Jundishapur J Microbiol. 2017 May; 10(5):e13098. doi: 10.5812/jjm.13098.
Published online 2017 May 17. **Research Article**

Antibiotic Resistance Trends and The ESBL Prevalence of *Escherichia coli* and *Klebsiella* spp Urinary Isolates in In-and Outpatients in a Tertiary Care Hospital in Istanbul, 2004 - 2012

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Received 2016 November 16; **Revised** 2017 April 05; **Accepted** 2017 April 05.

Abstract

Background: Extended spectrum beta-lactamase (ESBL) producing organisms causing urinary tract infections are increasing in incidence and becoming a serious health problem due to their resistance to large number of antibiotics.
Objectives: To investigate the ESBL prevalence of *Escherichia coli* and *Klebsiella* spp. which are isolated from urine samples for both in and outpatients with their resistance profiles.
Methods: From 2004 to 2012, a total of 13975 isolates (12897 *E. coli*, 1078 *Klebsiella* spp.) were included in this study. The antibiotic susceptibility was tested using Kirby–Bauer disk diffusion method and Vitek2 System (bioMerieux, France) according to CLSI.
Results: Our data showed a significant increasing in ESBL prevalence from 12.5% to 44.7% ($P < 0.001$) for inpatients; from 9.6% to 22.8% ($P < 0.001$) for outpatients in *E. coli* and from 25% to 60.5% ($P < 0.003$) for inpatients; from 12% to 25% ($P < 0.095$) for outpatients in *Klebsiella* spp. For *E. coli*, the increase was significantly high in both of males and females ($P < 0.001$). However, for *Klebsiella* spp. it was significantly high in male patients ($P < 0.001$). The resistance rates of antibiotics for the ESBL producing *E. coli*, and *Klebsiella* spp. showed a significant increase. These rates were higher than 70% for fluoroquinolones, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole. Even carbepenem resistance reached to 7% in the outpatients and 15% in inpatients for ESBL producing *Klebsiella* spp.
Conclusions: Our study demonstrated a significant increase in the prevalence of ESBL producing *E. coli* and *Klebsiella* spp. and a remarkable carbapenem resistance trend in the ESBL producing *Klebsiella* spp. isolated from urine samples.

Keywords: Urinary Tract Infection, ESBL, Antibiotic Resistance, Outpatient, Inpatient

1. Background

Urinary tract infections (UTIs) are among the most common infections which diagnosed in both hospitalized cases and community setting (1-3). The prevalence of extended spectrum beta-lactamases (ESBL) producing uropathogenic bacteria has increased in the recent years (4-6). These microorganisms derived from urinary tract that commonly cause sepsis, respiratory tract, and intra-abdominal infection (7).

Extended-spectrum of beta-lactamases are enzymes that able to inactivate beta-lactam antibiotics such as penicillins, cephalosporins, and monobactams by hydrolysis. Extended-Spectrum of Beta-Lactamases can be transferred basically on plasmids, hydrolyze third generation cephalosporins which are inhibited by clavulanic acid, tazobactam or sulbactam (7). The ESBL enzymes are predominantly found in *Escherichia coli* and *Klebsiella* spp. however, may also be found in other species of *Enterobacteriaceae* (4, 5, 8). The treatment of infections caused by ESBL-producing bacteria is often complicated by the concomitant resistance to other classes of antibiotics such as fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole (9).

2. Objectives

This study aimed to investigate the ESBL prevalence of *E. coli* and *Klebsiella* spp. which isolated from urine samples from in and outpatients with their resistance profiles during nine years period; in order to provide the microbiolog-

ical point of view in anti-infective management of urinary tract infections.

3. Methods

Our data was collected from the Haydarpasa Numune training and research hospital (HNEAH), which has a 725 bed capacity, in the period between January 2004 and December 2012, retrospectively. The patients were demonstrated clinical symptoms compatible with urinary tract infection from the outpatient clinics and inpatients after 48 hours of hospitalization. Ethical approval was granted by Haydarpasa Numune training and research hospital ethical committee (HNEAH-KAEK/09).

