Rwanda Journal ISSN 2305-2678 (Print); ISSN 2305-5944 (Online)
DOI : http://dx.doi.org/10.4314/rj.v1i1.1F

Antimicrobial Resistance and Extended-Spectrum β-Lactamase (ESBL) Producing Clinical Isolates from Urinary Tract Infection at two teaching hospitals in Rwanda

Claude Mambo Muvunyi[1], Florence Masaisa[1], Claude Bayingana[1], Léon Mutesa[2], André Musemakweri[3]

[1]Department of Clinical Biology, Centre Hospitalier Universitaire-Butare and Kigali, National University of Rwanda, Butare, Rwanda
[2]Medical Research Center, Rwanda Biomedical Center, Ministry of Health, Kigali, Rwanda
[3]Department of Internal Medicine, Centre Hospitalier Universitaire-Butare, National University of Rwanda, Butare, Rwanda

Corresponding author: Claude Mambo Muvunyi, E-mail: cmuvunyi@nur.ac.rw

Abstract

The objective of this study was to obtain data on susceptibility patterns of pathogens responsible for UTIs. In addition, for the first time, the prevalence and risk factors of ESBL-producing strains in Rwanda are described. Urinary isolates from symptomatic UTI patients' cases attending to the University Teaching Hospital-Kigali and University teaching Hospital-Butare were identified by conventional methods. Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method. Isolates resistant to third generation cephalosporin were tested for ESBL production by double disk synergy test method. Escherichia coli was the most common uropathogen, accounting for infections in 119 (60.7%) of the 196 cases; and occurred significantly more frequently in urines from outpatients (70.6%) than from inpatients (50%). Antimiocrobial agents commonly used for the treatment of UTI in Rwanda such as nalidixic acid, nitrofurantoin and ciprofloxacin are far from effective except Fosfomycin-trometamol and imipinem. ESBLs were detected in 38.3 % (36/94) of the strains from inpatients (13.8% of E.coli) and 5.9% (6/102) of all strains from outpatients (1.9% of E.coli). The use of ciprofloxacin and third-generation cephalosporin in the preceding 6 months (OR: 3.05; 95% CI 1.42–6.58; P = 0.04 and OR: 9.78; 95% CI 2.71–35.25; P = 0.01 respectively); and being inpatient (OR: 2.27; 95% CI 1.79–2.89; P < 0.001) were independently associated with ESBL production. In conclusion, ESBL producers are, as in many countries, frequent in enterobacteriaceae in Rwanda. On the basis of our findings, we suggest that antimicrobial agent such as fosfomycin-trometamol could be alternative therapy for uncomplicated UTI, and should be introduced in the national guidelines.

Keywords: *Urinary tract infection, antimicrobial resistance, ciprofloxacin, ESBL production, Rwanda*

Introduction

Background

Urinary tract infections (UTIs) are among the most common bacterial infections both in the community and hospital setting. In the majority of cases, antibiotics are given empirically before the final bacteriology results are available. Therefore, area-specific monitoring studies to document the micro-organisms causing UTIs and their antimicrobial susceptibility is mandatory for helping the selection of an effective empirical treatment (Smith and Coast 2002). An increasing rate of antibiotic resistance among pathogens responsible for UTIs has caused growing concern worldwide (Kahlmeter and Menday 2003; Zhanel et al. 2006). Mechanisms of resistance against β-lactam antibiotics in gram-negative bacilli include production of TEM- and AmpC β-lactamases, porin deficiency and efflux mechanisms and, more recently, Extended-Spectrum βlactamases (ESBL). Microorganisms responsible for UTIs such as *E. coli* and *Klebsiella spp.* remain the major ESBL-producing organisms isolated worldwide, but these enzymes have also been identified in several other members of the Enterobacteriaceae family and in certain non-fermenters (Jacoby and Munoz-Price 2005).

The aim of this prospective study was to obtain data on susceptibility patterns of pathogens responsible for both community and hospital UTIs in Rwanda to antimicrobial agents currently used to treat UTIs. In addition, for the first time, the prevalence and risk factors of ESBL-producing strains in Rwanda are described in this study.

