

Impact of Antibiotic usage on resistance in Microorganisms; Urinary Tract Infections with E-coli as a case in point

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Introduction

The introduction of quinolones was a breakthrough in the treatment of infectious diseases. Nalidixic acid was the first quinolone introduced in 1960 and newer quinolone agents (oxalinic acid, piperidic acid and cinoxacin) were added in 1970. More potent fluoroquinolones (ciprofloxacin and norfloxacin) were developed in 1980s, having a broad antimicrobial activity and oral availability making them the leading antimicrobial agents against a wide variety of infectious diseases.^{1,2}

Resistance to fluoroquinolone was first reported in 1990s and it has increased ever since.^{3,4} Frequent use of ciprofloxacin has been associated with resistance not only to quinolones but also to other antimicrobial agents like cloxacillin in Staph aureus as it leads to expression of high-level oxacillin-resistance in these organisms. A recently published paper has discussed that exposure to levofloxacin and ciprofloxacin is a significant risk factor for isolation of MRSA.^{5,6} Association between use of quinolones and resistance has been reported throughout the world.^{8,9}

Current International recommendations state that Institutional antimicrobial prescription policies must be developed in order to control the utilization of antibiotics to prevent antimicrobial resistance. The development of such policies however necessitate that resistance and antimicrobial usage data be analyzed according to location and areas with excessive utilization and rising resistance be identified and targeted in order to decrease consumption and hence resistance.

Increasing resistance to ciprofloxacin amongst microorganisms has been noticed in our hospital. In this study we sought to find out the utilization of quinolones in different clinical services and to correlate it with the resistance trend seen in microorganisms. Urinary isolates of E-coli from these areas have been used as representative

organisms to correlate resistance to quinolones seen against antimicrobial load.

Material and Methods

The study was conducted in a 350 bedded teaching hospital backed by a highly sophisticated clinical laboratory. Microbiological results of all clinical specimens were stored in the database of Information System Department (ISD) of the hospital.

Setting and Study period

Data of all urinary samples, received between January 1995 and December 2002, was retrieved and analyzed. Specimens yielding E-coli were included in this study,

Antibiotic utilization data

Antimicrobial usage data was obtained through our hospital pharmacy service. Consumption was expressed as unit utilized per year for quinolones in different medical, surgical and pediatric units of the hospital for the period of 1997- 2002.

Microbiological methods

All urinary samples were inoculated on Cystin Lactose Electrolyte Deficient (CLED) medium (oxid) using a 0.01ul calibrated loop such that growth of >10³ colonies of a pathogen considered significant, plates were incubated at 37°C for 24-48 hours. Isolates showing >10³ colonies were identified using standard microbiological techniques. E-coli were identified on the basis of triple sugar iron agar (TSI), sulphide indole motility (SIM) test medium, citrate utilization and urea production.^{10,11}

Antimicrobial susceptibility testing

Antibiotic susceptibility of E-coli was tested using Kirby Bauer disc diffusion method.²² The panel of antibiotics routinely tested for urinary strains of E-coli

included Ampicillin (10ug), Trimethoprim-sulphamethoxazole (25ug), Amikacin (30ug), Nitrofurantoin (300ug), Gentamicin (10ug), Ceftriaxone (30ug), Ofloxacin (5ug), Nalidixic acid (30ug) and Pipemedic acid (20ug) (Oxoid).¹²

Statistical analysis

Data was analyzed using SPSS; Significance of correlation between percent resistance and drug utilization was assessed using linear regression analysis.

Results

During the study period, 32,722 urinary specimens were processed. E.coli was the most frequent isolate (53%) in the total positive specimens followed by Pseudomonas (17%) and Enterobacter (9.3%). Over the study duration a steady increase in the number of ofloxacin resistant E.coli was noted, 24% in 1995 to 55% in 2002. On categorization of the data according to the clinical services, maximum number of these resistant strains were isolated from patients in Medical units; 41% in 1997 increasing to 70% in 2002, followed by Surgery (35%to 54%) Surprisingly, the ofloxacin resistant strains of E.coli were also isolated from urinary samples of Pediatric unit where use of quinolone is limited (Figure 1).

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The ofloxacin utilization data of hospital pharmacy for the period of 1997-2002 revealed a sharp increase in the consumption of ofloxacin in our hospital (Figure 2). There was a statistically significant correlation between the increase in the incidence of ofloxacin resistant strains of E.coli and the ofloxacin utilization in the hospital (Figure 3).

Table. Resistance in urinary E-coli from all isolates (1995-2002)

Antibiotic	% Resistance								Pvalue
	per year								
	1995	1996	1997	1998	1999	2000	2001	2002	
Amikacin	1.86	1.52	2.04	2.37	2.48	2.49	3.65	3.35	<0001
Gentamicin	14.53	16.24	19.00	20.91	23.44	28.58	32.58	32.02	<0001
Nitrofurantoin	8.05	11.58	13.22	15.15	12.30	12.93	10.85	12.54	<0001
Ampicillin	66.97	70.14	72.98	72.08	74.00	76.05	78.87	77.55	<0.0001
Co-trimoxazole	68.08	71.00	75.60	72.90	74.30	73.07	74.17	71.11	<0.0001
Ceftriaxone	12.11	13.04	15.40	17.30	19.90	25.49	31.85	33.74	<0001
Ofloxacin	24.00	26.80	2.57	33.40	37.06	42.50	47.60	49.50	<0001
Pipemedic acid	34.34	35.80	39.03	44.82	49.42	55.64	62.48	65.47	<0001
Nalidixic acid	32.28	34.98	38.58	43.28	48.64	53.66	64.00	62.89	<0001

The trend in antimicrobial resistance shows a strong positive relation to quinolone utilization in Medical (R =0.970) and Pediatrics units (R=0.980) while the association for the surgical unit was relatively weak positive estimated to be (R = 0.705). Furthermore in addition to increasing resistance to quinolones a significant gradually increasing trend was observed for almost all other antibiotics tested including Ampicillin, Ceftriaxone, Cotrimoxazole, Gentamicin and Amikacin has also been noted (Table).

