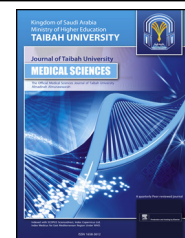




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Brief Communication

Prevalence of fluoroquinolone resistance in *Escherichia coli* in an Indian teaching hospital and adjoining communities



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Abstract

Multidrug resistant (MDR) strains of the Gram-negative pathogenic bacterium, *Escherichia coli*, particularly fluoroquinolone-resistant strains, are the major causative agents for hospital acquired (HA) infections, as well as epidemics linked to gastrointestinal (GI) and urinary tracts in the non-hygienic communities of most developing countries. The prevalence of multidrug resistance among 1642 strains of *E. coli*, isolated from clinical samples of patients with GI infections in a hospital over 39 months (November 2009–January 2013) is recorded, along with sensitivity patterns to 23 currently used antibiotics, including third-generation cephalosporins and fluoroquinolones with disc-diffusion method. A total of 1642 strains of *E. coli* were isolated from the clinical samples, of which 810 isolates were from CA samples and 832 isolates were from hospitalized patients during the study period. Of the 810 CA isolates, 567 strains were resistant to fluoroquinolone antibiotics; of the 832 HA isolates, 575 strains were fluoroquinolone-resistant, independently. Minimum inhibitory concentration values of fluoroquinolones (ciprofloxacin and levofloxacin) against the isolated *E. coli* strains confirmed the resistance in the current/coveted treatment options. Patients with other bacterial infections had relatively higher chances of becoming infected with fluoroquinolone-resistant *E. coli* strains. The data presented epitomize

the daunting state of the infection-dynamics of fluoroquinolone-resistant *E. coli* in hospitals and adjoining communities.

Keywords: *Escherichia coli*; Fluoroquinolone resistance; Hospital and community infection; Multidrug resistance

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Introduction

Suffering from several chronic uncontrollable bacterial infections often leads to terminal diseases, at least in immune-compromised/aged individuals, if the innards become infected. On analysis, it is found that drug-resistant bacteria are the causative organism of morbidity and mortality.¹ Indeed, pathogenic bacteria gain multidrug resistant (MDR) traits due to their simple genomes, and concomitantly natural consortia of bacteria help mediate their evolutionary exchanges of genetic materials.² As antibiotics are microbial in origin, targeted microbes develop resistance to the applied antibiotics intrinsically; the mutation frequency of antibiotic-resistance is recorded as one in $10^6 - 10^8$ cells.³ However, small and clean a hospital be it may, the chance of spreading pathogenic bacteria to health personnel, who often serve as reservoirs along with hospitalized patients, should be ample aside from the spread from fomites and devices.⁴ Furthermore, nosocomial infections of patients with burn and surgical injuries, as well as life-threatening urinary tract infections or even enteropathogenic episodes, frequently lead to

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bacteraemia/septicaemia.^{5,6} Any infection in a patient must be controlled forthwith, and the surveillance of a group of pathogens can be undertaken at a hospital for the estimation required for assurance on prescribed antibiotics. The evolutionary capabilities of a few pathogenic Gram-negative (GN) bacteria are so versatile that notorious pan-drug resistant (PDR; some strains of these bacteria are resistant to almost all contemporary antibiotics) strains have emerged; they are identified mainly as *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*.^{7,8} The outbreak of accredited MDR bacterial strains and their rapid spread affects the cost of hospitalization and the public health sector, leading to the urgency behind the implementation of some avant-garde drugs as antimicrobials.

Fluoroquinolones are broad-spectrum antibiotics that are used to treat several GN and Gram-positive (GP) bacterial infections. Since 1960, fluoroquinolones have become prevalent in the treatment of urinary, respiratory, gastrointestinal, urogenital, intra-abdominal, and skin infections. *E. coli* infections, especially in the urinary and gastrointestinal tracts, are frequently addressed with fluoroquinolones. The emergence of quinolone-resistance during treatment was first reported in *Staphylococcus aureus*, particularly along with the methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa*. Fluoroquinolone resistance emerged rapidly and spread to Gram-positive (GP) and GN bacteria in hospitals,⁹ with minimal inhibitory concentration (MIC) values inhibiting 90% of pathogen growth specifically over a broad range from ≤ 0.015 up to ≥ 128 mg/L fluoroquinolones.⁹ Drug-resistant subpopulations of pathogens became prevalent two decades ago and have remained almost unnoticed. Recent surveillance studies demonstrated that fluoroquinolone resistance rates have continued to increase, affecting patient management and necessitating the need for changes in the current treatment guidelines.¹⁰