The mid-stream urine specimens were transported to the Microbiology laboratory, and were then inoculated in 5% sheep blood agar (Oxoid, United Kingdom) and MacConkey agar (Oxoid, United Kingdom) by a calibrated loop of 10 μL using the streak plate method immediately. The growth of the $\geq 10^5$ colony forming units per milliliter in the culture was included in our study. The duplicate isolates from the same patient were excluded.

Overall, 13975 clinical isolates were analyzed and identified by standard culture, biochemical characteristics and differentiated to species level using BBL Enteric/Nonfermenter ID Kit (Becton Dickinson, USA) and Vitek2 system (bioMerieux, France). Antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method and microbroth dilution assay using Vitek2 system (bioMerieux, France) according to clinical and laboratory standards institute (CLSI) criteria (10). The following antibiotics (Antimicrobial Susceptibility Disks, Oxoid^{TM}, United Kingdom) were tested: amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, gentamicin, amikacin, cefoperazone-sulbactam, ciprofloxacin, levofloxacin, ertapenem, imipenem, meropenem and nitrofurantoin. The isolates were classified as susceptible, intermediate or resistant, according to the breakpoints established by the CLSI (10). The ESBL producers were detected by CLSI double disk diffusion method of three different beta-lactam antibiotics (ceftazidim 30 μg, cefotaxime 30 μg and cefuroxime 30 μg) with and without clavulanic acid (11). The quality control was performed by testing *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603.

3.1. Statistical Methods

The statistical analysis was performed with hypothesis test in the NCSS (Number cruncher statistical system) 2007&PASS (power analysis and sample size) 2008 Statistical Software (Utah, USA) program. In addition, in order to evaluate the study, data with definitive statistical methods (average, standard deviation, median, frequency, and ratio), for the comparison of the qualitative data Pearson Chi Square test, the Yates continuity correction test, the Fisher's Exact test, and for the trend analysis, the Chi-Square test linear-by-linear test were used. The Pearson's correlation analysis was used for the correlation between the ESBL proportions in years. The results were considered in 95% confidence intervals, and statistically significant with P values < 0.05.

4. Results

A total of 212,521 urine samples of patients were analyzed and 27,901 of them were positive for growth. The overall study included 12,897 *E. coli* and 1078 *Klebsiella* spp. isolates that 9,299 (72.1%) *E. coli* and 629 (58.3%) *Klebsiella* spp were isolated from female patients. 2461 *E. coli* (19%) and 365 (33.8%) *Klebsiella* spp. were isolated from inpatients. 1715 (13.3%) *E. coli* and 202 (18.7%) *Klebsiella* spp isolates were ESBL producers.

4.1. Escherichia coli

Between 2004 and 2012, the prevalence of ESBL producing *E. coli* increased significantly from 12.5% to 44.7% ($P < 0.001$) for inpatients and from 9.6% to 22.8% ($P < 0.001$) for outpatients. This time trend was linear and has an increasing rate in the ESBL producing isolates which were observed in all patient groups (Figure 1). Of the 2641 inpatients, 1906 (72.1%) with 7393 of the outpatients 5,323 (72%) being female. When 2004 was compared to 2012 for ESBL prevalence, there was a significant increase in both sexes ($P < 0.001$) (Table 1).

The resistance to the first line choice nitrofurantoin for the uncomplicated UTI was as low as 3% for the non-ESBL producer and 10% for the producing *E. coli* isolates in 2012, regardless of being an in or outpatient. The resistance rates of amoxicillin-clavulanic acid increased significantly in inpatients; especially for the ESBL producers. The susceptibility of the non-ESBL producer isolates fluoroquinolones and trimethoprim-sulfamethoxazole was significantly increased. The highest susceptibility detected for amikacin and there was no resistance to carbapenems. For all the antibiotics tested, the ESBL-producing *E. coli* isolates showed lower susceptibility rates compared to the non-producers ($P < 0.05$). There was no significant resistance difference as far as ESBL-producer isolates were concerned in both in- and outpatients. The resistance trends over these years are presented with details in Table 2.