Discussion

Our study demonstrates increasing prevalence of ofloxacin resistance in the urinary E-coli isolates. Furthermore, we also show a clear association of this increasing incidence of resistance with the increasing utilization of ofloxacin amongst in-patients.

Prior use of the fluoroquinolones has been identified as a risk factor for the emergence of the resistant strains.^{10,11} Our results support this association indirectly. Majority of the resistant strains emerged from the medical wards that cater for patients with a variety of underlying diseases such as febrile neutropenia, typhoid fever and chronic urinary tract infections. Ciprofloxacin is often used in these patients either empirically or as first line drug. Similarly, the statistically significant increase in per unit utilization of the fluoroquinolones in the medical unit also supports this association.

Use of quinolones in pediatric patients had been restricted due to the published reports of quinolone association with arthropathy, gait abnormalities and articular cartilage lesions in weight bearing joints of juvenile animals.¹³ Gradual increase in quinolones usage in pediatric patients has been observed in the recent years mainly due to presence of multidrug resistant organisms where quinolones may be the only treatment option. Increased usage in pediatric population may also be attributed to the recently published data about relative safety of quinolone use in children.^{14,15} This increased use has lead to a sharp increase in quinolone resistance observed in this group of patients as well.

Fluoroquinolones are the most potent broad-spectrum antibiotics effective in treatment of wide range of infections.^{16,17} They exert their antibacterial effects by inhibition of bacterial topoisomerase enzymes, namely, DNA gyrase (bacterial topoisomerase II) and topoisomerase IV.¹⁷ In gram-negative organisms, DNA gyrase is considered the primary target for all quinolones.^{18,19} Alteration of target enzyme appears to be the most dominant factor in expression of resistance to quinolones, other mechanisms include, decreased up-take and most recently known, plasmid mediated efflux mechanisms.^{17,18,20}

A previously published study from this region has reported the presence of ESBL producing E-coli strains resistant to all β -Lactam antibiotics except cephamycins and carbapenems.²¹ Recently published studies have reported expression of multiple efflux transporter mechanisms like MexA-MexB-OprM and MexC-MexD-OprJ, RND-type multidrug efflux pumps AcrB and AcrD in E.coli conferring resistance to multiple classes of

antibiotics including beta lactams, imipenam and tetracycline as also evident in our study.^{22,23} Such phenomenon may well explain the fact that the majority of ofloxacin resistant strains of E-coli also showed resistance to other unrelated antibiotics.

In conclusion, increasing E- coli resistance against most antibiotics needs continued local and national surveillance. Particularly the demonstration of a direct association of utilization with increasing resistance against quinolones points towards the need for improvement in antibiotic prescription policies to curtail such a trend.

Our study has moreover identified specific areas where antimicrobial prescription policies require review. In health care centers with limited resources it may be difficult to implement control policies across board; it is thus important to identify specific areas requiring greater attention. Presence of multidrug resistant organisms indicates a need for restricted and judicious use of antimicrobial agents. Empirical treatment of infections should be based on local surveillance data.

Limitation and Suggestion

As this study was conducted in a single hospital, results can not be generalized at national level, but authors believe that a similar scenario must be prevailing in other secondary and tertiary care hospitals therefore we suggest that further multicenter studies should be carried out to assess the severity of the problem which will facilitate in development of guidelines for appropriate antibiotics usage.

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Abstract

Objective: In this study, we sought to establish a correlation between consumption of fluoroquinolones in our hospital and the emergence of ofloxacin resistant strains of *E.coli* in the urinary specimens. Data of all urinary samples, received at Aga Khan University Hospital between January 1995 and December 2002, was retrieved and analyzed. Specimens yielding *E-coli* as an isolate were included in this study.

Methods: *E. coli* Isolates showing >10³ colonies were identified using standard microbiological techniques. Antibiotic susceptibility of *E-coli* was tested using Kirby Bauer disc diffusion method. Antimicrobial usage data, obtained through hospital Pharmacy as unit utilized per year for Quinolones in different medical and surgical units was available only for the period of 1997- 2002.

Results: Among 32,722 urinary specimens *E.coli* (53%) was the most frequent isolate. Steady increase in the number of ofloxacin resistant *E.coli* was noted, 24% in 1995 to 55% in 2002. Maximum quinolone resistant *E coli* have been observed in Medical units, 41% in 1997 increasing to 70% in 2002, followed by Surgery units (35% to 54%) and Pediatrics (12 to 38%). Sharp increase in ofloxacin consumption in our hospital, 1997 (28613 units) to 2002 (96880 units) has been observed. Trends in quinolone resistance correlate significantly with utilization in the same period as shown by linear regression.

Conclusion: *E- coli* resistance against most antibiotics has been on a rise particularly for quinolones. The utilization of quinolones correlates with increasing resistance in our hospitalized patients (JPMA 54:472;2004).