Indian J Med Res 144, October 2016, pp 604-610
DOI: 10.4103/0971-5916.200897

Quick Response Code:

Risk factors associated with fluoroquinolone-resistant enterococcal urinary tract infections in a tertiary care university hospital in north India

Tuhina Banerjee & Shampa Anupurba

Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Received January 12, 2014

***Background & objectives*: Fluoroquinolone resistance in both Gram-positive and Gram-negative bacteria has increased with the widespread use of fluoroquinolones. Fluoroquinolone resistance in Gram-negative bacilli has been widely studied, though staphylococci and enterococci are also notably resistant. Enterococci being the second most common cause of healthcare-associated urinary tract infections (UTIs) fluoroquinolones are often the drug of choice. This study was undertaken to assess the risk factors associated with fluoroquinolone-resistant enterococcal UTI in a tertiary level health facility in north India.**

***Methods*: A total of 365 patients with UTI caused by enterococci were studied over a period of two years. Patients with ciprofloxacin-resistant and susceptible UTI were considered as cases and controls, respectively. Resistance profile of the isolates against common antibiotics was studied by minimum inhibitory concentration (MIC) determination. Mechanisms for fluoroquinolone resistance was studied by efflux pump inhibitor activity and multiplex PCR targeting the *qnr* genes.**

***Results*: A total of 204 (55.89%) cases and 161 (44.1%) controls were identified. The fluoroquinolone-resistant isolates were significantly resistant to ampicillin, high strength aminoglycosides and vancomycin. The majority (78%) of the resistant isolates showed efflux pump activity. Treatment in indoor locations, presence of urinary catheters and pregnancy along with recent exposure to antibiotics especially fluoroquinolones, third generation cephalosporins and piperacillin-tazobactam were identified as independent risk factors.**

***Interpretation & conclusions*: Our results showed that fluoroquinolone resistance in enterococcal UTI was largely associated with indoor usage of antibiotics and use of indwelling devices. Knowledge of risk factors is important to curb this emergence of resistance.**

Key words Ciprofloxacin - enterococci - India - resistant - risk - urinary tract infection

Fluoroquinolones were introduced for clinical use with the prediction of very less chances of emergence of resistance due to intrinsically low minimum inhibitory concentrations (MICs) against most of the organisms[1]. However, with the gradual but widespread use of fluoroquinolones owing to their added advantages

like availability in oral formulations and potent broad spectrum activity[2], reports of fluoroquinolone resistance came into focus. Initially the major attention was on Gram-negative bacilli. With time, increasing fluoroquinolone resistance in Gram-positive organisms, particularly *Staphylococcus aureus,* and enterococci became evident. Global surveillance data reveal that fluoroquinolone resistance increased in both healthcare and community-acquired urinary tract infections (UTIs), with more than 50 per cent resistance in some parts of the world, particularly Asia[3].

Based on the status report of the Global Antibiotic Resistance Partnership, the units of antibiotics sold annually was highest for quinolone antibacterials[4]. Only a few studies have analyzed fluoroquinolone resistance in the country and most of these are in Gram-negative organisms[5-7]. Fluoroquinolone resistance against enterococci has been reported to be above 70 per cent[8,9]. This study was undertaken to analyze the risk factors associated with fluoroquinolone-resistant UTI caused by enterococci in a tertiary care hospital in north India.

Material & Methods

The study was conducted prospectively for two years (January 2011-December 2012) in the 1200-bedded Sir Sunderlal Hospital and Department of Microbiology, Institute of Medical Sciences, Varanasi, India. All patients with ciprofloxacin-resistant enterococcal UTI were considered as cases and ciprofloxacin susceptible enterococcal UTI as controls. The study was approved by the Institute Ethical Committee, and informed written consent was taken from each patient.

Urine samples were collected from patients with signs and symptoms along with clinical diagnosis of UTI. Samples were consecutive, non-duplicate, either midstream clean catch urine collected by the patients following instructions or urine collected from Foley catheter, suprapubic aspiration, nephrostomy tube under aseptic conditions. Samples were plated on cystine lactose electrolyte deficient agar (HiMedia, Mumbai, India) and incubated overnight at 37°C. Presumptive identification of enterococci was made by Gram's staining, bile esculin hydrolysis, and growth in 6.5 per cent sodium chloride[10], followed by confirmation with growth at *p*H 9.6 and pyrrolidonyl β-naphthylamide (PYR broth, HiMedia) hydrolysis based on Facklam Collin's classification[11]. Isolates were further speciated by an array of biochemical tests including carbohydrate fermentation media containing mannitol, sorbitol, sorbose, arabinose, raffinose, lactose, sucrose (Sigma, USA) and pyruvate (HiMedia) as per standard protocol[11].