4.2. Klebsiella spp.

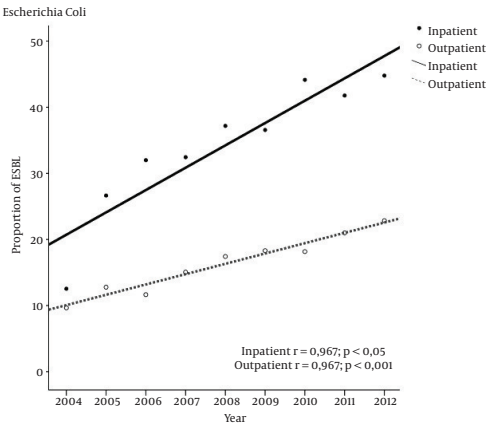
During 2004 to 2012, the prevalence of ESBL producing *Klebsiella* spp. increased from 25% to 60.5% ($P < 0.003$) for

Table 1. Number and Proportion of ESBL Producing *Escherichia coli* and *Klebsiella* spp Urinary isolates, 2004 and 2012[a]

	Escherichia coli			*Klebsiella* spp		
	2004	2012	P Value[b]	2004	2012	P Value[b]
All	99/947 (10.5)	400/1803 (22.2)	0.001[c]	13/78 (16.7)	74/188 (39.4)	0.001[c]
In-outpatient						
Inpatients	33/263 (12.5)	180/402 (44.8)	0.001[c]	7/28 (25.0)	46/76 (60.5)	0.003[c,d]
Outpatients	66/684 (9.6)	320/1401 (22.8)	0. 001[c]	6/50 (12.0)	28/112 (25.0)	0.095[d]
Sex						
Female	68/631 (10.8)	468/1414 (33.1)	0.001[c]	3/24 (12.5)	37/118 (31.4)	0.105[d]
Male	31/316 (9.8)	132/389 (33.9)	0.001[c]	10/54 (18.5)	37/70 (52.9)	0.001[c]
Sex-inpatient						
Inpatients-Female	27/193 (14.0)	129/293 (44.0)	0.001[c]	3/10 (30.0)	24/42 (57.1)	0.167[e]
Inpatients-Male	6/70 (8.6)	51/109 (46.8)	0.001[c]	4/18 (22.2)	22/34 (64.7)	0.009[c,d]
Sex-outpatient						
Outpatients-Female	41/438 (9.4)	239/1121 (21.3)	0.001[c]	0/14 (0.0)	13/76 (17.1)	0.207[e]
Outpatients-Male	25/246 (10.2)	81/280 (28.9)	0.001[c]	6/36 (16.7)	15/36 (41.7)	0.038[d]

[a] Values are expressed as No. (%).
[b] Pearson ki kare test.
[c] P < 0.001.
[d] Yates Continuity Correction.
[e] Fisher's Exact test.

Figure 1. Increasing in ESBL Producing Rates in *Escherichia coli* Both In-and Outpatients, 2004 - 2102



P, significance for correlation; r, Pearson's correlation index. Means and standard error of the means of ESBL producing rates from 2004 to 2012. The linear regression showed significant increase over time for *E. coli* both in-and outpatients (P < 0.001) for both, correlation was linear (P < 0.001) for both.

inpatients and from 12% to 25% (P < 0.095) for outpatients (Table 1). The increase was significant and linear in both groups (P < 0.005) (Figure 2). 195 inpatients (53.4%) and 434 outpatients (60.8%) were female. The ESBL prevalence significantly increased in the male inpatients in particular (P < 0.001) from 2004 to 2012.