Methodology

This study was conducted in both outpatients and inpatients with UTIs at University Teaching Hospital-Kigali and University teaching Hospital-Butare; two tertiary teaching hospitals after obtaining approval from the Research Ethics Committee of the Faculty of Medicine (FoMREC). The study was conducted in two parts. In the first part, between June and November 2009 a total of 1012 urine from patients received in the clinical microbiology laboratories of the two participating hospitals were analysed. For each patient, data were prospectively collected through a standardized questionnaire and

their medical records were checked when necessary. Risk factors for ESBL positive were as follows: age, sex, presence of a urinary catheter; prior UTI, prior urinary catheter, hospitalization during the previous year; and antibiotic exposure during the preceding 6 months. Each specimen was cultured using a 0.001 ml calibrated loop to inoculate blood agar and MacConkey agar plates, incubated at 37°C for 18-24 hours and the number of colonies was counted. Significant bacteriuria was defined as greater than 10^5 colony forming units/ml of a single pathogen.

Antimicrobial susceptibility testing was performed using disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLI 2008). Antibiotic disks were obtained from Oxoid (Oxoid ltd. England). Quality control was performed once weekly using test strains *E. coli* ATCC 25 922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Positive urine isolates obtained from the analysis were stored frozen at -80^0C and selected for the second part of the study.
In The second part of the study was done between February and August 2011, all stored isolates (196) were tested for ESBL production by the double disk synergy test (DDST) on Mueller-Hinton agar using ceftriaxone and ceftazidime placed at a distance of 15-20 mm apart from a disk containing amoxicillin plus clavulanic acid. A clear-cut extension of the inhibition zone around either ceftazidime or ceftriaxone disk towards the clavulanic acid-containing disk (also called "ghost zone") was interpreted as positive for ESBL production (CLI 2008). Confirmed ESBLs were characterized by PCR amplification using primer sets for CTX-M, SHV, and TEM genes, followed by sequencing of the PCR products at the the laboratory of Ghent University, in Belgium.

Data processing and statistical analysis were performed using SPSS software version 16.0. Differences in group proportions for categorical variables were assessed using Chi-square or Fisher's exact test. Logistic regression analysis was performed to identify risk factors ESBL production. A *p value* <0.05 was considered statistically significant.

Results

Of the 196 patients, 102 were outpatients and 94 were hospitalized patients. The mean age of the study population was 42 with a range of 18-90, and 71.9 % of the patients were female.

The distribution of uropathogens in outpatient and inpatients is shown in Table 1. *Escherichia coli* was the most common uropathogen, accounting for infections in 119 (60.7%) of the 196 patients; and occurred significantly more frequently in urines from outpatients (70.6%) than from inpatients (50%). The proportion of *Klebsiella* sp., *Proteus* sp. and *Citrobacter* sp. was relatively higher in inpatients (24.5, 6.5, 7.4 and 6.4%) than in outpatients (13.7, 5.9, 2, and 1%). *Pseudomonas aeruginosa* was only isolated from inpatients (3.2%). Gram-positive microorganism grew in only 3.1% of the positive cultures, and were not further included in the study

Table 1 : Bacterial isolates from urinary tract infections, in in- and outpatients.

	No. of isolates (%)		
Organisms	Outpatients	Inpatients	Total
E. coli	72 (70.6)	47 (50)	119(60.7)
Klebsiella spp.	14 (13.7)	23(24.5)	37 (18.9)
Proteus spp.	6 (5.9)	6 (6.4)	12 (6.1)
Enterobacter spp.	2 (2)	7 (7.4)	9 (4.6)
Citrobacter spp.	1 (1)	6 (6.4)	7 (3.6)
Acinectobacter spp.	2 (2)	1 (1.1)	3 (1.5)
P. aeruginosa	0 (0)	3 (3.2)	3 (1.5)
Gram-positive	5 (4.9)	1 (1.1)	6 (3.1)
Total	102 (52)	94 (48)	196 (100)

The antimicrobial susceptibility results of the most frequent uropathogens are summarized in the Table 2. Isolates from inpatients were significantly more resistant to antimicrobials than outpatients. 0ne hundred seventy seven (90.3%) of isolates were susceptible to fosfomycin, and 99.5% of isolates were susceptible to imipinem, which were the most effective drugs. Conversely, high resistance rates were detected among isolates from inpatients for amoxycillin

(98.9%), amoxycillin-clavulanic acid (72.3%), trimethoprim–sulphamethoxazole (90.4%), ciprofloxacin (57.4%), ceftriaxone (54.3%), ceftazidine (50%), nalidixic acid (74.6%) and nitrofuratoin (53.2%). For outpatients the corresponding resistance rates were 80,4%, 47.1%, 75.5%, 26.5%, 11.8%, 9.8%, 43, 3% and 37.3%.