Antimicrobial susceptibility testing: Fluoroquinolone resistance was determined by agar dilution method[12]. Resistance to ciprofloxacin (minimum inhibitory concentration, MIC ≥4 µg/ml) was taken as an indicator of fluoroquinolone resistance. Further, antimicrobial susceptibility testing was performed based on breakpoint MIC for ampicillin (MIC ≥16 µg/ml), high strength gentamicin (MIC ≥500 µg/ml), high strength streptomycin (MIC ≥2000 µg/ml), vancomycin (MIC ≥6 µg/ml) (HiMedia) for all the enterococcal isolates based on CLSI guidelines[12]. Susceptibility to nitrofurantoin (300 µg/disc) and linezolid (30 µg/disc) (HiMedia) was determined by Kirby-Bauer disc diffusion method[12].

Determination of alternative mechanisms of fluoroquinolone resistance: With the aim of studying efflux mediated ciprofloxacin resistance in the enterococcal isolates, MIC levels for ciprofloxacin was determined and compared by agar dilution method in the presence and absence of efflux inhibitor carbonyl cyanide m-chlorophenyl hydrazine (CCCP) (Sigma, USA). Stock solution of CCCP was prepared in dimethyl sulphoxide, and final concentration of 20 mg/ml was used[13]. Efflux pump activity was studied in 100 randomly chosen ciprofloxacin resistant isolates and 50 ciprofloxacin susceptible isolates. Two-fold or greater reduction in MIC levels was considered as indicative of efflux pump activity in the resistant isolates.

For determination of *qnr* mutants, two multiplex PCR were performed for the detection of *qnr A1, B1, S1* and *qep* genes and *qnr C, D* and *aac(6')-Ib-cr* genes as described elsewhere[14,15]. Briefly, for the first multiplex PCR, following reaction conditions were applied: initial denaturation at 95°C for two minutes; 30 cycles of 95°C for 20 sec, 52°C for one minute and 72°C for one minute 20 sec; and a final extension at 72°C for seven minutes. In the next multiplex, the annealing temperature was changed to 51°C.

Definition of cases and controls and data collection: All those patients with UTI due to fluoroquinolone-resistant enterococci were considered as cases, and those with UTI due to fluoroquinolone-susceptible enterococci were considered as controls. Colonization and infection were clearly differentiated based on the following criteria: the presence of signs and symptoms of UTI along with clinical diagnosis of the same,

including only those patients where *Enterococcus* as a single uropathogen was isolated in a significant number ($\geq 10^5$ cfu/ml) and isolation of the same organism on repeated culture. Only a single episode of enterococcal UTI per patient was included in the study.

Data were collected from hospital records and patients' treatment cards and history based on age, sex, department in the hospital where treatment was being undertaken, history of admission to long-term care facilities including oncology, burn and dialysis units within 30 days prior to the diagnosis of UTI, presence of urinary catheters, presence of other invasive devices such as nephrostomy tube, ureteric stents, intravenous catheters and presence of urological anomalies like renal functional disorders requiring dialysis or structural renal diseases like calculi and hydronephrosis, underlying chronic prostatitis in males and UTI in pregnancy.

Owing to lack of satisfactory history of prior antibiotic intake due to poor socio-economic and educational status of the patients to provide reliable treatment history as well as insufficient medical records, this factor could not be included in the study. To meet this end, 50 enterococcal isolates were collected from stool of non-hospitalized patients without any history of prior antibiotic intake for more than three weeks. Similarly, enterococcal isolates were also collected from stool of patients with prolonged hospitalization (greater than seven days) on antibiotics from the Intensive Care Unit. The fluoroquinolone-resistant enterococcal carriage rates in stool were compared between these two groups. In addition, information regarding previous exposure to antibiotics within past one month period was collected and compared from cases and controls hospitalized in the indoor units of the hospital during the study.

Statistical analysis: Continuous variables were expressed as median values. Categorical variables were expressed as proportion and compared using chi square test. To identify the potential independent risk factors for fluoroquinolone-resistant enterococcal UTI, a multivariate analysis was done. In the multivariate model, those variables with $P<0.05$ in univariate analysis were included. All the statistical analysis was performed by SPSS version 19 (Armonk, New York, USA).

