

## Current Trend in Antimicrobial Resistance of *E.coli* to Fluoroquinolones: A Comparative Study of Four Years

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### Abstract

Fluoroquinolones are being extensively used for the treatment of OPD patients suffering from various infective diseases. Extensive use of these of drugs has resulted in high drug resistance among common pathogens. We analyzed the current antibiotic resistance pattern of *E.coli* to fluoroquinolones for four consecutive years. 9186 samples were processed and 716 *E.coli* were isolated. Antimicrobial susceptibility testing was done in accordance with latest Clinical and Laboratory Standards Institute (CLSI) guidelines. 6644 were urinary samples followed by blood (1321), pus ((704), and miscellaneous (517). Maximum positivity was seen in pus samples (43.18%, 308/704) followed by miscellaneous (18.56%, 96/517), blood (18.01%, 238/1321). Antibiotic resistance trend, during 2016, revealed least resistance against levoflox (12%) followed by gatiflox (13%) and moxifloxacin (28%). Maximum resistance was seen against norflox (42%) and ciproflox (40%). Nitrofurantoin showed minimum resistance (8%) among all. We observed a pattern of decreasing resistance against fluoroquinolones for *E.coli* during the study period.

**Keywords:** Fluoroquinolones, *E.coli*, OPD patients.

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### INTRODUCTION

Fluoroquinolones are being extensively used for the treatment of OPD patients suffering from various infective diseases. Fluoroquinolones have been very patient friendly. Extensive use of these of drugs has resulted in development of drug resistance among common pathogens. Fluoroquinolones comprise a large and ever expanding group of synthetic antibiotics. Nalidixic acid, first among this, was synthesised in 1962. Many more have been developed since then. All have a common chemical structure 1, 8 naphthyridone or quinoline derivatives. Changes to various parts of molecules confer different properties and variation in antibacterial activities. It is difficult to classify them accurately due to varied properties. According to their antibacterial activity and spectrum, four broad groups are recognised. Commonly used fluoroquinolones are summarised in Table-1 along with their groups [1]. Bacterial topoisomerases are responsible for supercoiling of DNA in bacterial cells. One topoisomerase, DNA Gyrase, is a tetramer and have two pairs of  $\alpha$  and  $\beta$  sub units. Chromosomal mutations in the quinolone resistance-determining region (QRDR) of *gyrA* and *gyrB* which encode DNA gyrase subunits and *parC* and *parE* which encode topoisomerase IV

units are responsible for drug resistance in *E.coli*. Plasmid mediated quinolones resistance (PMQR) genes have been reported in GN bacteria including *E.coli*. This however causes low level of resistance and does not lead to MICs exceeding the breakpoints. Nalidixic Acid and other Fluoroquinolones act by binding  $\alpha$  sub unit of this topoisomerase, although another enzyme, topoisomerase IV is also targeted. Indeed topoisomerase IV seems to be main target in Gram positive organisms [2]. These Fluoroquinolones have played important role in decreasing illness and death associated with infectious diseases. Fluoroquinolones have been used in past for the treatment of upper and lower respiratory infections, urinary tract infections, acute shigellosis, enteric fever, mild to moderate ear infections and other common infections. These have been used as one of the main drug outpatient management. Irregular and off the counter issue of fluoroquinolones have led to marked drug resistance among pathogenic and commensal bacteria. Thus, selective pressure exerted by drug use has been the main cause for emergence of drug resistance. There are mainly four mechanism of drug resistance operating in various bacterias. These are: (1) Mutation at the level of the gene targets (*gyrA*, *gyrB*, *parC*, *parE*), (2) Decreased