The most common ESBLs identified among the isolates were members of the CTX-M15 with only one case of CTX-M14. Fig. 1.
The univariate and multivariate analysis of risk factors for ESBL positivity are shown in table 3. In univariate analysis, previous hospitalization, prior or presence of urinary tract catheterization, use of ciprofloxacin and third-generation cephalosporin in the preceding 6 months and being inpatient were significantly associated with ESBL production. In multivariate only the use of ciprofloxacin and third-generation cephalosporin in the preceding 6 months (OR: 3.05; 95% CI 1.42–6.58; P = 0.04 and OR: 9.78; 95% CI 2.71–35.25; P = 0.01 respectively); and being inpatient (OR: 2.27; 95% CI 1.79–2.89; P < 0.001) were independently associated with ESBL production.

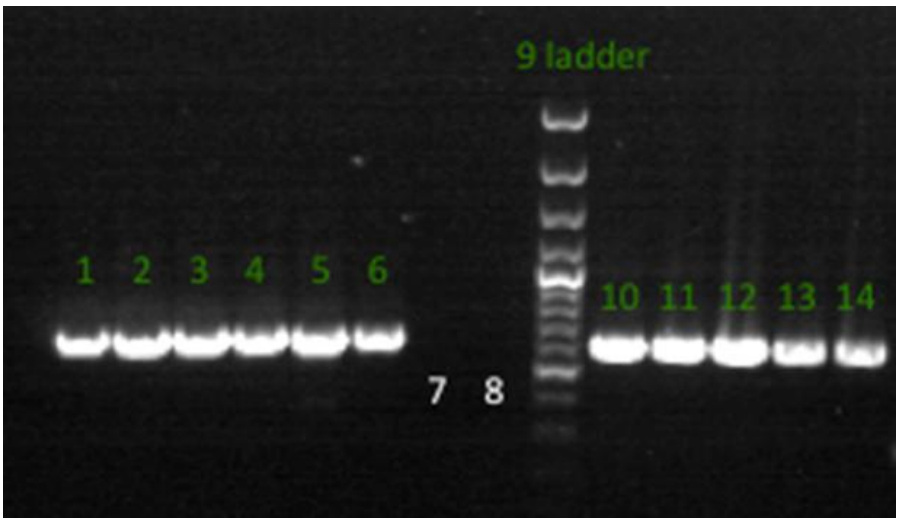

Fig.1.: *Electrophoresis of the amplified products of bla_{CTX-M} like genes by a multiplex PCR in a 2% agarose gellanes 1 to 7 and 10 to 14, clinical isolates. Lanes 7 and 8 are negative. Lane 9. DNA ladder molecular weight marker*

Table 2: Resistance rates to antimicrobial agents tested, in- and outpatients.

Antimicrobial agent		*Butare Hospital*	*University*	*Kigali Hospital*	*University*
	Overall (%) n= 196	Outpatients (%) n= 42	Inpatients (%) n= 14	Outpatients (%) n= 60	Inpatients (%) n= 80
Amoxicillin	175 (89.3)	31 (73.8)	13 (92.9)	51 (85)	80 (73.8)
Amoxicillin/ clavulanic acid	116 (59.2)	17 (40.5)	8 (50)	31 (51.7)	61 (76.3)
Cefalotin	158 (80.6)	7 (16.7)	4 (28.6)	18 (30)	42 (52.5)
Ceftriaxone	63 (32.1)	4 (9.5)	8 (57.1)	8 (13.3)	43 (53.8)
Ceftazidime	57 (29.1)	5 (11.9)	7 (50)	5 (8.3)	40 (50)
Ciprofloxacin	81 (41.3)	9 (21.1)	5 (35.7)	18 (30)	49 (61.3)
Norfloxacin	78 (39.8)	11 (26.2)	6 (42.9)	16 (26.7)	45 (56.3)
Ofloxacin	71 (36.2)	7 (16.7)	4 (28.6)	18 (30)	42 (52.5)
Nalidixic acid*	86 (56.2)	19 (45.2)	9 (64.3)	20 (41.6)	38 (77.5)
Nitrofurantoin	88 (44.9)	16 (38.1)	11 (78.6)	22 (36.7)	39 (48.8)
Trimethopin/sulfamethoxazole	162 (82.7)	30 (71.4)	10 (71.4)	47 (78.3)	75 (93.8)
Gentamicin	91 (46.4)	12 (28.6)	7 (50)	21 (35)	51 (63.8)
Amikacin	81 (41.3)	20 (47.6)	3 (21,4)	20 (33.3)	38 (47.5)
Piperacillin	152 (77.6)	26 (61.9)	11 (78,6)	39 (65)	76 (95)
Fosfomycin/ trometamol	19 (9.7)	3 (7.1)	5 (35.7)	5 (8.3)	6 (7.5)
Imipinem	1 (0.5)	0 (0)	0 (0)	0 (0)	1 (1.3)