Results

A total of 387 patients with enterococcal UTI were noted during the study period. However, 22 patients were not included due to insufficient data for analysis. Finally, 365 episodes of enterococcal UTI were studied comprising 204 fluoroquinolone-resistant cases (55.89%), and 161 fluoroquinolone-susceptible (44.1%) controls. The power of the study was 85 per cent.

Of the 365 enterococcal isolates, 169 were *Enterococcus faecalis*, 148 were *E. faecium* and 48 belonged to other enterococcal species (23 *E. durans*, 18 *E. hirae*, 2 *E. gallinarum*, 3 *E. dispar* and 2 *E. pseudoavium*).

The antimicrobial resistance profile of all the isolates is shown in Table I. Fluoroquinolone-resistant isolates were significantly resistant than the fluoroquinolone-susceptible isolates to ampicillin ($P<0.001$), high strength gentamicin ($P<0.001$), high strength streptomycin ($P<0.001$) and vancomycin ($P=0.01$). None of the isolates were resistant to linezolid.

Study of the mechanisms of resistance revealed that MIC levels decreased in 78 of the 100 ciprofloxacin resistant isolates in the presence of CCCP. The MIC levels decreased more than four-fold in 10 (10%) isolates, four-fold in 36 (36%) and two-fold in 32 (32%) isolates. No effect of efflux pump activity was seen in 22 resistant isolates. Among the susceptible isolates,

Table I. Antimicrobial resistance profile of the enterococcal isolates causing urinary tract infection among the cases and controls

Antibiotics	Number (%) of resistant isolates among the cases (n=204)	Number (%) of resistant isolates among the controls (n=161)
Ampicillin	53 (25.98)***	2 (1.2)
Nitrofurantoin	25 (12.25)	13 (8.07)
High strength gentamicin	173 (84.8)***	14 (8.6)
High strength streptomycin	107 (52.45)***	3 (1.8)
Vancomycin	18 (8.8)**	3 (1.8)
Linezolid	0	0

P **<0.01, *** <0.001 compared to controls

no effect of efflux pump inhibitors was seen. All the resistant isolates showing four-fold or greater reduction in MIC were isolated from cases with previous history of fluoroquinolone use either as monotherapy or as a combination with other drugs. None of the resistant isolates showed the presence of any *qnr* genes. None of the departments where the patients were located were significantly associated with fluoroquinolone-resistant UTI.

Demographic characteristics and associated factors of the cases and controls are shown in Table II. Median age (30 yr) was comparable in both groups. Univariate analysis revealed that indoor location of the patients [odds ratio (OR) = 2.01, *P*=0.002], indwelling urinary catheters (OR = 2.22, *P*=0.005) and UTI in pregnancy (OR = 3.79, *P*=0.017) were significant risk factors associated with fluoroquinolone resistant enterococcal UTI. In general, overall exposure to antibiotics (OR = 3.27, *P*=0.011) was significantly greater among the cases with recent exposure to fluoroquinolones, third generation cephalosporins and piperacillin-tazobactam, in particular, being specifically associated with fluoroquinolone-resistant enterococcal UTI (Table III). These factors were independently associated with fluoroquinolone resistance as revealed by multivariate analysis.

The presence of fluoroquinolone-resistant enterococci in stool as commensals in those without any recent antibiotic intake was nearly 20 per cent (11/50), whereas fluoroquinolone-resistant enterococcal carriage rate in stool of hospitalized patients was 68 per cent (34/50) (OR = 6.56, *P*<0.001).

Discussion

The most frequent infections caused by enterococci are UTI[9] and enterococci is also being reported as the most common urinary isolate after *Escherichia coli* and *Klebsiella* spp.[16,17]. Enterococcal fluoroquinolone resistance has been estimated to be 59 per cent in hospital acquired UTI and stated to be frequent in community-acquired UTI[3]. Consequently, identification of risk factors associated with this resistance is of prime importance. Based on local epidemiology and treatment practices, risk factors may vary. In this study, indoor location of patients, cases of complicated UTI, specifically presence of urinary catheter and UTI in pregnancy along with recent exposure to third-generation cephalosporins, fluoroquinolones and β-lactamase inhibitors (piperacillin-tazobactam, in particular) were found as independent risk factors for fluoroquinolone-resistant enterococcal UTI.