The resistance rate of the amoxicillin-clavulanic acid and cefoperazone-sulbactam to ESBL producing *Klebsiella* spp. increased in all patients. For inpatients, the susceptibility rates of fluoroquinolones in the ESBL producers decreased, whereas fluctuations were observed in other groups. There was no significant difference in the susceptibility rates for gentamicin, amikacin, and nitrofurantoin among the groups. Interestingly, the trimethoprim-sulfamethoxazole resistance significantly decreased for non-ESBL producers in outpatients, whereas it's significantly increased in other groups. In general, the ESBL producing isolates had increased resistance rates in all antibiotics when compared to the non ESBL producers. The ertapenem resistance among the ESBL-producing *Klebsiella* spp. isolates emerged in 2008 and 2009 in inpatients and outpatients, respectively. The carbepenem resistance reached to 7% in the outpatients and 15% in inpatients (Table 3).

5. Discussion

This is a nine-year surveillance study in order to evaluate the antibiotic resistance patterns and ESBL prevalence of *E. coli* and *Klebsiella* spp. which is recovered from UTI

Table 2. Trends in Antibiotic Resistance Among ESBL Producing and Non-ESBL Producing *E. coli* Both in-and Out Patient Between 2004 and 2012

Antibiotic	Patient	ESBL	2004	2005	2006	2007	2008	2009	2010	2011	2012	P Value	Trend
Amoxicillin-Clavulonic acid	Inpatients	[-]	27	23,9	22,8	25	27	24,3	29,4	33,2	32	*[a]	↑
		[+]	63,6	60,3	68,4	64,4	73,8	69,4	80,5	89,4	93,3	**[b]	↑
	Outpatients	[-]	22,3	14,5	19,8	19,9	21,2	19,7	22,1	23,3	27,9	**	↑
		[+]	47	50,4	46,5	45	47,4	39,4	37,8	44,9	50,6		↑
Cefoperazone-Sulbactam	Inpatients	[+]	27,3	28,8	27,4	13,7	15,5	14,9	15	18,9	26,7		↓
	Outpatients	[+]	16,7	16,8	18,9	10,6	9,6	6,9	5,2	6,6	13,1	**	↓
Amikacin	Inpatients	[-]	3,5	3,5	4,5	3,3	3,4	2,4	2,1	2,2	1,8		↓
		[+]	3	4,1	3,2	4,1	6,8	8,3	5,3	6,1	7,2		↑
	Outpatients	[-]	0,8	3,6	3,2	2	2,7	1,3	1	1	0,9	**	↑
		[+]	3	3,5	3,9	3,8	3,8	4,5	4,3	4,4	5,9		↑
Gentamicin	Inpatients	[-]	22,6	10,9	15,3	19,1	18,4	17,1	14,7	13,6	12,2	*	↓
		[+]	51,5	37	42,1	50,7	42,7	38	52,2	56,1	49,4	*	↓
	Outpatients	[-]	11	14,1	18,4	17,9	17,2	16,6	16,7	14	12,1		↑
		[+]	40,9	42,5	39,4	40	38,3	38,2	41,2	36,9	39,4		↓
Ciprofloxacin	Inpatients	[-]	44,3	43,8	45	47,4	44,8	38,1	35	33,7	31,1	**	↓
		[+]	72,7	75,3	83,2	83,6	81,6	71,9	69,9	75	72,8		↑
	Outpatients	[-]	32	36,6	36,8	33,1	34,9	32,6	31,5	29,1	20,5	**	↓
		[+]	75,8	72,6	72,4	80	77,5	67,5	66,1	68,2	70	*	↓
Levofloxacin	Inpatients	[-]	40,4	44,3	45,5	46,7	43,7	35,2	34,3	31	29,7	**	↓
		[+]	66,7	74	85,3	84,9	78,6	69,4	65,5	77,3	71,7		↑
	Outpatients	[-]	35,6	37,4	36,5	32,4	35	33,8	31	28,9	19,8	**	↓
		[+]	72,7	69,9	73,2	70	76,1	65,4	64,4	66,1	68,4		↓
Nitrofurantoin	Inpatients	[-]	13	11,4	5,9	4,6	6,9	4,3	4,2	3,8	2,3	**	↓
		[+]	6,1	12,3	17,9	17,8	14,6	15,7	12,4	11,4	10,6		↑
	Outpatients	[-]	9,9	9,3	7,7	5,3	5,8	5,6	4	3,6	2,2	**	↓
		[+]	19,7	13,3	9,4	11,3	11,5	13,8	12,4	9,9	9,7		↓
Trimethoprim-Sulfamethoxazole	Inpatients	[-]	52,2	50,7	52,5	46,7	52,3	45,2	41,3	42,9	32,4	**	↓
		[+]	45,5	52,1	78,9	67,1	73,8	65,3	72,6	74,2	68,9	*	↑
	Outpatients	[-]	59,9	47	47,1	45	42,8	45,2	40,2	40,7	27,8	**	↓
		[+]	66,7	54,9	59,1	67,5	61,2	62,6	56,7	54	62,5		↓