cellular uptake and or active expulsion/efflux, (3) Protection of DNA gyrase by plasmid encoded pentapeptides, (4) Inactivation of fluoroquinolones (rare). *E.coli* and *Klebsiella* are the two commensal of intestine which are responsible for hospital acquired infections both in wards and critical care setting. *E.coli* is sometimes used as a sentinel for monitoring antimicrobial drug resistance in faecal bacteria because it is found more frequently in a wide range of hosts and easily acquires resistance [3]. It is also a reliable indicator of resistance in salmonellae [4]. A retrospective analysis of *E.coli* from urine specimens collected from patients during 1997-2007 showed an increasing resistance trend for ciprofloxacin, trimethoprim/sulfamethaxazole, and amoxicillin/clavulanic acid [5]. Similarly a 30 year (1979-2009) follow up study on *E.coli* in Sweden showed an increasing resistance trend for ampicillin, sulphonamide, trimethoprim, and gentamicin [6]. 12-18% resistance were observed by Sreela S Namboodiri *et al.*, in their study in Accra Ghana [7]. Resistance pattern of *E.coli* strains varies considerably from one region to another region. Hence, there is a constant need to study and analyse the antibiotic sensitivity pattern in any health care institution for better care. The aim of over study was to analyse the antibiotic sensitivity pattern of *E.coli* against commonly used three groups of fluoroquinolones. Nalidixic acid is not much in use hence not being included in our study. Nitrofurantoin, though not a quinolone, but is commonly used as therapeutic agent for urinary infections in OPD hence it is being tested simultaneously and studied here. This study was carried out at a 550 bedded zonal hospital located in northern India.

## MATERIAL AND METHODS

The present study was carried out by analysing the data of all the clinical isolates of *E.coli* from March 2012 to March 2016 at a zonal hospital from OPD cases. A total of 9186 samples were processed, and directly inoculated on Blood agar, Mac Conkey Agar,

CLED and BacT/ALERT (Biomérieux) culture bottles by using standard culture protocols. Isolates were identified by using gram staining, morphology and biochemical characters. Antimicrobial susceptibility testing was done in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines by the Kirby-Bauer disk diffusion methods using norflox (10µg), ciprofloxacin (5µg), levoflox(5µg), gatiflox(5µg), gemiflox (5µg), moxiflox(10µg) and nitrofurantoin(300µg), (Hi-Media Laboratories). These isolates were simultaneously processed by Vitek 2 System (Biomérieux) which gave sensitivity results of relevant antibiotics along with MIC values. *E.coli* ATCC25922 was used as quality control strain.

## RESULTS

Out of 9186 samples, 6644 were urinary samples followed by blood (1321), pus ((704), and miscellaneous (517) that included various body fluids, tracheal aspirates, CSF etc. Culture positivity varied considerably. Maximum positivity was seen in pus samples (43.18%, 308/704) followed by miscellaneous (18.56%, 96/517), blood (18.01%, 238/1321). Urine had minimum positivity in spite of being in maximum numbers (13.13%, 873/6644) (Table-2). A total of 716 *E.coli* strains were isolated and processed giving a prevalence of 7.79% (716/9186). Maximum numbers of *E.coli* were isolated from urine (633) followed by miscellaneous (47), pus (18) and blood (18). Latest status of antibiotic resistance pattern depicted in Table-3. Among fluoroquinolones least resistance (12%) was seen against levoflox followed by gatiflox (13%) and moxifloxacin (28%). Maximum resistance was seen against norflox (42%) and ciproflox (40%) during 2016. Nitrofurantoin showed minimum resistance (8%) among all. Further, we analysed the last four year pattern of antibiotic resistance (Figure-1). In our study we observed a pattern of decreasing resistance against fluoroquinolones for *E.coli*.

**Table-1: Classification of Fluoroquinolones**

Group 1: Compound with narrow antibacterial spectrum directed mainly against Enterobacteriaceae: Nalidixic Acid, Oxolinic Acid.
Group 2: Fluoroquinolones (fluorine at 6- positions), which exhibit potent activity against Gram negative bacilli including <i>Ps.aeruginosa</i> and many Gram positive bacteria excluding streptococcus pneumoniae: Norfloxacin, Ciprofloxacin, Ofloxacin, Pefloxacin.
Group 3: Compounds with increased activity against <i>Str pneumoniae</i> and <i>Staph. aureus</i> : Levofloxacin, Gatifloxacin, Sparfloxacin.
Group 4: Compound with properties similar to those groups 2 and 3 and additional activity against anaerobes: Moxifloxacin, Gemifloxacin, Sitafloxacin.