The aim of this study was to evaluate the occurrence of fluoroquinolone resistance, particularly in *E. coli* causing nosocomial and community-acquired infections in patients over a period of 39 months (November 2009–January 2013). The antibiotic resistance patterns of these isolates against 23 antibiotics along with fluoroquinolones used in the study is crucial to hospital management, as empiric therapy is often needed for *E. coli* infections in the GI and urinary tracts.

Materials and Methods

Isolation, identification and antibiotic sensitivity of E. coli isolates

IMS and Sum hospital is a philanthropic teaching hospital situated in Bhubaneswar, the capital city of Odisha state, India. Patients from all walks of life, from slum-dwellers to socialites, attend this hospital. Clinical samples (urine and stool samples) collected from the outpatient department (OPD) were taken as community acquired (CA) samples. Collected clinical samples from wards, cabins, intensive care units (ICU) and neonatal intensive care units (NICU) of the hospital were referred to as hospital acquired (HA) samples for the isolation of *E. coli* strains. Of the total 12,846 clinical samples obtained over a period of 39 months,

only 7194 samples yielded both GN and GP bacteria. GN bacteria were cultured on MacConkey (MC) agar and cysteine deoxycholate electrolyte deficient (CLED) agar (HiMedia, Mumbai). The *E. coli* strain MTCC strain number 443, was used as the reference strain for all experiments. Antibiotic susceptibility tests were performed using Kirby–Bauer's disc diffusion method.¹¹ This study was performed after being approved by the institutional ethical board.

Determination of MIC values of fluoroquinolones antibiotics

The MIC values of two frequently used fluoroquinolone antibiotics, ciprofloxacin and levofloxacin against 50 drug-sensitive strains and 50 resistant strains of *E. coli* were determined by micro-broth dilution method using 96-well microtiter plates, as described elsewhere.¹² The results were interpreted using the standard breakpoint values suggested by the clinical and laboratory standards institute and the 2014 guidelines of the European committee of antimicrobial susceptibility testing.^{13,14}

Statistical analysis

Statistical analysis was performed using the Statistical Package for Medical Science version 17.0 (SPSS Inc., IL) and Microsoft Excel.

Results

Isolation and identification of E. coli strains

A total of 1642 strains of *E. coli* were isolated from clinical samples, of which 810 isolates were from CA samples and 832 isolates were from hospitalized patients, taken during the study period. Of the 810 CA isolates, 567 strains were resistant to fluoroquinolone antibiotics. Similarly, of the 832 HA strains, *E. coli* 575 strains were fluoroquinolone-resistant, independently. When grown on MC agar, medium-sized colonies coloured bright pink (due to inherent lactose fermentation) were seen, and when grow on CLED agar, large, round yellow coloured colonies were seen, confirming the presence of *E. coli*.

Antibiotic susceptibility pattern of E. coli isolates

The antibiotic susceptibility patterns of isolated *E. coli* strains were studied over the period of 39 months using 23 antibiotics. HA *E. coli* strains were found to be highly resistant to, in descending order, gentamicin (98%), cefepime (82%), nitrofurantoin (94%) and norfloxacin (87%) and were least resistant to imipenem (21%). Similarly, CA *E. coli* isolates were resistant to co-trimoxazole (94%), followed by gentamicin (91%), ciprofloxacin (89%), amikacin (87%) and cefotaxime (87%), and were least resistant to imipenem (17%) and levofloxacin (25%) (Table 1). The resistance percentage values clearly support that there is high occurrence of ESBL and fluoroquinolone-resistant strains of *E. coli* isolates in hospital and community settings. Moreover, resistance to aminoglycosides and carbapenems (imipenem and meropenem) were recorded, which would further complicate the treatment regimen for an infection, as

Table 1: Resistance percentages of *E. coli* to 23 antibiotics of eight classes^a.