[a] $P < 0.05$.
[b] $P < 0.01$.

among in and outpatients in a tertiary care hospital. Routinely, the data that collected in targeted clinics on antibiotic resistance, is remarkable for developing guidelines, which been used in our hospital since 2003.

The ESBL production among *Enterobacteriaceae* is a worldwide concern. The rates have been reported in a variety of studies as 1.7% to 19% (8, 11-14). In our study, the ESBL production rates were 13.3% and 18.7% for *E. coli* and *Klebsiella* spp., respectively. The studies reporting ESBL production rates in our country showed similar results (15, 16). Despite the common increasing in ESBL production in both of in and outpatients, this rates can vary among both regions and hospitals. The data on the prevalence of ESBL isolates among outpatients are rarely reported (17-19).

5.1. Escherichia coli

Extended-spectrum of beta-lactamases producing *E. coli* has become the most troublesome causative agent of UTIs. In 2012, the overall prevalence in our study among inpatients and outpatients was 44.7% and 22.8%, respectively. The lower rates were reported in outpatients from Turkey (16, 17, 20). Although in Netherlands, the prevalence of ESBL-producing *E. coli* in outpatients is lower than our country, there was a significant increasing from 2004 (0.1%) to 2009 (1%) (18). However, China (21) and Tanzania (22) have higher rates when compared to our study. The prevalence of ESBL-producing *E. coli* for inpatients in Switzerland is 6.6% (23), 10.4% in Saudi Arabia (8) and 52.9% in China (21). ESBL producing *E. coli* incidences was significantly higher in females (73.6%) than males (26.4%) at the end of study.

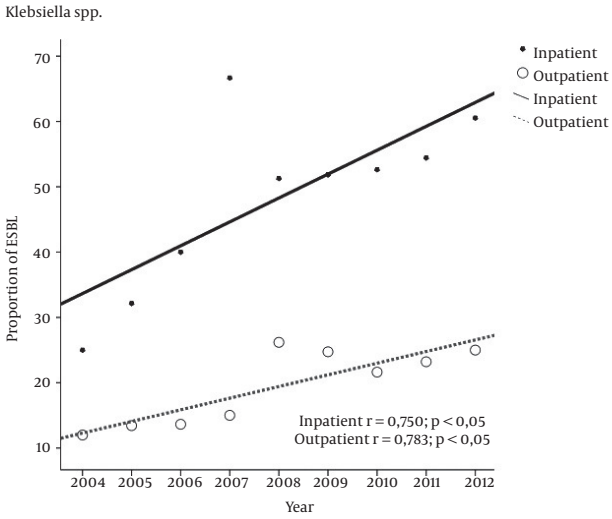
Table 3. Trends in Antibiotic Resistance Among ESBL Producing and Non-ESBL Producing *Klebsiella* spp. Both In-and Out Patient Between 2004 and 2012