Table 3: Univariate and multivariate analysis of risk factors for ESBL positivity in UTI isolates.

	Total n = 196	*ESBL positivity, n = 42 (%)*	*P value*
Univariant analysis			
Age over 50	56	14 (25)	0.441
Hospitalized (inpatient) Hospitalization in the last 12 months	94 70	36 (38) 26 (37)	<0.001 <0.001
UTI in last 12 months	37	10 (27)	0.357
Urinary catheter	40	20 (50)	<0.001
urinary catheter in last 12 months	43	21 (49)	<0.001
Use of other Antibiotics in the previous 6 months	88	22 (25)	0.271
Ciprofloxacin use in the previous 6 months	22	10 (45)	0.004
Third-generation Cephalosporin use in the previous 6 months	11	8 (73)	<0.001
Multivariant analysis			
Ciprofloxacin use in the previous 6 months			0.04
Third-generation Cephalosporin use in the previous 6 months			0.01
Hospitalized (inpatient)			<0.001

Discussion

The present study confirms that *E. coli* is still the most common uropathogen isolated from both in- and outpatients, but that various other bacteria cause infection, especially in inpatients (Table 1). These findings are in agreement with similar surveillance studies (Alos et al. 2005; Ruka et al. 2004; Randrianirina et al. 2007). Some

studies demonstrate a decline of *E. coli*, being replaced by other members of the Enterobacteriaceae. Our hospitalized patients had fewer *E. coli* and more *Klebsiella* spp. The low percentage of *E. coli* amongst hospital isolates in our study corresponded to that obtained by other investigators (Akram et al. 2007; Schaeffer et al. 2001). UTIs are more frequent in women than in men, which corresponds to our findings as71.9% of our patients were female (Akram et al. 2007).

As can be expected, and as reported in other studies, the antimicrobial resistance of hospital isolates was higher than in outpatients (Hryniewiez et al. 2001; Arslan et al. 2005). In our study, 75.5% of the outpatients isolate and 90.4% of the hospitalized isolates were resistant to Trimethoprim-sulfamethoxazole. Several other studies from the United States of America and worldwide indicate the emergence of trimethoprim-sulfamethoxazole resistance in a significant percentage (> 20%) of community-acquired UTI isolates (Akram et al. 2007; Karlowsky 2002). In a study of UTI from outpatients in Canada, Zhanel et al (Zhanel 2006) found resistance to amoxicillin and nitrofuratoin at rates of 41.0 and 0.1% respectively. Corresponding values in our study were 80.4 and 37.3%. In the present study, the higher proportion of outpatient and inpatient E. coli strains resistant to amoxycillin (86 vs. 100%), nitrofurantoin (26.4 vs. 29.8%), nalidixic acid (45.1 vs. 77.4%), amoxycillin-clavulanic acid (56 vs. 70.2%) and gentamycin (36.1 vs. 46.8%%), is similar to what was reported by Aboderin and coworkers (aboderin et al. 2009).

