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High Levels of Multidrug Resistance in Surgical Wound Isolates of *Escherichia coli*

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The objectives of this study were to determine antibiotic resistance of *E.coli* isolates and evaluate probable relationship between multidrug-resistance patterns and clinical specimens. In this study, 500 *E.coli* isolates obtained from different clinical specimens were collected from a research and private hospital in Turkey, during two years. Antibiotic resistance tests were performed by automated system. Results were available for *E.coli* isolates that had been tested against fourteen selected antimicrobial agents. Of these isolates 37.2% were resistant to three or more agents and considered as multidrug-resistant (MDR). Resistance rates for MDR *E.coli* isolates against cefazolin, ampicillin-clavulanate, ceftriaxone, ceftazidime, trimethoprim-sulfamethoxazole, ciprofloxacin, tobramycin and levofloxacin were 80.6%, 76.3%, 72.6%, 72%, 62.9%, 61.8% and 60.8% respectively. Surprisingly MDR was higher among wound isolates than the other clinical specimens. The phenotypes among MDR *E.coli* isolated from wound included resistance to all antimicrobial agent except for amikasin and imipenem. This was the common phenotype observed to be higher among surgery wards than others wards. This is the first report showing definitive relationship between MDR rates and wound samples in Turkey. These findings showed a need for regular monitoring of antimicrobial drug resistance and preventing inappropriate use of antibiotics by the patients applied to private hospitals.

Key words: *Escherichia coli*, multidrug-resistance, surgical wound.

Pathogenic *Escherichia coli* can cause variety of infectious diseases, including septicemia, newborn meningitis, intestinal and urinary tract infections (UTI)[1-3]. These infections caused by multidrug resistant isolates increased significantly over the last decade. These isolates also frequently occur in inpatients and may lead to dire consequences[4-6].

Antibiotic resistance mechanisms appeared to be increasing among *Enterobacteriaceae*. Much of the problem has been shown to be due to the presence of transferable plasmids encoding multidrug resistance among different these species[7]. *E.coli* has a multidrug-resistance to fluoroquinolones, beta lactamase inhibitors, co-trimoxazole and aminoglycosides. Treatment is difficult because of frequent resistance. This resistance leads to limit treatment options and may affect the prognosis of the *E.coli* infections[8,9]. Alternative treatment options of these infections included carbapenems, amikacin, and β-lactam inhibitor combinations[10]. Nowadays, carbapenems are commonly used to treat these infections but in time carbapenemase producers will also increase among MDR species inevitably.

The objectives of this study were to determine antibiotic resistance of 500 *E.coli* isolates obtained from different clinical specimens between January 2010 and December 2011 in a

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research and private hospital, Turkey and evaluate probable relationship between multidrug resistance patterns and clinical specimens or wards.

MATERIALS AND METHODS

Sample collection and analysis

In this study 500 *Escherichia coli* isolates were obtained from different clinical specimens including urine, wound, blood, abscess, tracheal secretion, steril body fluid, catheter, bile, bronchoscopic bronchoalveolar lavage and thorasynthesis liquid. The specimens were collected from a research and private hospital, Ankara, Turkey, between January 2010 and December 2011.

The identification of strains and their antibiotic resistance rates against aminoglycosides (amikacin, gentamicin and tobramycin), beta lactamase inhibitors (ampicillin-clavulanate and piperacillin-tazobactam), carbapenem (imipenem), cephalosporins (cefazolin, ceftazidime, ceftriaxone and cefotetan), quinolones (ciprofloxacin and levofloxacin), nitrofurantoin and trimethoprim sulfamethoxazole were determined by VITEK-32 automated system (bioMérieux, Fransa) and evaluated according to Clinical and Laboratory Standards Institute (CLSI) criteria. *Escherichia coli* ATCC 25922 were used as reference.

Statistical analysis

Clinical data were analyzed using SPSS 15.0 software package for Windows. Chi-square and Fisher's Exact Test were performed. A difference was considered highly significant if the probability that chance would explain the results was reduced to less than $p \leq 0.001$.