In general, three mechanisms of fluoroquinolone resistance have been proposed among which mutations at the *gyrA* and/or *ParC* genes is commonly implicated in enterococcal resistance[18]. Along with this, the presence of efflux pump reducing drug accumulation and plasmid-mediated quinolone resistance through *qnr* genes have also been described[19]. Although none

Table II. Risk factors associated with urinary tract infection caused by enterococci

Characteristics	Cases n=204 (%)	Controls n=161 (%)	Univariate OR (CI)	*P*	Multivariate OR (CI)	*P*
Median age (yr)	30 (one month to 88 yr)	30 (10 months to 82 yr)				
Male sex	116 (56.86)	79 (49.06)	1.36 (0.90-2.07)	0.138		
Indoor location	88 (43.13)	44 (27.32)	2.01 (1.29-3.14)	0.002	1.67 (1.32-1.97)	0.04
Age >50 yr	53 (25.98)	49 (30.43)	0.80 (0.50-1.27)	0.346		
Prior admission in long-term care facilities	39 (19.11)	22 (13.66)	1.01 (0.56-1.80)	0.973		
Indwelling catheters	51 (25)	21 (13.04)	2.22 (1.27-3.88)	0.005	1.46 (1.23-1.55)	0.006
Invasive devices	25 (12.25)	14 (8.6)	1.46 (0.73-2.92)	0.276		
Underlying urological anomaly	35 (17.15)	20 (12.42)	1.46 (0.80-2.64)	0.211		
Chronic prostatitis	25 (25/116, 21.55)	18 (18/79, 22.78)	1.10 (0.58-2.11)	0.751		
Pregnancy	18 (18/88, 20.54)	4 (4/82, 4.8)	3.79 (1.25-11.45)	0.017	2.67 (1.52-8.45)	0.020

OR, odds ratio; CI, confidence interval

Table III. Exposure to antibiotics among the cases and controls

Antibiotics	Cases n=88 (%)	Controls n=44 (%)	Univariate OR (CI)	*P*	Multivariate OR	*P*
Metronidazole	36 (40.9)	13 (29.54)	1.65 (0.76-3.51)	0.20		
Third generation cephalosporin	58 (65.9)	17 (38.63)	2.72 (1.27-5.84)	0.009	3.84 (0.8-7.21)	0.001
Fluoroquinolones	67 (76.13)	20 (45.45)	3.8 (1.77-8.26)	0.0006	2.9 (1.24-8.09)	0.001
Vancomycin	13 (14.7)	3 (6.81)	2.13 (0.57-7.96)	0.257		
Imipenem	29 (32.95)	9 (20.45)	1.87 (0.79-4.42)	0.148		
Piperacillin-tazobactam	41 (46.59)	7 (15.9)	4.61 (1.85-11.45)	0.001	5.29 (1.02-12.68)	0.03
Amikacin	34 (38.63)	11 (25)	1.88 (0.84-4.22)	0.122		
Linezolid	9 (10.22)	1 (2.2)	4.98 (0.6-39.96)	0.137		
All antibiotics[#]	78 (88.63)	31 (70.45)	3.27 (1.29-8.23)	0.011	2.74 (1.47-7.62)	0.001

[#]Any of the mentioned antibiotics either alone or in combination. OR, odds ratio; CI, confidence interval

of the *qnr* genes was detected in the study isolates, efflux activity contributed considerably to resistance in the enterococcal isolates. Prominent efflux activity was seen in those isolates collected from patients with prior fluoroquinolone use. This finding is in concordance with another study[20], suggesting that exposure to fluoroquinolones often poses as an important risk factor for increased expression of these efflux pumps mediating resistance.

According to Infectious Diseases Society of America guidelines, ciprofloxacin is the drug recommended for treatment of UTI, due to increasing evidence of resistance with trimethoprim/sulphamethoxazole[21]. However, in cases of enterococcal UTI, fluoroquinolones are often the first drug because of intrinsic inactivity against trimethoprim/sulphamethoxazole[22]. Enterococcal resistance to fluoroquinolones has already been documented in several studies from India[8,9,18,23]. In addition, we found simultaneous significant resistance of these isolates to other groups of antibiotics namely the aminoglycosides and vancomycin, thus narrowing the therapeutic options. Like another study[24], nitrofurantoin proved to be a better alternative for these resistant strains, but not without its side effects. Therefore, along with rising prevalence of fluoroquinolone resistance, deciding on the effective empirical treatment adds to the existing problem in these UTI patients.