Antibiotic group	Antibiotics ($\mu\text{g}/\text{disc}$)	Resistance (in %) <i>E. coli</i>	
		HA	CA
Aminoglycosides	Amikacin 30	81	87
	Gentamicin 10	98	91
	Netillin 30	74	65
β -lactams	Amoxycylav 30	82	85
	Ampicillin 10	67	71
	Piperacillin 100	76	71
	Piperacillin/tazobactam 100/10	79	65
Carbapenems	Imipenem 10	21	17
	Meropenem 10	55	41
Cephalosporins	Cefepime 30	94	79
	Cefixime 30	75	71
	Cefotaxime 30	84	87
	Ceftazidime 30	76	79
	Ceftriaxone 30	82	73
	Cefuroxime 30	78	84
Fluoroquinolones	Ciprofloxacin 5	78	89
	Gatifloxacin 5	82	74
	Levofloxacin 5	32	25
	Norfloxacin 10	87	75
	Ofloxacin 5	79	74
Monobactam	Aztreonam 30	83	75
Sulfonamides	Co-trimoxazole 25	84	94
Synthetic drug	Nitrofurantoin 300	94	86

^a Data presented are means of triplicate values; HA, Hospital acquired; CA, Community acquired. Total number of *E. coli* isolates 1642.

these are the latest generation of broad spectrum antibiotics used against drug resistant pathogenic bacteria, as well as in the empiric therapy of critically ill patients.

MIC values

For 50 fluoroquinolone sensitive *E. coli* strains, it was found that when ciprofloxacin was used, the MIC range was 0.5–1.0 $\mu\text{g}/\text{ml}$ for 33 strains, and the remaining 17 strains had MIC values of ≤ 0.25 $\mu\text{g}/\text{ml}$; similarly, for levofloxacin, the MIC range was 0.5–1.0 $\mu\text{g}/\text{ml}$ for 35 strains, and the remaining 15 strains had MIC values of ≤ 0.25 $\mu\text{g}/\text{ml}$. Further, with ciprofloxacin, of the 50 fluoroquinolone-

resistant *E. coli* strains, 47 isolates had a MIC range of 2–256 $\mu\text{g}/\text{ml}$, and the remaining three isolates had a MIC value of ≥ 512 $\mu\text{g}/\text{ml}$. Likewise, with levofloxacin, all 44 fluoroquinolone-resistant *E. coli* strains had a MIC range of 8–256 $\mu\text{g}/\text{ml}$, and the remaining six isolates had a MIC value of ≥ 512 $\mu\text{g}/\text{ml}$ (Table 2).

Univariate analysis of fluoroquinolone sensitive and resistant isolates of *E. coli*

Univariate analysis of fluoroquinolone sensitive and resistant isolates of *E. coli* was performed using four variables, number of isolates, sex, comorbidities and other

Table 2: Detection of MIC values of 50 *E. coli* isolates in with two fluoroquinolone antibiotics^a.

Breakpoint ($\mu\text{g}/\text{ml}$)	Ciprofloxacin		Levofloxacin	
	FL-S (n = 50)	FL-R (n = 50)	FL-S (n = 50)	FL-R (n = 50)
≤ 0.25	17	—	15	—
0.5	10	—	22	—
1	23	—	13	—
2	—	—	—	—
4	—	—	—	—
8	—	18	—	—
16	—	17	—	—
32	—	—	—	01
64	—	—	—	17
128	—	02	—	22
256	—	10	—	04
≥ 512	—	03	—	06

^a Fq-S, fluoroquinolone-sensitive; Fq-R, fluoroquinolone-resistant.

infections. For fluoroquinolone-resistant levels and sensitive level strains as determined from the MIC values, *E. coli* had a 0.9589 times greater chance of being picked up by any patient in a hospital setting (Table 3). Males had a 1.0848 times greater chance of picking up fluoroquinolone-resistant isolates of both bacteria; these values were not statistically significant. People with non-infectious comorbidities had a greater chance of picking up fluoroquinolone-resistant isolates. The data recorded for non-infectious or infectious comorbidities were highly significant for *E. coli* ($p < 0.05$) (Table 3).

Discussion

The resistance percentage values clearly supported the occurrence of ESBL-producing and fluoroquinolone-resistant strains of *E. coli* isolates in both hospitals and community settings. Moreover, resistance to aminoglycosides (amikacin, gentamicin, netillin) and carbapenems (imipenem and meropenem) was recorded, which would further complicate the treatment regimen, as these are the latest generation of broad spectrum antibiotics used against drug-resistant pathogenic bacteria, as well as in the empiric therapy of critically ill patients. Resistance percentages of 32–89% were recorded against five fluoroquinolone antibiotics and were further confirmed by the MIC values. The univariate analysis revealed invariably that patients with other bacterial infections had a relatively higher chance of picking up fluoroquinolone-resistant strains of *E. coli* infection.