RESULTS

Results were available for 500 *E.coli* isolates that had been tested against 14 selected antimicrobial agents of different classes. Among the beta lactam antibiotics, imipenem was found to be the best efficient antibiotic against all *E. coli* isolates (100%), followed by amikacin and nitrofurantoin (99.2%) showed in Table 1. Of these isolates 37.2% (186 of 500) were multidrug resistant. All MDR *E.coli* isolates were highly resistant to cefazolin (80.6%), ampicillin-clavulanate (76.3%), ceftriaxone (72.6%), ceftazidime (72%), trimethoprim-sulfamethoxazole (62.9%), ciprofloxacin (61.8%), tobramycin and levofloxacin (60.8%), gentamisin (45.7%), piperacillin-tazobactam (29.6%) and cefotetane (12.4%). Whereas, all these isolates were found to be more effective with significant percent susceptibility against imipenem (100%), nitrofurantoin (98.4%) and amikacin (97.8%). A statistically significant difference has been found for the resistance of all *E.coli* and MDR *E.coli* strains against all antibiotics in this study except imipenem, amikacin and nitrofurantoin ($p<0.001$).

Table 1. Antimicrobial resistance of *E. coli* isolated from clinical samples

Antimicrobial agent	% Total isolates (no.)	% MDR isolates (no.)	p value
Amicasin	0.08 (4)	2.2 (4)	0.222
Gentamicin	18.8 (94)	45.7 (85)	<.001
Tobramycin	23.8 (119)	60.8 (113)	<.001
AMC	37.2 (186)	76.3 (142)	<.001
PPT	11.4 (57)	29.6 (55)	<.001
Imipenem	0	0	1.000
Cefazolin	30.2 (151)	80.6 (150)	<.001
Cefotetane	4.6 (23)	12.4 (23)	<.001
Ceftazidime	26.8 (134)	72 (134)	<.001
Ceftriaxone	27 (135)	72.6 (135)	<.001
Ciprofloxacin	24 (120)	61.8 (115)	<.001
Levofloxacin	23.4 (117)	60.8 (113)	<.001
TMS	35.2 (176)	62.9 (117)	<.001
Nitrofurantoin	0.08 (4)	1.6 (3)	0.396

AMC; Ampicillin-clavulanate, PPT; Piperacillin-tazobactam, TMS; Trimethoprim sulfamethoxazole

Table 2. Resistance to one or more antimicrobials among 500 *E.coli* isolates tested against thirteen antimicrobials

Number of antibiotics to which isolates were resistant	Total % of clinical isolates (no.)	% Clinical isolates (no.) resistant to:												
		Aminoglycosides			β-lactamase inhibitors		Cephalosporins			Quinolones			Others	
		Amk	Gm	To	Amc	Ppt	Cz	Ctt	Caz	Cft	Cp	Lvx	Tms	Ntf
0	44.6 (223)													
1	10.2 (51)	0	0	0	33.3 (17)	0	0	0	0	0	2 (1)	0	62.7 (32)	2 (1)
2	8 (40)	0	22.5 (9)	15 (6)	67.5 (27)	5 (2)	2.5 (1)	0	0	0	10 (4)	10 (4)	67.5 (27)	0
3[a]	5.8 (29)	0	27.6 (8)	20.7 (6)	34.5 (10)	10.3 (9)	31 (9)	0	13.8 (4)	13.8 (4)	41.4 (12)	37.9 (11)	65.5 (19)	3.4 (1)
4[a]	2.8 (14)	0	14.3 (2)	21.4 (3)	64.3 (9)	28.6 (4)	50 (7)	0	28.6 (4)	28.6 (4)	50 (7)	50 (7)	64.3 (9)	0
5[a]	5 (25)	0	28 (7)	36 (9)	68 (17)	32 (8)	76 (19)	8 (2)	68 (17)	68 (17)	40 (10)	40 (10)	32 (8)	4 (1)
6[a]	4.4 (22)	0	50 (11)	50 (11)	68.2 (15)	31.8 (7)	86.4 (19)	13.6 (3)	68.2 (15)	68.2 (15)	50 (11)	45.5 (10)	68.2 (15)	0
7[a]	5.4 (27)	0	33.3 (9)	70.4 (19)	92.6 (25)	7.4 (2)	100 (27)	14.8 (4)	100 (27)	100 (27)	70.4 (19)	70.4 (19)	40.7 (11)	0
8[a]	7 (35)	0	60 (21)	88.6 (31)	91.4 (32)	40 (14)	100 (35)	14.3 (5)	94.3 (33)	97.1 (34)	68.6 (24)	68.6 (24)	74.3 (26)	2.9 (1)
9[a]	4.4 (22)	4.5 (1)	72.7 (16)	100 (22)	100 (22)	40.9 (9)	100 (22)	18.2 (4)	100 (22)	100 (22)	90.9 (20)	90.9 (20)	81.8 (18)	0
10-12[a]	2.4 (12)	25 (3)	91.7 (11)	100 (12)	100 (12)	66.7 (8)	100 (12)	41.7 (5)	100 (12)	100 (12)	100 (12)	100 (12)	91.7 (11)	0

