Antimicrobial Resistance and Sensitivity among Isolates of *Escherichia coli* from Patients in Kahramanmaras, Turkey

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To determine the level of resistance to the widely used antibiotics in clinical isolates of *Escherichia coli*. 74 isolates were collected from different hospitals in Kahramanmaras and recorded at specimens. Antibiotic resistance was determined by agar disc diffusion method using Mueller-Hinton agar according to Clinical and Laboratory Standards Institute recommendations and the production of β-lactamase was detected with the iodometric slide test. This study was carried out in the laboratory of Kahramanmaras Sütçü Imam University, Biology Department. The results indicated that resistance rate of antibiotics was in the range of 91% Penicilin (PEN), 65% Tetracycline (TET) and Amoxicillin (AMX), 62% Cefazolin (CEFX), 59% Streptomycine (STR), 34% Ofloxacin (OFL), 32% Chloramphenicol (CHL) and Ceftriaxone (CEFT), 27% Gentamycin (GEN), 24% Cefoxitin (CFX), 18% Nitrofurantoin (NIT), %12 Meropenem (MER). Among the 74 isolates of *E.coli* were showed 38(51%) beta lactamase activity and 36(49%) isolates of *E.coli* were showed no beta lactamase activity. Out of 74 isolates, 50(68%) isolates showed Multiple Antibiotic Resistance three to twelve antibiotics.

**Key words:** *Escherichia coli*, Antibiotic, Resistance, Beta lactamase activity.

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*Escherichia coli* is one of the main causes of nosocomial infections in humans. *E.coli* is also a common inhabitant of the human and animal gut and is considered an indicator of fecal contamination in food. Recently it is though that *E.coli* and certain other foodborne illnesses can sometimes trigger serious health problems months or years after patient survived that initial bout. The organisms is of clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections. Virulent strains of *E.coli* can cause gastroenteritis, urinary tract infections, blood stream infections and neonatal meningitis¹. 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 patients²-³. In rare cases, virulent strains are also responsible for Haemolytic Uremic Syndrome (HUS), peritonitis, mastitis septicemia, and gram negative pneumoniae¹. It is one of the organisms most frequently isolated from different clinical cases of diarrhea and others⁴-⁶. Antibiotic resistance is a major, increasing problem in clinical microbiology as a whole and in the therapy of hospital infections in particular. *E. coli* and other Enterobacteriaceae are more resistant to β-lactams⁵-⁸. *E.coli* often carry multi drug resistant plasmids and under stress readily transfer those plasmids to otherspecies⁹-¹¹.

We aimed in the present study to determine the status of antimicrobial resistance, underlying conditions, and determination of *E.coli* isolates with beta-lactamase from different hospitals in Kahramanmaras Turkey.
MATERIALS AND METHOD

Isolation of bacterial strains and identification

74 isolates were collected from hospital patients in Kahramanmaras and recorded at specimens. Mac Conkey agar and EMB agar (Eosin Metilen Blue) agar used for E. coli isolation. Isolates were considered to be presumptive Escherichia spp. gram-negative bacill, 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 H₂S, IMVIC (Indol, Metil Red, Voges-Proskauer and Citrate)12,13.

Antibiotic resistance activity

Antibiotic resistance was determined by an agar disc diffusion test14 using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute15 recommendations. Twelve 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 (MER, 10µg/ml), Amoxicillin (AMO, 20µg/ml), Penicillin (PEN, 10µg/ml), Nitrofrantoin (NIT, 30µg/ml), Cefazolin (CEF, 30µg/ml), Cefoxitin (CEF, 30µg/ml), Ceftriaxone (CEFT, 30µg/ml), Gentamycin (GEN, 10µg/ml), Tetracycline (TET, 30µg/ml), Streptomycin (STR, 10µg/ml), Chloramphenicol (CHL, 30µg/ml), Ofloxain (OFL, 5µg/ml). The isolates those grown in inoculation were evaluated as resistant and the others were evaluated as susceptible15. 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.

β-lactamase production

The production of β-lactamase was detected with the iodometric slide test16-17. Initially, solution of all samples will turn purple. An indication of β-lactamase production is clearing of solution, clearing of purple color to white within 5 min. But the entire mixture does not have to clear; clearing of definite clumps or areas is sufficient to denote a positive result. Starch and iodine react in solution to produce a purple color.

Multiple Antibiotic Resistance Index

For all isolates, we calculated the MAR index 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 index 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 ≤ 0.2 observed when antibiotics are seldom or never used18,19.

RESULTS AND DISCUSSION

The resistance of E. coli isolates to antimicrobial agents (n=74) gave high resistance rates found that E. coli isolates diffusion tests for penicillin (91%), tetracycline and amoxicillin (65%), cefazolin (%62), streptomycine (59%), oflaxain(34%), chloramphenicol and ceftriaxone (32%) and gentamycin (27%). The most effective drugs against E. coli were meropenem (%12), nitrofurantoin (18%) and cefoxitin(24%). The results were given table 1.

