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Antimicrobial Resistance and Sensitivity among Isolates of *Esherichia coli* from Urine Samples in Denizli, Turkey

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ABSTRACT

Objective: The research was carried out with isolate and determines the antimicrobial sensitivity in E. coli from urinary tract infections in special hospital in Denizli and recorded at specimens.
Methods: Urine samples (n=21) were collected from patients with signs and symptoms of Urinary tract infections. Bacteria were isolated and identified by conventional biochemical profile. Antibiotic resistance pattern of E. coli against different antibiotic was determined by Kirby-Baur method.
Results: The results revealed that sensitivity rate of antimicrobial agents were in the range of meropenem (100%), norfloxacin and ciprofloxacin (86%), cefotaxime (80%), aztreonam (76%). None of the samples showed no resistance to amikacin, ceftazidime, aztreonam, amoxicillin/clavulanic acid, and meropenem. Out of 21 isolates, 3(14%) isolates showed Multiple Antibiotic Resistance ten to thirteen antibiotics.
Conclusion: It is concluded that most of the urinary tract infections in human are caused by E.coli exhibited highest resistance to meropenem (100%), followed by norfloxacin and ciprofloxacin (86%).

Key words: Escherichia coli, Antibiotic, Resistance, Prevalence, MDR

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INTRODUCTION

Antibiotic resistance is recognized worldwide as a major problem in the management of patients hospitalized with serious infections (Swartz, 1994). Turkish hospitals also face increasing numbers of antibiotic-resistant organisms including *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, Methicillin resistance in *Staphylococcus aureus*, *Escherichia coli* (Toroglu and Keskin, 2011, Toroglu *et al*., 2013, Yakupoguları *et al*., 2006).

E. coli are gram negative, facultative bacteria that ferment glucose and are members of the family Enterobacteriaceae (Feng and Weagant, 2009). They are mainly allocated in the intestine of animals and forms part of the normal intestinal flora that maintains the physiology of a healthy animal (Conway and Macfarlane, 1995). Thus, most *E.coli* strains are nonpathogenic but pathogenic strains that cause gastrointestinal illness in humans and opportunistic ones that normally affect immune compromised patients exists (Nataro and Kaper, 1998). For example, more than 80% of urinary tract infections occur in outpatients and *E.coli* accounts for more than 50% of the infections in these patients (Blomgran *et al*.,2004, Jha and Bapat, 2005). In rare cases, virulent strains are also responsible for Haemolytic Uremic Syndrome (HUS), peritonitis, mastitis septicemia, and gram-negative pneumonia Olowe *et al*., 2003). It is one of the organisms most frequently isolated from different clinical cases of diarrhea and others (Okeke *et al*.,1999, Tobih *et al*., 2006).

We aimed in these research to determine the status of antibiotic resistance, underlying conditions, and isolation of *E.coli* isolates with from a special hospital in Denizli, Turkey.

SUBJECTS AND METHODS

Isolation of bacterial strains and identification: 21 isolates were determined from special hospital patients in Denizli July and August in 2013 and recorded at specimens. Mac Conkey agar and EMB agar (Eosin Methylene Blue) agar used for *E.coli* isolation. Isolates were considered to be presumptive *Escherichia* spp. Gram-Negative bacilli, mucoid colonies and lactose positive. Confirmation of isolates was performed by using classic chemical tests (motility test, ure hydrolysis, acid production from mannitol, production of H_2S, IMVIC (Indol, Metil Red, Voges-Proskauer and Citrate) (Prakash *et al*., 2011, Cowan and Steel, 1970).