Antibiotic	Patient	ESBL	2004	2005	2006	2007	2008	2009	2010	2011	2012	P Value	Trend
Amoxicillin-Clavulonic acid	Inpatients	[-]	23,8	21,1	40	50	47,4	38,5	40,7	33,3	40		↑
		[+]	42,9	66,7	60	75	90	85,7	80	81,4	95,7	**[a]	↑
	Outpatients	[-]	20,5	17,2	21,1	26,5	38,7	41,4	36,8	40,7	40,5	**	↑
		[+]	50	44,4	66,7	66,7	77,3	69,6	75	76,9	89,3	**	↑
Cefoperazone-Sulbactam	Inpatients	[+]	14,3	33,3	30	25	40	35,7	53,3	51,2	54,3	**	↑
		[+]	16,7	22,2	33,3	50	36,4	47,8	41,7	42,3	50		↑
Gentamicin	Outpatients	[-]	19	10,5	20	50	31,6	30,8	22,2	22,2	20		↑
		[+]	28,6	33,3	30	50	20	28,6	36,7	37,2	39,1		↑
	Inpatients	[-]	18,2	12,1	15,8	14,7	12,9	11,4	17,2	19,8	20,2		↑
		[+]	33,3	22,2	33,3	33,3	36,4	30,4	33,3	38,5	35,7		↑
Amikacin	Outpatients	[-]	9,5	10,5	6,7	0	15,8	7,7	7,4	8,3	6,7		↓
		[+]	14,3	22,2	20	25	25	21,4	13,3	14	10,9		↓
	Inpatients	[-]	4,5	5,2	2,6	2,9	6,5	4,3	3,4	5,8	6		↑
		[+]	16,7	22,2	16,7	16,7	13,6	8,7	8,3	11,5	7,1		↓
Levofloxacin	Inpatients	[-]	23,8	26,3	33,3	50	42,1	46,2	48,1	38,9	36,7		↑
		[+]	42,9	44,4	50	75	60	50	60	76,7	71,7	*[b]	↑
	Outpatients	[-]	29,5	24,1	23,7	23,5	29	21,4	20,7	24,4	36,9		↑
		[+]	66,7	55,6	66,7	66,7	63,6	43,5	45,8	61,5	60,7		↓
Ciprofloxacin	Inpatients	[-]	28,6	21,1	33,3	50	47,4	53,8	51,9	38,9	40		↑
		[+]	57,1	55,6	50	75	65	57,1	60	76,7	69,6		↑
	Outpatients	[-]	31,8	25,9	26,3	26,5	30,6	20	19,5	25,6	35,7		↑
		[+]	66,7	55,6	66,7	66,7	59,1	47,8	50	61,5	64,3		↓
Nitrofurantoin	Inpatients	[-]	33,3	31,6	46,7	50	52,6	46,2	33,3	44,4	43,3		↑
		[+]	42,9	33,3	50	50	60	57,1	50	65,1	78,3	**	↑
	Outpatients	[-]	43,2	34,5	31,6	47,1	48,4	40	47,1	46,5	42,9		↓
		[+]	83,3	55,6	66,7	50	45,5	47,8	58,3	57,7	75		↓
Trimethoprim-Sulfamethoxazole	Inpatients	[-]	23,8	26,3	33,3	50	47,4	38,5	29,6	22,2	16,7		↓
		[+]	57,1	44,4	60	75	60	71,4	80	74,4	71,7	*	↑
	Outpatients	[-]	47,7	41,4	36,8	35,3	43,5	30	25,3	22,1	23,8	**	↓
		[+]	66,7	55,6	83,3	66,7	54,5	60,9	79,2	73,1	71,4		↑
Ertapenem	Inpatients	[+]	0	0	0	0	20	21,4	13,3	18,6	15,2		↓
	Outpatients	[+]	0	0	0	0	0	13	8,3	19,2	10,7		↓
Imipenem	Inpatients	[+]	0	0	0	0	0	14,3	10	14	15,2		↑
	Outpatients	[+]	0	0	0	0	0	0	8,3	3,8	7,1		↓
Meropenem	Inpatients	[+]	0	0	0	0	0	14,3	10	14	13		↓
	Outpatients	[+]	0	0	0	0	0	0	8,3	3,8	7,1		↓