[a]37.2% (186 of 500) of isolates were resistant to three or more antimicrobials and defined as MDR.

Amc = ampicillin-clavulanate; Amk = amikacin; Caz = ceftazidime; Cft = ceftriaxone; Cp = ciprofloxacin; Ctt = cefotetane; Cz = cefazolin; Gm = gentamicin; Lvx = levofloxacin; Ntf = nitrofurantoin; Ppt = piperacillin-tazobactam; Tms = trimethoprim sulfamethoxazole; To = tobramycin.

Table 3. Distribution of MDR *E.coli* from various clinical specimens

Clinical specimens	Total no. of isolate	MDR isolates (%)
Wound	24	22 (91.7)
Others*	13	11 (84.6)
Blood	14	8 (57.1)
Abscess	13	5 (38.5)
Urine	436	140 (3.2)

Others*; bile, body fluid, bronchoscopic bronchoalveolar lavage, catheter, thorasynthesis liquid, tracheal secretion

Among 500 *E.coli* isolates, a 44.6% majority of these was susceptible to all the agents studied (Table 2) and 10.2% were resistant to a single agent predominantly trimethoprim-sulfamethoxazole. MDR isolates accounted for 37.2% (n=186) of the 500 isolates. The majority of MDR isolates (n=186; 15.6%) were resistant to three antimicrobials, and these accounted for 5.8% of all isolates.

Rates of multidrug resistance were identified among the wound isolates (91.7%),

Table 4. Multidrug resistance phenotypes of *E.coli* isolates in wound samples

Antibiotimicrobial agents	Wards*
AMC, CZ, NTF	Emergency Service
GM, CZ, CAT, CFT	Surgery
GM, TO, CP, LVX, TMS	Obstetrics and Gynecology
GM, TO, CP, LVX, TMS	Surgery
CZ, CTT, CAZ, CFT, TMS	Surgery
TO, AMC, CZ, CAZ, CFT	Surgery
GM, TO, CZ, CAZ, CFT	Surgery
AMC, PPT, CZ, CAZ, CFT, TMS	Anesthesiology and Reanimation
AMC, CZ, CAZ, CFT, CP, LVX, TMS, NTF	Surgery
GM, TO, AMC, PPT, CZ, CAZ, CFT, TMS	Gastroenterology
GM, TO, AMC, PPT, CZ, CAZ, CFT, TMS	Obstetrics and Gynecology
TO, AMC, CZ, CAZ, CFT, CP, LVX, TMS	Gastroenterology
TO, CZ, CTT, CAZ, CFT, CP, LVX, TMS	Surgery
TO, CZ, CTT, CAZ, CFT, CP, LVX, TMS	Surgery
TO, AMC, CZ, CTT, CAZ, CFT, CP, LVX, TMS	Gastroenterology
GM, TO, AMC, CZ, CAZ, CFT, CP, LVX, TMS	Surgery
GM, TO, AMC, PPT, CZ, CTT, CAZ, CFT, TMS	Obstetrics and Gynecology
GM, TO, AMC, PPT, CZ, CTT, CAZ, CFT, TMS	Obstetrics and Gynecology
GM, TO, AMC, CZ, CTT, CAZ, CFT, CP, LVX, TMS	Cardiovascular Surgery
GM, TO, AMC, PPT, CZ, CAZ, CFT, CP, LVX, TMS	Surgery
GM, TO, AMC, PPT, CZ, CAZ, CFT, CP, LVX, TMS	Surgery
GM, TO, AMC, PPT, CZ, CAZ, CFT, CP, LVX, TMS	Surgery