Antibiotic resistance activity

Antibiotic resistance was determined by an agar disc diffusion test (Bauer *et al*., 1996) using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute (CLSI., 2005) recommendations. Twenty different antibiotics were used. For antibiotic resistance determination, the isolates were grown in Luria- Bertani (LB) broth until the turbidity equal to the 0.5 Mc Farland standart. Cultures were swabbed on to the Mueller–Hinton agar and all isolates were tested against Meropenem (MEM, 10µg/ml), Piperacillin/ tazobactam (TZP, 110µg/ml), Ampicillin/Sulbactam (SAM, 20µg/ml), Amikacin (AK, 30µg/ml), Ceftazidime (CAZ, 30µg/ml), Tobramycin (TOB,10µg/ml), Amoxycillin /clavulanic acid (AMC, 30µg/ml), Gentamycin (CN, 10µg/ml), Aztreonam (ATM., 30µg/ml), Cefepime (FEP PM, 30µg/ml), Cefotaxime (CTX, 30µg/ml), Cefuroxime (CXM, 30µg/ml), Ceftriaxone (CRO, 30µg/ml), Sulphamethazol/Trimetroprim (SXT,25µg/ml), Ciprofloxacin (CIP,5µg/ml), Cefoperozone (CFP, 75µg/ml), Cephazolin (CZ, 30µg/ml), Norfoflaxain (NOR, 10µg/ml), Ampicillin (AM, 10µg/ml), Cefixime (CFM, 5µg/ml).

The isolates those grown in inoculation were evaluated as resistant, and the others were evaluated as susceptible (Bauer *et al*., 1996). The antibiotic discs were dispensed sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were incubated at 37°C, and the diameters of the inhibition zones were measured after 18 h. All susceptibility tests were carried out in duplicate and were repeated twice if discordant results had been obtained.

Multiple Antibiotic Resistance Index:

For all isolates, we calculated the MAR ındex values (a/b, where a represents the number of antibiotics the isolate was resistant to, b represents the total number of antibiotics the isolate tested against). A MAR ındex value ≥ 0.2 is observed when isolates are exposed to high-risk sources of human or animal contamination, where antibiotics use is common; in contrast a MAR index value <or = 0.2 observed when antibiotics are seldom or never used (Krumperman, 1985, Matyar *et al*., 2008).

Results The sensitivity of *E.coli* isolates to antimicrobial agents (n=21) gave high sensitive rates found that *E.coli* isolates diffusion tests for meropenem (100%), norfloxacin and ciprofloxacin (86%), cefotaxime (80%), aztreonam (76%). None of the samples showed no resistance to amikacin, ceftazidime, aztreonam, amoxicillin/clavulanic acid, and meropenem. The results were given table 1.

DISCUSSION

Carbapenems, mainly meropenem, sensitivity rate of meropenem was showed in 100 %. Some researchers have reported meropenem sensitivity rate to *E.coli* from 100% to 90.9% (İnan and Gurler, 2004, Yılmaz *et al*., 2010, Barisic *et al*., 2003). Our results were similar to Yılmaz *et al*.,(2010) who also reported that percentage of meropenem sensitivity was 100% in Turkey. It can be suggested that meropenem can be used for infections based on *E.coli*.

Table 1: Antibiotic resistance pattern of *Escherichia coli* isolated from urine samples

Antibiotics	Sensitive	Intermediate	Resistance
MEM	21(%100)	(%0)	(%0)
NOR	18(%86)	(%0)	3(%14)
CIP	18(%86)	(%0)	3(%14)
CTX	17(%80)	3(%14)	1(%5)
ATM	16(%76)	5(%24)	(%0)
CAZ	15(%71)	5(%24)	(%0)
FEP PM	15(%71)	5(%24)	1 (%5)
CFM	13(%62)	5(%24)	3(%14)
CRO	12(%57)	6(%29)	3(%14)
TZP	12(%57)	9(%42)	(%0)
SXT	11(%57)	4(%19)	6(%29)
CFP	10(%48)	8(%4)	3(%14)
CXM	9(%42)	8(%38)	4(%19)
AMC	8(%38)	13(%62)	(%0)
AK	7(%33)	14(%67)	(%0)
CN	6(%29)	12(%57)	5(%21)
TOB	4(%19)	16(%76)	1(%5)
CZ	4(%19)	12(%57)	5(%21)
SAM	3(%14)	17(%80)	1(%5)
AM	1(%5)	10(%21)	10(%48)

