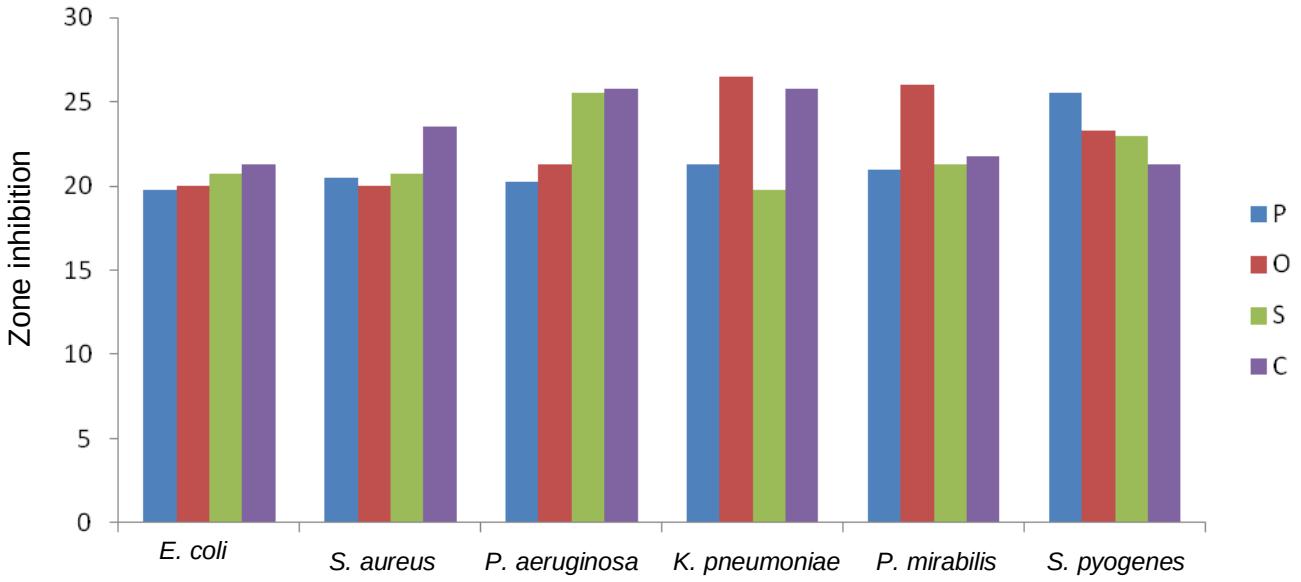

Figure 2. Overall average of drugs on urine isolates only. P: Pefloxacin; O: ofloxacin; S: sparfloxacin; C: ciprofloxacin.

remained almost constant throughout the years at ≤2%. *Enterococci* demonstrated frequently resistance against fluoroquinolones although resistance rates decreased between 2002 and 2009 (AMR Report, 2009). The Antimicrobial Resistance Epidemiological Survey on Cystitis (ARESC) study revealed that in uropathogens collected in nine European countries and Brazil from 2003 to 2006 ciprofloxacin resistance in *E. coli* was recorded in >10% of all the isolates in Brazil, Spain, Italy, and Russia; in the remaining European countries, ciprofloxacin resistance ranged from 1.4% in France to 6.7% in Poland (Naber et al., 2008; Schito et al., 2009; Neuzillet et al., 2012). Though extended spectrum beta-lactamase production was not verified in our isolates, it has been however reported that increase to fluoroquinolone resistance is aided by its production (Azap et al., 2010). Increasing fluoroquinolone resistance in pneumococci paralleled increased usage of fluoroquinolones in general or 2nd generation quinolones in particular (Chen et al., 1999; Waites and Brown, 2003; Bhavnani et al., 2005; Pletz et al., 2011). Occasionally, fluoroquinolone resistance resulted in clinical failures in patients with pneumococcal pneumonia having been previously treated empirically with oral fluoroquinolones (Ho et al., 1999; Urban et al., 2001; Davidson et al., 2002; Pottumarthy et al., 2005; Fuller and Low, 2005). In total, there were 20 ciprofloxacin and levofloxacin treatment failures reported till January 2005 and reviewed by Fuller and Low (2005). Susceptibility testing of *P. aeruginosa* isolates from cystic fibrosis (CF) patients revealed that ciprofloxacin resistance in Europe ranged from 13.7% in Bulgaria (Strateva et al., 2009) to approximately 30% in the UK, Spain, Germany, and Italy (Schulin, 2002; Pitt et al., 2003; Morosini et al., 2005; Manno et al., 2005); 37.4% of the US isolates were ciprofloxacin-resistant (Burns et al., 2000). One of the most important features of bacterial resistance to fluoroquinolones is the ability to accumulate several mutations, affecting both DNA gyrase and bacterial permeability and resulting in strains associated with very high MICs (e.g. MICs of ciprofloxacin of 32 to 1,024 sg/ml). Such strains have been observed among isolates of *S. aureus,* Enterobacteriaceae species, and *P. aeruginosa* (Truong et al., 1995; Lehn et al., 1996). Widely varying percentages of resistance to fluoroquinolones have been associated with particular bacterial species, clinical settings, origin of strains, geographic locations, and local antibiotic policies (Acar and Goldstein, 1997). The continued increase in fluoroquinolone resistance rates affects patient management and necessitates a change in some current guidelines for the treatment of, for example, urinary tract infections (Peterson, 2004; Han et al., 2010; Wagenlehner et al., 2011).

Conclusion

Although all fluoroquinolones used in this study showed potency against the clinical isolates for the period of study, this does not negate the need for periodic monitoring of the efficacy of these drugs, as well as strict compliance to drug usage policies.

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