Resistance in *E. coli* is caused mainly by 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 subunits. Moreover, plasmid-mediated quinolone-resistant (PMQR) genes have been reported in GN bacteria, including *E. coli*. The acquisition of PMQR genes alone results in a low level of fluoroquinolone resistance and does not lead to MICs exceeding the breakpoints of these agents.¹⁵

The impact of prescribing ciprofloxacin on the emergence of fluoroquinolones resistance in uropathogenic *E. coli* was analysed in 72 general practices in west Ireland. Over a 4.5 year period (from April 2004 to September 2008), susceptibility and prescribing data were collected and analysed by a multilevel model with ciprofloxacin-resistance as the outcome and prescribing as the predictor. The analysis

revealed that in “mean” practices with one prescription per month, ciprofloxacin resistance was low (3%), whereas in practices with 10 prescriptions per month, ciprofloxacin resistance amounted to 5.5%.¹⁶ Similar results were recorded in patients with UTI monitored over a 6-year period in the USA.¹⁷ In 1999, the initial therapy for UTI was switched to levofloxacin. Prescriptions increased from 3.1 to 12.7 per 1000 visits; in parallel, fluoroquinolone resistance increased from 1% to 9%. Risk factors for the acquisition of fluoroquinolone-resistant *E. coli* were hospitalization (or for each week of hospitalization = 2.0), and levofloxacin use within the previous year (OR-value 5.6). Similar risk factors were recorded independently from many other countries.⁹ In a population-based survey of 3996 persons in Indonesia, fluoroquinolone-resistant *E. coli* was prevalent in the faecal flora of 6% of patients at hospital admission and 23% of patients at discharge, but not among healthy relatives or patients visiting primary healthcare centres. Molecular typing showed extensive genetic diversity with only limited clonality among isolates. This finding suggested that the independent selection of resistant mutants occurs frequently. Fluoroquinolone-resistant isolates exhibited a higher rate of spontaneous mutation, but low virulence profiles, than FQ-susceptible isolates from the same population.¹⁸

As in several developing countries, there is no ideal antibiotic policy in India. Such a study should strengthen the epidemiological database and would help foster prudent decisions in the country’s forthcoming antibiotic policies. Secondly, countries belonging to zones other than Western Europe and North America would benefit from this study to estimate the global load of MDR bacteria in terms of recently used antibiotics, such as carbapenems and fluoroquinolones. This matter is an important aspect of global medicine, as MDR bacteria remain pervasive in communities and hospitals. The limitation of the study is the lack of molecular diagnosis of isolated strains, as has been done elsewhere.¹⁵ Thus, the data presented epitomize the daunting state of the infection-dynamics of fluoroquinolone-resistant *E. coli* in hospitals and adjoining communities.

In conclusion, a total of 1642 strains of *E. coli* were isolated, of which 810 isolates were from CA samples and 832 isolates were from hospitalized patients, in 39 months. Of the 810 CA isolates, 567 strains were resistant to fluoroquinolone antibiotics; of the 832 HA isolates, 575 strains were fluoroquinolone-resistant, independently. This clearly indicated that fluoroquinolone resistance was of similar magnitude in CA and HA clinical samples. The MIC values of

Table 3: Univariate analysis of fluoroquinolone-sensitive and resistant isolates of *E. coli*^a.

Variables		Fq-S	Fq-R	<i>p</i> -value	Odd ratio	Range
Strains	CA	243	567	0.6954	0.9589	0.7770 – 1.1833 ^b
	HA	257	575			
Sex	Male	275	605	0.4495	1.0848	0.8784 – 1.3398 ^b
	Female	225	537			
Comorbidities	Present	154	686	<0.0001	0.2959	0.2365 – 0.3701
	Absent	346	456			
Other infections	Present	297	345	<0.0001	3.3799	2.7160 – 4.2060
	Absent	203	797			

^a Fq-S, fluoroquinolone-sensitive; Fq-R, fluoroquinolone-resistant.