Among the beta lactam antibiotics, penicillin resistance rate was the highest (91%). The bactericidal antibiotics which inhibits bacterial cell wall synthesis, are penicillins. These bacteria offer resistance to penicillins by production of lactamases and by permeability barrier of the cell surface20.

The fact that over 65% of E. coli isolates were resistant to amoxicillin is of great importance and means the antibiotic can not be used as empirical therapy for hospital patients infection. High frequency resistance to amoxicillin was also reported among E. coli isolates in Iran 83.7%21; in Cameroon 89.2%-79.5%22; in Croatia 42%23; in Portugal and Spain 54.3%24, as well as in USA 39%25 and UK 38.4%26. Our results were in complyance with previous researchers.

As for the resistance rate of tetracycline, it was 65% (table1). Some researchers have reported
that tetracycline resistance rate from 44 to 94%\textsuperscript{5,27,28}. Our results were in compliance with previous researchers.

As for the resistance rate of cefazolin, it was 62% (table1). Some researchers have reported that cefazoline resistance rate from 12% to 78\textsuperscript{29-33}. Our results were in compliance with previous researchers.

With respect to resistance of streptomycine, it was 59% (table1). Some researchers have reported E. coli resistance rate to streptomycin 0\textsuperscript{28,34-35} to 28.08%\textsuperscript{34-35}. In contrast to previous researchers, our results were very high percentage (59%). Many factors may have contributed to such high rates of resistance including misuse of antibiotics by health care professionals or non-skilled practitioners, misuse of antibiotics by the general public, and inadequate surveillance due to lack of information arising from routine antimicrobial susceptibility testing, like reports from other developing countries\textsuperscript{4}.

Fluoroquinolones are antibiotics that are very effective against many gram negative microorganisms, including E. coli. The resistance rate was in ofloxacin with 34% (table1). Ofloxacin resistance has been reported to be 13% and 60% in different studies\textsuperscript{28,29,36}. Koksaldi-Motor et al (2010)\textsuperscript{37} reported that resistance of quinolones have increased year by year. Our results are similar to that reported by Ates (2007)\textsuperscript{36} who also reported that E. coli showed resistance of 30% to ofloxacin.

In her study, a total of 200 common pathogenic bacteria were recovered from patient with urinary tract infections E. coli, Klebsiella, Streptococi, Enterobacter, Proteus, Coagulase negative Staphylococci, Staphylococcus aureus, Enterococi, Pseudomonas, Citrobacter, Serratia species were obtained from this study.

In respect of resistance rate of chloramphenicol, it was 32% (table1). Some researchers have reported that chloramphenicol resistance rate from 2.17 to 60%\textsuperscript{34,35,38}. Our results were in accordance with previous researchers.

Taking into account the total number of clinical isolates in the present study period 32% of

### Table 1. Antibiotic resistance pattern of Escherichia coli isolated from clinical samples

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pen</td>
<td>7(%9)</td>
<td>-</td>
<td>67(%91)</td>
</tr>
<tr>
<td>Amx</td>
<td>17(%23)</td>
<td>9(%12)</td>
<td>48(%65)</td>
</tr>
<tr>
<td>Tet</td>
<td>19(%26)</td>
<td>7(%9)</td>
<td>48(%65)</td>
</tr>
<tr>
<td>Cef</td>
<td>23(%31)</td>
<td>5(%7)</td>
<td>46(%62)</td>
</tr>
<tr>
<td>Str</td>
<td>24(%32)</td>
<td>6(%8)</td>
<td>44(%59)</td>
</tr>
<tr>
<td>Ofl</td>
<td>47(%64)</td>
<td>2(%3)</td>
<td>25(%34)</td>
</tr>
<tr>
<td>Chl</td>
<td>45(%61)</td>
<td>5(%7)</td>
<td>24(%32)</td>
</tr>
<tr>
<td>Ceft</td>
<td>39(%53)</td>
<td>11(%15)</td>
<td>24(%32)</td>
</tr>
<tr>
<td>Gen</td>
<td>46(%62)</td>
<td>8(%11)</td>
<td>20(%27)</td>
</tr>
<tr>
<td>Cefx</td>
<td>54(%73)</td>
<td>2(%3)</td>
<td>18(%24)</td>
</tr>
<tr>
<td>Nit</td>
<td>59(%79)</td>
<td>2(%3)</td>
<td>13(%18)</td>
</tr>
<tr>
<td>Mer</td>
<td>63(%85)</td>
<td>2(%3)</td>
<td>9(%12)</td>
</tr>
</tbody>
</table>