Meropenem =MEM, Piperacillin/ tazobactam =TZP, Ampicillin/Sulbactam= SAM, Amikacin =AK, Ceftazidime =CAZ, Tobramycin= TOB,, Amoxycillin /clavulanic acid =AMC, Gentamycin =CN, Aztreonam =ATM., Cefepime= FEP PM, Cefotaxime =CTX, Cefuroxime=CXM, Ceftriaxone= CRO ,Sulphamethazol/Trimetroprim =SXT,Ciprofloxacin=CIP,Cefoperazone=CFP, Cephazolin= CZ, Norfoflaxain=NOR, Ampicillin=AM, Cefixime= CFM

When it comes to norfloxacin, sensitivity rate, it was %86. Many researchers tested sensitivity of norfloxacin to *E.coli* (Karki *et al*., 2001, Ay *et al*., 2003).

Sensitivity rate of ciprofloxacin was showed in 86%. Many researchers have tested the sensitivity of ciprofloxacin to *E.coli* (Turnidge *et al*., 2002, Aiyegoro *et al*., 2007, Mansouri *et al*., 2002). Our results were similar to Mansouri *et al*.,(2002) who also reported that percentage of ciprofloxacin sensitivity was 84.62% in Iran. It can be suggested that ciprofloxacin can be used for infections based on *E.coli*.

As for the sensitivity rate of cefotaxime, it was 80%. Many researchers have tested resistance of cefotaxime to *E.coli* (Jones *et al*., 2004, Gönüllü *et al*., 2008. Our results were similar to Gonullu *et al* (2008) who also reported that sensitivity rate of cefotaxime was 84%.

Sensitivity rate of aztreonam was showed in 76 %. Many researchers have tested the resistance of aztreonam to *E.coli* (Cho *et al*., 2011, Gonlugur *et al*., 2004, Igbal *et al*., 2002) Our results were similar to Iqbal *et al* (2002) who also reported that aztreonam sensitivity rate was 74% .

When it comes to ceftazidime sensitivity rate, it was 71%. Some researchers have reported that ceftazidime sensitivity rate from 99.6% to 4.5% (Rhonberg and Jones, 2007, Ozsahin *et al*., 2005). Our results were in compliance with previous researchers

As for the sensitivity rate of cefepime, it was 71%. Some researchers have reported sensitivity rate cefepime to *E.coli* in clinical samples (Iqbal *et al* 2002, Ashgar, 2006, Albayrak and Kaya, 2009, Kumarasinghe, 2001). Our results were similar to Iqbal *et al* (2002) who also reported that cefepime sensitivity rate was 80% .

As for sensitivity rate of cefixime was 62%. Cefixime has a broad antibacterial spectrum and it is active against a wide variety at gram-negative organisms, including *E.coli*. Our results were similar to Iqbal *et al.* (2002) who also reported that cefixime sensitivity rate was 61% .

As for sensitivity rate of ceftriaxone, it was 57%. Many researchers have tested the resistance of ceftriaxone to *E.coli*. According to previous studies resistance of *E.coli* was from 0%to 100% (Koken *et al*., 2008, Matute *et al*., 2004, Ateş, 2007, Yuluğkıral, 2007, Kalantar *et al*., 2008, Uzun *et al*., 2006).

As for the sensitivity rate of piperacillin/ tazobactam, it was 57%. Tazobactam seems to be the most promising beta-lactamase inhibitor, which has unlike clavulanic acid and sulbactam, its own antibiotic activity (Blahova *et al*., 1995).

Sensitivity rate of *sulfamethoxazole*/trimethoprim was showed in 52 %. Some researchers have reported *sulfamethoxazole*/trimethoprim sensitivity rate to *E.coli* in clinical samples (Gonlugur *et al*., 2004, Eryılmaz *et al*., 2010, Rawat *et al*., 2010) Our results were similar to Gonlugur *et al*.(2004)[33] who also reported that *sulfamethoxazole* /trimethoprim sensitivity rate was 55%.