^b statistically not significant.

ciprofloxacin and levofloxacin used *in vitro* could help the current/coveted treatment options. Patients with other bacterial infections had relatively higher chances of becoming infected with fluoroquinolone-resistant *E. coli* strains.

Contributions

S Rath conducted the experiments and recorded the results. S Rath and RN Padhy edited and drafted the final manuscript.

Conflicts of interests

The authors report no conflicts of interests.

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Ethical approval

Not required.

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References

1. Khan AU, Raffaele Z, editors. *Multidrug resistance: a global concern*. USA: Bentham Publishers; 2012.
2. McMurry LM, Levy SB. The periplasmic protein *mppA* is not involved in regulation of *marA* in *Escherichia coli*. *Antimicrob Agents Chemother* 2011; 55: 4939.
3. Gillespie SH, Basu S, Dickens AL, O'Sullivan DM, McHugh TD. Effect of subinhibitory concentrations of ciprofloxacin on *Mycobacterium fortuitum* mutation rates. *J Antimicrob Chemother* 2005; 56: 344–348.
4. Sydnor ERM, Perl TM. Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev* 2011; 24: 141–173.
5. Bean DC, Krahe D, Wareham DW. Antimicrobial resistance in community and nosocomial *Escherichia coli* urinary tract isolates, London 2005–2006. *Ann Clin Microbiol Antimicrob* 2008; 7: 10–18.
6. Taneja J, Mishra B, Thakur A, Dogra V, Loomba P. Nosocomial blood-stream infections from extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from GB Pant Hospital, New Delhi. *J Infect Dev Ctries* 2010; 4: 517–520.
7. Souli M, Kontopidou FV, Papadomichelakis E, Galani I, Armaganidis A, Giamarellou H. Clinical experience of serious infections caused by Enterobacteriaceae producing VIM-1 metallo-beta-lactamase in a Greek University Hospital. *Clin Infect Dis* 2008; 46: 847–854.
8. Falagas ME, Kanellopoulou MD, Karageorgopoulos DE, et al. Antimicrobial susceptibility of multidrug-resistant gram-negative bacteria to fosfomycin. *Eur J Clin Microbiol Infect Dis* 2008; 27: 439–443.
9. Dalhoff A. Global fluoroquinolone resistance epidemiology and implications for clinical use. *Interdisc Perspect Infect Dis* 2012; 2012: 1–37. <http://dx.doi.org/10.1155/2012/976273>. Article ID 976273.
10. Keddy KH, Smith AM, Sooka A, Ismail H, Oliver S. Fluoroquinolone-resistant typhoid, South Africa. *Emerg Infect Dis* 2010; 16: 879–880.
11. Rath S, Dubey D, Sahu MC, Debata NK, Padhy RN. Surveillance of multidrug resistant *Escherichia coli* in community and a hospital from Odisha. *Asian Pacif J Trop Dis* 2014; 4: 140–149.
12. Rath S, Padhy RN. Prevalence of community and hospital acquired multidrug resistant *Klebsiella oxytoca* and *Klebsiella pneumoniae* in an Indian tertiary care hospital. *J Infect Publ Health* 2014; 7: 496–507.
13. CLSI. Clinical and Laboratory Standards Institute. *Performance standard for antimicrobial susceptibility testing: twenty-first informational supplement*; 2014. Document M200-S21; USA: Wayne, PA.
14. EUCAST. The European committee of antimicrobial susceptibility testing. *Breakpoint tables for interpretation of MICs and zone diameters*, 3; 2014. p. 1.
15. Sato T, Yokota S, Uchida I, Okubo T, Ishihara K, Fujii N, Tamura Y. A fluoroquinolone-resistant *Escherichia coli* clinical isolate without quinolone resistance-determining region mutations found in Japan. *Antimicrob Agents Chemother* 2011; 55: 3964–3965.
16. Vellinga A, Murphy AW, Hanahoe B, Bennett K, Cormican M. A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice. *J Antimicrob Chemother* 2010; 65: 1514–1520.
17. Johnson L, Sabel A, Burman WJ, et al. Emergence of fluoroquinolone-resistance in outpatient urinary *Escherichia coli* isolates. *Am J Med* 2008; 121: 876–884.
18. Kuntaman K, Lestari ES, Severin JA, et al. Fluoroquinolone-resistant *Escherichia coli*, Indonesia. *Emerg Infect Dis* 2005; 11: 1363–1369.