Fluoroquinolones are considered to be the most commonly used antibiotics in long-term care facilities and the second most common in hospitals in the US[1]. An increased ciprofloxacin and levofloxacin resistance in clinical isolates in the UK has been probably associated with increased use of enrofloxacin and flumaquine in animals in the study region[25]. It was earlier noted that about 15 years back, even in countries of restricted antibiotic use like Spain, nearly 29 per cent of indoor fluoroquinolone administration as a first-line drug was done following accepted guidelines[26] suggesting indiscriminate use in hospital settings. In India also ciprofloxacin and levofloxacin are most commonly used drugs for UTI[16]. Besides, ciprofloxacin was the most common antibiotic prescribed in outdoor settings of a tertiary care hospital in western India, thus implying its widespread use[27].

Recent hospitalization, catheterization, fluoroquinolone usage, invasive procedures, recurrent UTI, old age, underlying chronic conditions, and urological anomalies have been found responsible for fluoroquinolone resistance in UTI, mostly from Gram-negative organisms[28]. Studies on fluoroquinolone-resistant enterococcal UTI have concluded that recent exposure to fluoroquinolones[18] and other antibiotics[29], along with recent hospitalization (less than or equal to two weeks) are significant risk factors. In this study, complicated UTI due to catheterization and pregnancy were found to be significant independent risk factors. Enterococci usually interact with the host with initial colonization followed by long-term persistence and gradual infections[30]. This might account for increasing UTI in patients with indwelling catheters and pregnancy, both conditions promoting initial colonization.

Our study had certain limitations. First, risk factors could not be assigned based on hospital acquired and community acquired UTI as done in the majority of the well-designed studies[28,29]. Second, specific antibiotics could not be attributed for enterococcal UTI for all the patients studied. However, use of fluoroquinolones and

other antibiotics were significantly higher among the indoor patients, and this factor along with prolonged hospitalization also resulted in increased colonization with fluoroquinolone-resistant enterococci in this study. Fluoroquinolone usage has been shown to be directly associated with fluoroquinolone-resistant organisms carriage in stool[29].

In conclusion, indoor location, indwelling urinary catheters and pregnancy were found to be independent risk factors. In addition, recent exposure to fluoroquinolones, third generation cephalosporins and piperacillin-tazobactam were also identified as independent risk factors contributing to resistance. Avoiding unnecessary usage of antibiotics both in hospitals and community along with limited use of catheters can make situations better. More studies on the likely causes of the emergence of fluoroquinolone resistance and strategies for control will provide a better understanding of the situation.

Acknowledgment

The authors thank Shri Joel Filgona, IMS, BHU and Dr Amitabha Bhattacharjee, Assam University, Silchar, for helping in the study on mechanisms of drug resistance.