### Table 2. Multiple Antibiotic Resistance Index of 74 Escherichia coli strains

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>Total Isolates</th>
<th>Multiple Antibiotic Resistance Index (MAR)</th>
<th>Beta lactamase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>52</td>
<td>0.08(6i), 0.17(3i), 0.25(4i), 0.33(5i), 0.42(8i), 0.5(8i)</td>
<td>29(+)</td>
</tr>
<tr>
<td>Drain</td>
<td>1</td>
<td>0.5(1i)</td>
<td>1(+)</td>
</tr>
<tr>
<td>Abscess</td>
<td>4</td>
<td>0.33(3i), 0.67(1i)</td>
<td>3(+), 1(-)</td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>0.67(1i), 0.33(1i)</td>
<td>2(-)</td>
</tr>
<tr>
<td>Tissue</td>
<td>1</td>
<td>0.42(1i)</td>
<td>1(+)</td>
</tr>
<tr>
<td>Wound</td>
<td>6</td>
<td>0.17(1i), 0.25(1i), 0.42(2i), 0.58(1i), 0(1i)</td>
<td>6(-)</td>
</tr>
<tr>
<td>Appendicitis</td>
<td>1</td>
<td>0.33(1i)</td>
<td>1(+)</td>
</tr>
<tr>
<td>Vaginal fluid</td>
<td>4</td>
<td>0.08(1i), 0.5(1i), 0.75(1i), 0(1i)</td>
<td>3(+), 1(-)</td>
</tr>
<tr>
<td>Probe tip</td>
<td>1</td>
<td>0.42(1i)</td>
<td>1(+)</td>
</tr>
<tr>
<td>Abdominal Internal Fluid</td>
<td>1</td>
<td>0.08(1i)</td>
<td>1(+)</td>
</tr>
<tr>
<td>Tracheolaringal aspirate</td>
<td>1</td>
<td>0.42(1i)</td>
<td>1(-)</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td></td>
<td>38(+), 36(-)</td>
</tr>
</tbody>
</table>

i: isolates +: Present, -: Absent
the *E. coli* species were resistant to cefoxitin (table1). Some researchers have reported resistance rate of cefoxitin 2%-32.1% in different studies. Our results were similar to Pieboji et al (2004)22 who also reported that gram negative bacilli isolates (n=522), obtained from a wide range of clinical specimens (urine, pus and blood) from inpatients and outpatients at Yaounde Central hospital between March 1995 and April 1998, were evaluated for resistance to antibiotics.

As for resistance rate of ceftriaxone, it was 32% (table1). Many researchers have tested resistance of ceftriaxone to *E. coli*. According to previous studies resistance of *E. coli* was from 0% to 100% 29,31,36,38,42-45. Our results were in compliance with previous researchers.

Among the aminoglycosides group, gentamycin resistance rate was 27% (table1). Some researchers have reported gentamycin resistance rate to *E. coli* from 1.5% to 54%37,40,46,47. Our results were similar to Cho et al (2011)41 who also reported that gentamycin resistance rate was 29.2% patients with diarrhea. 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 occured different locations37.

In this study hospitalized patients *E. coli* isolates were found to be sensitive to nitrofurantoin 18% resistant (table1). Nitrofurantoin still exhibited low resistance frequencies in all countries investigated (0-25 %) despite many years of usage 24,30,32,48-50. Our results were similar to Ay et al (2001)48 who also reported that nitrofurantoin resistance rate was 22% in hospitalized patients.

Carbapenems, mainly meropenem, resistance rate of meropenem was showed in 12% (table1). Some researchers have reported meropenem resistance rate to *E. coli* from 0% to 9.1%29,33,45. Our results were similar to Uzun et al.,(2006)45 who also reported that percentage of meropenem resistance was 9.1% in Turkey. It can be suggested that meropenem can be used for infections based on *E.coli*.

Among the 74 isolates of *E.coli* were showed 38 (51%) beta lactamase activity and 36(49%) isolates of *E.coli* were showed no beta lactamase activity (tableII). The main mechanism of bacterial resistance to the β-lactam class of antibiotics consists of the production of - β lactamases, which are hydrolytic enzymes with the ability to inactivate these antibiotics, before they reach the penicillin-binding proteins located at the cytoplasmic membrane51. Some researchers have reported beta lactamase activity rate to *E.coli* from 6.5% to 65.94%27,42,52-54. Our results were similar to Kumar et al (2011)54 who also reported that 57.69% beta lactamase activity rate to *E.coli*.

In present study, the lowest MAR index was 0.008 obtained from urine, vaginal fluid and abdominal internal fluid samples. In contrast to the highest MAR index was 1 obtained from urine. The lowest MAR index was 0 obtained from tissue samples, none of the isolates showed no resistance all tested antibiotics. Out of 74 isolates, 50 (68%) isolates showed Multiple Antibiotic Resistance three to twelve antibiotics. The MAR index were determined 0.25 and above (table 2). Some researchers have reported Multi Drug Resistance rate to *E.coli* from 2% to 97%55-61. Our results were in complyance with previous researchers. Study shows that multiple resistance is a common hospital pathogens. Higher resistance exhibited to penicillin, tetracycline and amoxicillin may have accounted due to increased use of these drugs in this area and referral hospital status of our hospital and prior treatment with multiple antibiotics.

In conclusion, it is suggested that meropenem and nitrofurantoin could be better for treatment of infections based on *E.coli* according to the present study. Penicillin, tetracycline and amoxicillin were non-advisable antibiotics for *E.coli* infections according to the MAR results. Also, the high number of multidrug resistant isolates gives rise to concern. Regular monitoring of antimicrobial drugs. Resistance seems to necessary to improve our guidelines for empirical antibiotic therapy.

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