We documented a remarkable high resistance to ciprofloxacin, which is of great concern because fluoroquinolones are the drugs of choice for first-line empiric treatment of both community and hospital acquired UTI in settings where resistance to trimethoprim/ sulfamethoxazole exceeds 20% and they have become more commonly prescribed as first-line antibiotic therapy in the last few years (Goettsch et al. 2000). Resistance rates for ciprofloxacin against uncomplicated and complicated UTI strains were reported as 8.5% and 19.5% respectively by Alos and coworkers (Alos et al. 2006). Recently, Arslan and co-workers reported 17% and 38% resistance rates for the uncomplicated and complicated UTI strains,

respectively (Arslan et al. 2005). Our rates were found to be much higher: 26.5% for the inpatient UTI strains, and 57.4% for the outpatient UTI strains.

Resistance rates in isolates of outpatients were also higher compared to studies from other countries for the majority of antimicrobials except third-generation cephalosporin ceftriaxime and ceftazidime. However, the resistance to third-generation cephalosporins was significantly higher in strains from inpatients (Table 2). Randrianirina and coworkers have reported an increasing resistance rate to the third-generation cephalosporin in their study in patients aged 65 years (Randrianirina et al. 2007). The findings of the present study indicate that β -lactams, trimethoprim/sulfamethoxazole, nitrofurantoin and ciprofloxacin should no longer be used as empirical treatments of UTI in Rwanda, because of their high rate of resistance. Alternatives must be recommended, especially for empirical treatments of uncomplicated UTI (cystitis) in outpatients.

Fosfomycin-trometamol and imipinem were found to be the most effective antimicrobials, 99 and 93% E. coli isolates tested being susceptible respectively. As a result, fosfomycin-trometamol and imipinem may be the drugs of choice for empirical therapy of UTIs based on the *in vitro* data. Previous studies have reported fosfomycin-trometamol resistance rates of 0.3% in 288 and no resistance in 100 E. coli strains (Randrianirina et al. 2007). Schito in Pakistan have recorded 86.9 % of isolates being susceptible to imipinem (Schito 2003). Fosfomycin-trometamol has been recommended as a reliable empirical treatment for uncomplicated UTI because of its easy use (single dose), its good tolerance, and its efficacy (aboderin et al. 2009). Unfortunately, this antibiotic is not yet available in Rwanda. The high susceptibility to imipenem observed in our study is a clear indication that carbapenem resistance is still almost absent in Enterobacteriaceae isolated from UTI in the region. This can be explained by the infrequent use of this antibiotic in the developing world because of its cost and limited availability. For the first time in our laboratory, we performed phenotypic confirmation tests for ESBL production. The prevalence of ESBLs both in outpatients and inpatients has been investigated and varies among countries in several studies (Peterson and Bonomo 2005;

Falagas et al. 2009). In all of these, *E. coli* and *Klebsiella* spp. accounted for the vast majority of isolates (Mulvey et al. 2004). Our study showed a higher percentage of ESBL positivity among *E. coli* isolates when compared to reports from Canada (0.26%), Europe (1.3%) and Tunisia (2.7%) (Falagas et al. 2009). In agreement with our data, Russian, Cameroonian and Korean studies revealed that overall prevalence of ESBLs among *E. coli* isolates was 15.8%, 14.3% and 9.3%, respectively (Mulvey et al. 2004). Studies conducted by Akram et al (Akram et al. 2007) showed a higher percentage, than our study, of *E. coli* producing ESBL. The most common ESBLs identified among the isolates were members of the CTX-M15 with only one case of CTX-M14. Fig. 1. CTX-M-type ESBLs, primarily CTX-M-15, have emerged as the predominant type of ESBL produced by common gram-negative rods in our health care system. Prior use of antibiotics, especially ciprofloxacin and third-generation cephalosporins and in- hospital stay, both major risk factors retained in our multivariate analysis, were also found to be independent risk factors for ESBL positivity in previous studies (Colodna et al. 2004; Skippen et al. 2006).

Conclusion

Gram-negative organisms, especially *E.coli* were the most common organisms isolated. We found that antibiotics commonly used for the treatment of UTI in Rwanda such as nalidixic acid, nitrofurantoin and ciprofloxacin are far from effective. ESBL producers are, as in many countries, frequent in enterobacteriaceae in Rwanda. On the basis of our findings, we suggest that antimicrobial agent such as fosfomycin-trometamol could be alternative therapy for uncomplicated UTI, and should be introduced in the national guidelines.