[a] $P < 0.01$.
[b] $P < 0.05$.

The resistance to different antibiotics in ESBL producing *E. coli* is frequent and at least in part, due to the fact that genes coding is located on the same plasmids (6). In both inpatients and outpatients, roughly 70% of ESBL producing *E. coli* were resistant to both trimethoprim-sulfamethoxazole and fluoroquinolones. Several studies have been shown fluoroquinolones and trimethoprim-sulfamethoxazole to be highly active against *E. coli* (24-28) in contrast to dramatically decreasing susceptibility rates among ESBL-producers (20, 21, 25, 29). Fluoroquinolone resistance was reported 85% in ESBL producing *E. coli* isolates in a study in Nepal in 2012 (29) and in 2010 Italian study reported this rate to be even higher (85% - 90%) in ESBL-producing *E. coli* isolates (30). The trimethoprim sulfamethoxazole and fluoroquinolones susceptibility rates in non-ESBL-producing *E. coli* were between 20% and 30% in recent studies. These drugs are not useful for empirical treatment however, if the specific ESBL-producing microorganism is finally susceptible, these antibiotics could be used. The nitrofurantoin susceptibility rates for both non-ESBL and ESBL-producing *E. coli* were 98% and 90%, which were the same as those that observed in similar stud-

Figure 2. Increasing in ESBL Producing Rates in *Klebsiella* spp. Both In-and Outpatients, 2004 - 2102



P, significance for correlation; r, Pearson's correlation index. The linear regression showed an increasing over the time for *Klebsiella* spp in inpatients (P = 0.021), in outpatients (P = 0.004); correlation was linear for both (P = 0.021), (P = 0.004) respectively.

ies (21, 29, 31). A study conducted in Brazil reported a higher resistance rate of 35% for nitrofurantoin in ESBL-producing *E. coli* (19). Also a review article from Turkey showed similar susceptibility rates, just as in our study for *E. coli* (20), suggesting the use of nitrofurantoin for the first-line empirical oral treatment of community-acquired uncomplicated UTIs.

The resistance rate of amoxicillin-clavulanic acid was 90% in inpatients and 50% in outpatients respectively, for ESBL-producing *E. coli*; therefore it may not be a good choice in our region for UTIs. Some other studies showed similar results but some found higher susceptibility rates in ESBL-producing *E. coli* isolates (18, 21, 29).

Amikacin was found to have a high activity against all *E. coli*, however gentamicin had higher resistance rates in ESBL-producing *E. coli* as shown in other studies (13, 29, 30). Our findings showed that all *E. coli* exhibited a 100% susceptibility to carbapenems, therefore they were the most effective choice of drug against ESBL producing *E. coli*. However, it is a concern for spreading of carbapenemase producers which will increase the incidence of MDR bacteria.

5.2. Klebsiella spp.

The prevalence rates of ESBL producing *Klebsiella* spp. are increasing worldwide. Our rates in 2012, with an increasing trend, reached to 60.5% and 25% for both inpatients and outpatients, respectively. Similar results were reported in a previous study (14). An approximation frequency of 24% was observed in Turkey (16), 41.5% in Tanzania (22) and 16.5% in Nepal (29). The rates of ESBL producing *Klebsiella* spp. increased in male inpatients in particular, in our study.