**Table-2: Culture positivity among various samples**

Types of samples	Total samples	Positive (%)	Number of <i>E.coli</i> isolates
Pus	704	308 (43.75%)	18
Urine	6644	873 (13.13%)	633
Blood	1321	238 (18.01%)	18
Miscellaneous	517	96 (18.56%)	47

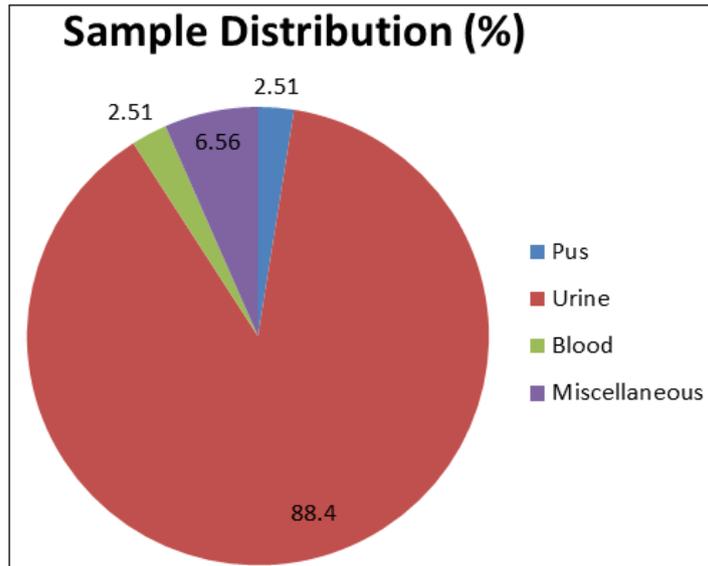


Fig-1: Sample Distribution (%)

Table-3: Antibiotic resistance pattern (%) in various studies

Antibiotics	Current 2016	Shakti <i>et al</i> 2009-13 <sup>10</sup>	Nirjan V & Malini M 2014 <sup>8</sup>	S.Chaudhary 2014 <sup>9</sup>	John David 2005-09 <sup>15</sup>
Nitrofurantoin	08	14	17.9	19.6	-
Norflo	42	75	74.2	-	66
Ciproflo	40	89	75	49	42
Levoflo	12	25	-	47.6	-
Gatiflo	13	74	-	-	-
Moxiflo	28	-	-	-	-