As for the sensitivity rate of cefoperazone, it was 48 %. Some researchers have reported sensitivity rate cefoperazone to *E.coli* in clinical samples (Ozsahin *et al*., 2005, Dean *et al*., 2008)

Sensitivity rate of cefuroxime was showed in 42 %. Some researchers have reported sensitivity rate cefuroxime to *E.coli* in clinical samples (İnan and Gurler 2004, Duman *et al*., 2010). In our study resistance of cefuroxime was detected 19%. Our results were similar to Inan and Gurler (2004) who also reported that resistance of cefuroxime showed 14 % in *E.coli* strains isolated from children with urinary tract infections.

As fort the sensitivity rate of amoxicillin–clavunat, it was %38. Some researchers have reported amoxicillin–clavunat sensitivity rate to *E.coli* (Uzun *et al*., 2006, Kader and Kumar, 2004).

As for the sensitivity rate of amikacin, it was 33%. Some researchers have reported amikacin sensitivity rate to *E.coli* (Karki *et al*., 2001, Ekim *et al*., 1998, Çetin *et al*., 2006, Giray *et al*., 2012). In our study resistance of amikacin was detected 0%. Our results were similar to Giray *et al*., (2012) who also reported that resistance of amikacin showed 0 % in *E.coli* strains obtained from urology outpatient clinic of Ege Medical Faculty in Izmir.

Among the aminoglycosides group, gentamycin sensitivity rate was 29%. Some researchers have reported gentamycin sensitivity rate to *E.coli* from 1.5% to 54% (Koksaldi-Motor *et al*., 2010, Kutlu, 2007, Pieboji *et al*., 2004, Kalem *et al*., 2008). Our results were compliance with previous researchers. Koksaldi-Motor *et al*., (2010) reported that when it compared to previous year's data susceptibility of *E.coli* isolated from urine to gentamycin were decreased and also different resistance rate occurred different locations.

As for the sensitivity rate of tobramycin, it was 19%. Some researchers have reported tobramycin sensitivity rate to *E.coli* (Gonlugur *et al*., 2004, Sucu *et al*., 2004). Our results were similar to Gonlugur *et al*. (2004) who also reported that tobramycin sensitivity rate was 16.6%.

As for the sensitivity rate of cefazolin, it was 19%. Some researchers have reported that cefazolin sensitivity (Vlieghe, 2009, Arıkan *et al*., 1995, Frederick, 2011). Our results were in compliance with previous researchers.

As for sensitivity rate of ampicillin/sulbactam was 14%. Many researchers have tested sensitivity ampicillin/sulbactam to *E.coli* (Toroglu *et al*., 2013, Khan and Zaman, 2006).

As for sensitivity rate of ampicillin was 5%. Many researchers have tested the resistance of ampicillin to *E.coli* (Leblebicioğlu *et al*., 1994, Rawat *et al*., 2010, Ahmed *et al*., 2000). Our results were similar to Rawat *et al.* (2004) who also reported that aztreonam sensitivity rate was 5.71% from Kumaun region.

In the present study, the lowest MAR index was 0 obtained from urine samples from female and male. In contrast to the highest MAR index was 0.65 obtained from a female. Out of 21 isolates, 3 (14%) isolates showed Multiple Antibiotic Resistance ten to thirteen antibiotics. The MAR ındex were determined 0.25 and above (Table 2). Some researchers have reported Multi-Drug Resistance rate to *E.coli* from 2% to 97% (Al- Mardeni *et al*., 2009, Mathai *et al*., 2008, Al-Tawfiq, 2006). Our results were in compliance with previous researchers. Study shows that multiple resistance is a common hospital pathogen.

Table 2: Multiple antibiotic resistance (MAR) index among 21 *Escherichia coli* urine samples

Source of isolates	Total isolates	MAR index
Male	1	0,5
Male	2	0.15
Male	1	0.45
Female	2	0.05
Female	1	0.65
Female	1	0.05
Female	3	0.1
Female	9	0
Male	1	0
Total	21	

In conclusion, it is suggested that meropenem, norfloxacin and ciprofloxacin, cefotaxime, aztreonam could be better for the treatment of infections based on *E.coli* according to the present study. Ampicillin/sulbactam and ampicillin were not-advisible antibiotics for *E.coli* infections according to results of the present study.

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Authors Contributions

All the authors have contributed significantly in study design, experimentation, data analysis and manuscript drafting.

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