The resistance to antibiotic classes was even higher than in *E. coli*. The ESBL-producing *Klebsiella* spp. resistance rates in all patients in 2012 were above 60% for all of the antibiotics tested, except for cefaperazone-sulbactam, aminoglycosides, and carbapenems. The resistance rates were reasonably high and maximally resistant (95.7%) to amoxicillin-clavulanic acid. A high resistance of 78.3%, 71.7%, and approximately 70%, were shown in nitrofurantoine, trimethoprim-sulfamethoxazole and fluoroquinolones, respectively. A moderate resistance of 50.4% was shown towards cefoperazone-sulbactam and 39.1% to gentamicin. The amikacin (10.9% resistance) was the most effective antibiotic. A study in Tanzania also showed similar results (22). In other studies which conducted in Italy (30), Brazil (14), and Nepal (29), the resistance rates were lower compared to our results.

The carbapenem resistance was alarming against the ESBL-producers. It's increased from 0% to 15% for the inpatients. Some of studies that conducted in other countries have not reported such high resistance for carbapenems in UTIs (25, 26). Recently, carbapenems have been increasingly used as an empirical treatment for complicated UTIs and these results affect in promoting the selection of drug-resistant bacteria and an increasing prevalence of fungal infections and flora imbalance.

Non-ESBL producing *Klebsiella* spp. had a remarkable increasing resistance for nitrofurantoine compared to *E. coli*. On the contrary, the trimethoprim-sulfamethoxazole resistance was decreased from 47.7% to 23.8% due to the limited using. Therefore, it can be a preferred choice in UTIs. Similar results were reported from Netherlands (26). However, a study carried out in Tanzania, reported a high rate for the trimethoprim-sulfamethoxazole and a lower rate for nitrofurantoine (22).

The major limitation in our study was that we could not classify complicated or uncomplicated UTIs due to the lack of information on the database. Moreover, the molecular characterization of the ESBL isolates could not be studied. This is a study that was conducted for long-term as it needed nine years in order to evaluate the antibiotic resistance of ESBL-producing and non-producing *E. coli* and *Klebsiella* spp. isolates which were recovered from both in and outpatients in both of sexes.

In conclusion, our study demonstrated a significant increase in prevalence of ESBL producing *E. coli* and *Klebsiella* spp. during 2004 to 2012 for UTIs. The ESBL prevalence of *E. coli* significantly increased in both sexes and also

in-outpatients, however for the *Klebsiella* spp., it's significantly increased just in male inpatients. The resistance rates of antibiotics, especially fluoroquinolones, showed a significant increase for ESBL producing *E. coli* and *Klebsiella* spp. The carbapenem resistance was alarming against the ESBL producing *Klebsiella* spp. The nitrofurantoin may be the first line choice for the uncomplicated UTI caused by *E. coli*. The trimethoprim-sulfamethoxazole may be a good choice for treatment of non-ESBL *E. coli* and *Klebsiella* spp. Regular surveillance studies need to be performed in order to select adequate empirical antibiotic regimens and controlling the antibiotic resistance, especially monitoring of ESBLs.

Footnotes

Authors' Contribution: Seniha Senbayrak, Efe Serkan Boz and Simin Cevan designed and conducted the study. Seniha Senbayrak, Efe Serkan Boz, Simin Cevan, Nilgun Dosoglu, Naz Cobanoglu, and Ismail Davarci coordinated and performed all the laboratory analyses. Seniha Senbayrak, Efe Serkan Boz, Asuman Inan and Derya Ozturk Engin analyzed and interpreted all of the data. Seniha Senbayrak, Efe Serkan Boz, Simin Cevan, Ozgur Dagli and Sebahat Aksaray wrote a first draft of paper. All authors read, commented on and approved the final manuscript.

Conflict of Interest: The authors declare that no conflicting of interest.

Financial Disclosure: The authors declare that no competing financial interests.

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