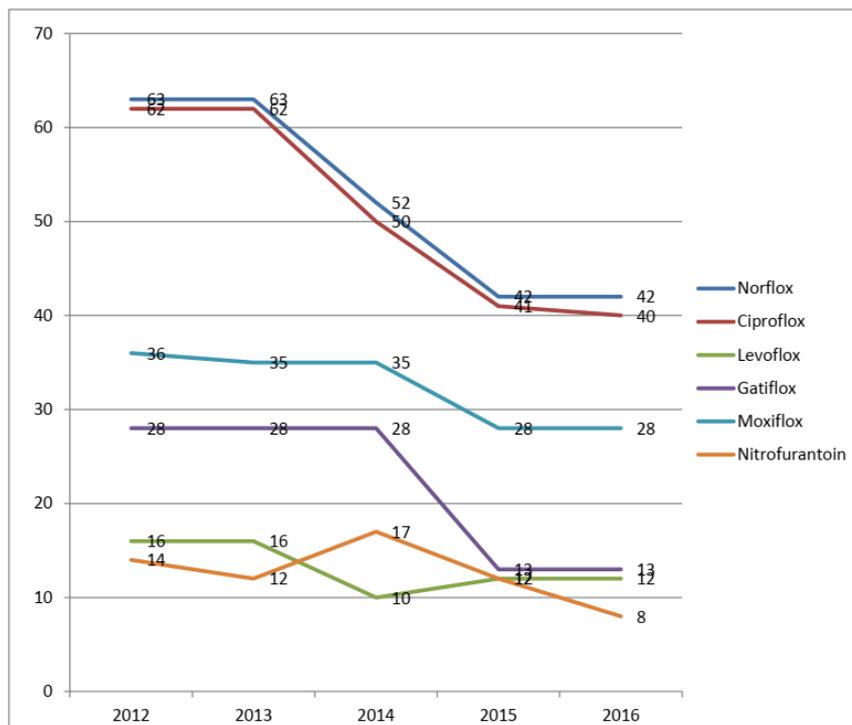


Fig-2: Trend in resistance patterns of fluoroquinolone to E.coli from 2012-2016

## DISCUSSION

*E.coli* is one of the commonest pathogens responsible for various types of infections both in community and in hospital. The organism is slowly developing multi-drug resistance including fluoroquinolones. Reduced susceptibility of fluoroquinolones to *E.coli* is a great concern in any health care setting. The burden of infectious disease is very high in developing countries like India and many times patients are treated with broad spectrum orally administered antibacterials. *E.coli* is also having very high drug resistance to sulphonamides, tetracycline and ampicillin. Hence, monitoring of drug resistance against *E.coli* is very relevant. Such studies have always given clinically relevant information. We have also observed few interesting trends in our study. Maximum numbers of *E.coli* isolates were from urinary samples followed by body fluids, blood and pus samples. Our urine culture positivity (13.13%) was low as we included samples from OPD cases as compared to other similar to the study done by Niranjana V & Malini A, who had urine culture positivity of 18.5%. 85.91% positivity in urine samples were observed in a Bangladesh study [9]. Fluoroquinolones from all the groups are now very much in use along with cephalosporins. This over use had led to higher drug resistance. We observed very high resistance to group II fluoroquinolones; norflox, ciproflox (63%, 62%) during the 2012 and least resistance against group III 12% and 28% against levoflox and gatiflox respectively. Group IV, moxiflox and gemiflox fluoroquinolones showed intermediary resistance (53%-28%). At our centre group II fluoroquinolones are not in much use since last three years. This has reflected in a decrease in resistance from 63% to 42% and from 62% to 40% during the current year (2016) against norflox and ciproflox respectively. We observed lower drug resistance to norflox and ciproflox (Table-3) as compared to 85% and 88.4% respectively in India [8]. We observed lesser resistance during the year 2013 as compared to the study carried by Shakti Rath et al covering the period 2009 to 2013 [10]. Current trend of resistance is similar to the study done by M Ajij *et al.*, They observed 53.6% resistance to Fluoroquinolones against the *E.coli* isolates from healthy individuals [11]. Further, we observed very less resistance against nitrofurantoin (8%) in all urinary isolates and minimum variation in resistance pattern during the study period of four years (14% to 8%). Our results are similar to other similar studies where resistance was observed from 6.4% to 6.1% resistance during the period 1989 to 1998 [12]. Hence nitrofurantoin still has a big role in management of OPD cases. Resistance to levoflox is increasing in Asia and it has ranged from a low of 43.9% in 2006 to a high of 61.6% in 2008 [13]. However in our study it ranged from 16% in 2012 to 12% in 2016. Resistance against levoflox is low as of now as compared with ciproflox as its use is restricted to ICU setting and in severely ill cases. However, it is going to increase in future as levoflox has been used indiscriminately for the

treatment of OPD cases as well in ICU set ups during current decade. In some areas resistance has already increased to 26% as being observed by D Rukanova and *et al.*, in their study [14]. In Bangladesh very high resistance to levoflox (47.6%) has been observed [9]. In our study we observed that resistance against ciprofloxacin is low (40%) as compared to many other studies as mentioned [8-10] but resistance was nearly similar to the study done by John David and Rilwanu Aminu [15].

We observed a rapid decrease in resistance during initial three years and slow decrease in resistance during last year. Levoflox is not being prescribed to OPD patients as a routine at our centre. Normally gatifloxacin and moxiflox are not tested for their resistance as these are not commonly prescribed being toxic in nature and mainly used in ear and ophthalmic preparations. Even these antibacterials have shown significant resistance against *E.coli*. Resistance to gatiflox (13%) and moxiflox (28%) is being observed. New drug gemiflox is also being tested and shown results nearly similar to its group (23%).

## CONCLUSION

*E.coli* has developed drug resistance to all the groups of Fluoroquinolones. There has been a decrease in resistance against commonly used fluoroquinolones during the last four years follow up. If proper HICC protocol is followed along with drug holiday, spread of drug resistance can be overcome. Judicious use of fluoroquinolones can prevent further increase in bacterial resistance against these especially of levoflox.

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