Acknowledgements

We thank the staff in Bacteriology unit at Kigali and Butare Teaching Hospitals for their technical assistance. In particular, we thank Carine Uwera, Innocent Nzabahimana and Jean Baptiste Mugema for their excellent technical support in data collection and processing.

This study was approved by Research Ethics Committee of the Faculty of Medicine (code: 04/FoMREC/09) and supported by a Grant from SIDA/SAREC Research Commission, National University of Rwanda.

References

1. Aboderin OA, Abdu AR, Odetoyin BW, Lamikanra A, 2009. Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections. J Natl Med Assoc 101: 1268-73.
2. Akram M, Shahid M, Khan AU, 2007. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann Clin Microbiol Antimicrob 6: 4.
3. Alos JI, Serrano MG, Gomez-Garces JL, Perianes J, 2005. Antibiotic resistance of Escherichia coli from community-acquired urinary tract infections in relation to demographic and clinical data. Clin Microbiol Infect 11: 199-203.
4. Arslan H, Azap OK, Ergonul O, Timurkaynak F, 2005. Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey. J Antimicrob Chemother 56: 914-8.
5. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; Eighteenth informational supplement 100-S18. Clinical and laboratory Standards Institute, Wayne, PA, USA.
6. Colodner R, Rock W, Chazan B, Keller N, Guy N, Sakran W, Raz R, 2004. Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. Eur J Clin Microbiol Infect Dis 23: 163-7.
7. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L, 2003. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrob Agents Chemother 47: 3724-32.
8. Falagas ME, Karageorgopoulos DE, 2009. Extended-spectrum beta-lactamase-producing organisms. J Hosp Infect 73: 345-54.
9. Goettsch W, van Pelt W, Nagelkerke N, Hendrix MG, Buiting AG, Petit PL, Sabbe LJ, van Griethuysen AJ, de Neeling AJ, 2000. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the netherlands. J Antimicrob Chemother 46: 223-8.

10. Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W, 2001. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. J Antimicrob Chemother 47: 773-80.
11. Jacoby GA, Munoz-Price LS, 2005. The new beta-lactamases. N Engl J Med 352: 380-91.
12. Kahlmeter G, Menday P, 2003. Cross-resistance and associated resistance in 2478 *Escherichia coli* isolates from the Pan-European ECO.SENS Project surveying the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections. J Antimicrob Chemother 52: 128-31.
13. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF, 2002. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. Antimicrob Agents Chemother 46: 2540-5.
14. Mulvey MR, Bryce E, Boyd D, Ofner-Agostini M, Christianson S, Simor AE, Paton S, 2004. Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella spp*. in Canadian hospitals. Antimicrob Agents Chemother 48: 1204-14.
15. Paterson DL, Bonomo RA, 2005. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18: 657-86.
16. Raka L, Mulliqi-Osmani G, Berisha L, Begolli L, Omeragiq S, Parsons L, Salfinger M, Jaka A, Kurti A, Jakupi X, 2004. Etiology and susceptibility of urinary tract isolates in Kosova. Int J Antimicrob Agents 23 Suppl 1: S2-5.
17. Randrianirina F, Soares JL, Carod JF, Ratsima E, Thonnier V, Combe P, Grosjean P, Talarmin A, 2007. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in Antananarivo, Madagascar. J Antimicrob Chemother 59: 309-12.
18. Schaeffer AJ, Rajan N, Cao Q, Anderson BE, Pruden DL, Sensibar J, Duncan JL, 2001. Host pathogenesis in urinary tract infections. Int J Antimicrob Agents 17: 245-51.
19. Schito GC, 2003. Why fosfomycin trometamol as first line therapy for uncomplicated UTI? Int J Antimicrob Agents 22 Suppl 2: 79-83.
20. Skippen I, Shemko M, Turton J, Kaufmann ME, Palmer C, Shetty N, 2006. Epidemiology of infections caused by extended-

spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella spp*.: a nested case-control study from a tertiary hospital in London. J Hosp Infect 64: 115-23.
21. Smith RD, Coast J, 2002. Antimicrobial resistance: a global response. Bull World Health Organ 80: 126-33.
22. Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Weshnoweski B, Johnson J, Noreddin A, Low DE, Karlowsky JA, Hoban DJ, 2006. Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents 27: 468-75.