* Surgery; cardiovascular and general surgery
AMC = ampicillin-clavulanate; CAZ = ceftazidime; CFT = ceftriaxone; CP = ciprofloxacin; CTT = cefotetane; CZ = cefazolin; GM = gentamicin; LVX = levofloxacin; NTF = nitrofurantoin; PPT = piperacillin-tazobactam; TMS = trimethoprim sulfamethoxazole; TO = tobramycin.

followed by others (84.6%) (bile, body fluid, bronchoscopic bronchoalveolar lavage, catheter, thorasynthesis liquid, tracheal secretion) (Table 3).

16 phenotypes in wound samples identified are listed in Table 4. The phenotypes among MDR *E.coli* isolated from wound included resistance to aminogly cosides except amikasin, beta lactamase inhibitors, cephalosporins, quinolones, nitrofurantoin and trimethoprim-sulfamethoxazole. It shows that among 22 *E. coli* wound isolates, the most active antibiotics were the amikasin and imipenem. In addition this was the many of phenotypes is observed to be among surgical wards 59.1% (13 of 22).

DISCUSSION

MDR Enterobacteriaceae includes the most common causative agents of nosocomial and hospital acquired infections[11,12]. *E.coli* mainly cause UTI, surgical wound infections and neonatal meningitidis. There is a relationship of drug resistance to phylogenetic groups of *E. coli* isolates from wound infections[13]. Rates of beta-lactam, fluoroquinolone, and multidrug resistance among *E. coli* isolates have been reported from many parts of the world[8,14]. Our study revealed that, multidrug resistance to fluoroquinolones, aminoglycosides, beta lactamase inhibitors, trimethoprim sulfamethoxazole and first, second and third generation cephalosporins. The resistance rates for all these antibiotics were significantly higher in MDR isolates than the other isolates ($p<0.001$).

Aminoglycosides are therapeutic alternatives to multidrug resistance *E.coli*. Gentamicin was inactive against these isolates in this study and amikacin exhibited similar activity to imipenem. Fluoroquinolone-resistance is typically encoded chromosomally. This resistance against quinolones in our study may reflect significant antibiotic pressure in the environment rather than co-carriage of this resistance gene on plasmids. Ciprofloxacin and ampicillin-clavulanic acid have commonly been used as oral therapeutic option for MDR isolates[15].

In the present study, the percentage of wound isolates especially isolated from surgery wards demonstrating MDR was extremely high as compared to rates reported in the Brazil[16]. Infections caused by MDR isolates are often treated with amikasin, sulphonamides, tigecycline, quinolones, colistin or fosfomycin and treatment options are limited for these infections[8, 14, 17]. The most successful antibiotics evading MDR Enterobacteriaceae are reported to be carbapenems, amikasin and fosfomycin. Nevertheless, excessive use of these agents due to MDR infections has caused to emerge these resistance in other nosocomial pathogens. Besides, Vardakas *et al*., (2012) investigated carbapenems versus alternative antibiotics. As it is well-known phenomenon that imipenem is still the most active agent against MDR *E.coli*[18].

In a previous study, site specific multi drug resistance rate was found to be 75.7% for wound swabs[19]. In comparison, the specimens with the highest number of MDR *E.coli* isolates were wound (91.7%) in our study.

CONCLUSIONS

Our study revealed that there is a definitive relationship between multidrug resistance rates and wound samples or surgical wards. These associations could best be explained by transmission via some materials like catheter used during or after surgery. Spreading of MDR isolates is related to a multitude of infections in hospitalized patients admitted to surgical wards with a longer duration of hospital stay. These findings showed a need for regular monitoring of antimicrobial drug resistance and preventing inappropriate use of antibiotics by the patients applied to private hospitals.

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