***Conflicts of Interest*:** None.

References

1. Lautenbach E, Fishman NO, Bilker WB, Castiglioni A, Metlay JP, Edelstein PH, *et al.* Risk factors for fluoroquinolone resistance in nosocomial *Escherichia coli* and *Klebsiella pneumoniae* infections. *Arch Intern Med* 2002; *162* : 2469-77.
2. Neu HC. Ciprofloxacin: an overview and prospective appraisal. *Am J Med* 1987; *82* : 395-404.
3. Dalhoff A. Global fluoroquinolone resistance epidemiology and implications for clinical use. *Interdiscip Perspect Infect Dis* 2012; 2012 : 976273.
4. Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JP, Gupta U, *et al.* Rationalizing antibiotic use to limit antibiotic resistance in India. *Indian J Med Res* 2011; *134* : 281-94.
5. Mehta G, Goyal R. Emerging fluoroquinolone resistance in *Neisseria meningitidis* in India: cause for concern. *J Antimicrob Chemother* 2007; *59* : 329-30.
6. Thamizhmani R, Bhattacharya D, Sayi DS, Bhattacharjee H, Muruganandam N, Ghosal SR, *et al.* Emergence of fluoroquinolone resistance in *Salmonella enterica* serovar Typhi in Andaman and Nicobar Islands, India. *Indian J Med Res* 2012; *136* : 98-101.
7. Nair S, Unnikrishnan M, Turner K, Parija SC, Churcher C, Wain J, *et al.* Molecular analysis of fluoroquinolone-resistant *Salmonella* Paratyphi A isolate, India. *Emerg Infect Dis* 2006; *12* : 489-91.
8. Mohanty S, Dhawan B, Gadepalli RS, Lodha R, Kapil A. Case report vancomycin - resistant *Enterococcus faecium* VanA phenotype: first documented isolation in India. *Southeast Asian J Trop Med Public Health* 2006; *37* : 335-7.
9. Karmarkar MG, Gershom ES, Mehta PR. Enterococcal infections with special reference to phenotypic characterization and drug resistance. *Indian J Med Res* 2004; *119* : 22-5.
10. Ross PW. *Streptococcus* and *Enterococcus*. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie and McCartney practical medical microbiology*, 14th ed. London: Churchill Livingstone; 1996. p. 263-74.
11. Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* 1989; *27* : 731-4.
12. Clinical Laboratory and Standards Institute. *Performance standard for antimicrobial susceptibility testing.* Twenty first informational supplement M100:31(1). Wayne, PA: CLSI; 2011.
13. Jonas BM, Murray BE, Weinstock GM. Characterization of emeA, a NorA homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2001; *45* : 3574-9.
14. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 2007; *60* : 394-7.
15. Hassan WM, Hashim A, Domany R. Plasmid mediated quinolone resistance determinants *qnr, aac(6')-Ib-cr,* and *qep* in ESBL-producing *Escherichia coli* clinical isolates from Egypt. *Indian J Med Microbiol* 2012; *30* : 442-7.
16. Mandal J, Acharya NS, Buddhapriya D, Parija SC. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli*. *Indian J Med Res* 2012; *136* : 842-9.
17. Manjunath GN, Prakash R, Annam V, Shetty K. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore. *Int J Biol Med Res* 2011; *2* : 504-7.
18. Yasufuku T, Shigemura K, Shirakawa T, Matsumoto M, Nakano Y, Tanaka K, *et al.* Mechanisms of and risk factors for fluoroquinolone resistance in clinical *Enterococcus faecalis* isolates from patients with urinary tract infections. *J Clin Microbiol* 2011; *49* : 3912-6.
19. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005; *41* (Suppl 2) : S120-6.
20. Swaminathan S, Alangaden GJ. Treatment of resistant enterococcal urinary tract infections. *Curr Infect Dis Rep* 2010; *12* : 455-64.
21. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). *Clin Infect Dis* 1999; *29* : 745-58.

22. Wisell KT, Kahlmeter G, Giske CG. Trimethoprim and enterococci in urinary tract infections: new perspectives on an old issue. *J Antimicrob Chemother* 2008; *62* : 35-40.

23. Anupurba S, Banerjee T, Drug resistance in clinical isolates of enterococci with special reference to vancomycin, from North India. *J Pure Appl Microbiol* 2012; *6* : 807-12.

24. Butt T, Leghari MJ, Mahmood A. *In-vitro* activity of nitrofurantoin in *Enterococcus* urinary tract infection. *J Pak Med Assoc* 2004; *54* : 466-9.

25. van der Bogaard AE, Willems R, London N, Top J, Stobberingh EE. Antibiotic resistance of faecal enterococci in poultry, poultry farmers, and poultry slaughterers. *J Antimicrob Chemother* 2002; *49* : 497-505.

26. Ena J, López-Perezagua MM, Martínez-Peinado C, Cia-Barrio MA, Ruíz-López I. Emergence of ciprofloxacin resistance in *Escherichia coli* isolates after widespread use of fluoroquinolones. *Diagn Microbiol Infect Dis* 1998; *30* : 103-7.

27. Lallan BK, Hiray RS, Ghongane BB. Drug prescription pattern of outpatients in a tertiary care teaching hospital in Maharashtra. *Int J Pharm Biol Sci* 2012; *3* : 225-9.

28. van der Starre WE, van Nieuwkoop C, Paltansing S, van't Wout JW, Groeneveld GH, Becker MJ, *et al.* Risk factors for fluoroquinolone-resistant *Escherichia coli* in adults with community-onset febrile urinary tract infection. *J Antimicrob Chemother* 2011; *66* : 650-6.

29. Rattanaumpawan P, Tolomeo P, Bilker WB, Fishman NO, Lautenbach E. Risk factors for fluoroquinolone resistance in *Enterococcus* urinary tract infections in hospitalized patients. *Epidemiol Infect* 2011; *139* : 955-61.

30. Cattoir V, Leclercq R. Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? *J Antimicrob Chemother* 2013; *68* : 731-42.

Reprint requests: Dr. Shampa Anupurba, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, Uttar Pradesh, India
e-mail: shampa_anupurba@yahoo.co.in