**Introduction**

Urinary tract infections are the most common community and nosocomial (hospital acquired) infections requiring antibiotic therapy.\(^1\) Gram-negative bacilli of Enterobacteriaceae family in particular, *Escherichia coli* and *Klebsiella pneumoniae* are the most commonly reported agents causing this infection.\(^1\) Frequent failure to treatment is often attributed to inappropriate use of antibiotics.\(^2\) Extended spectrum β-lactamase (ESBL) producing bacteria isolated from UTI are resistant to many antibiotics. For this reason, different antibiotic options are sometimes required for successful treatment.

The increasing frequency of ESBL producing *E. coli* and *K. pneumoniae* strains is a major problem in terms of community health. These strains are usually resistant to many other antibiotics, that reduce the chance of successful treatment thus/therefore. ESBL producing resistant bacterial infections have higher morbidity and mortality.\(^1,2\)

Most frequently detected mechanism of antibiotic resistance detected in *K. pneumoniae* and *E. coli* operates by the enzymatic action of beta lactamase which cleaves the amide bond of beta lactam ring of the antibiotic.\(^3\) Genes encoded this enzyme are spread by transferring from bacterium to bacterium via plasmid and transposon. ESBL enzymes, the largest beta lactamases in the spectrum, can also disrupt third-generation cephalosporins and aztreonam. Because of the spread of ESBL enzyme genes among species through plasmids, these bacteria produce outbreaks and cause difficulties in treatment. Although, ESBL producing strains are resistant to penicillins, cephalosporins, and aztreonam, they may become susceptible during routine antibiogram process. However, even if they are sensitive in vitro, infections of these strains may not respond to treatment with these antibiotics. For this reason, these antibiotics are not effective in the treatment of patients and cause time loss. In accordance with the recommendation of the Clinical and Laboratory Standards Institute (CLSI), ESBL producing microorganisms must be detected in microbiology laboratories. However, determining of the prevalence of ESBL producing *E. coli* and *Klebsiella* strains will guide the selection of antibiotics in the treatment of infections associated with these strains.\(^3\)

Nowadays, the increase of resistance to antibiotics such as
β-lactams and β-lactamase inhibitor combinations, quinolones, trimethoprim-sulfamethoxazole and β-lactam antibiotics commonly that were used in the empirical treatment of community-acquired UTI.

The search for alternative drugs in treatment was led to empirical treatment of community-acquired UTI with lactam antibiotics commonly that were used in the quinolones, trimethoprim-sulfamethoxazole and β-lactams with a very low incidence of resistance. In addition to its pharmacokinetic and pharmacodynamic advantages, it has important advantages such as being in vivo activity, clinical efficacy, highly tolerability and reliability.5

The purpose of this study is to determine Fosfomycin resistance rates and ESBL rates of E. coli and Klebsiella spp. that were isolated from urine cultures of inpatients and outpatients that were treated at various outpatient clinics and clinics of our hospital.

Materials and Methods

The ESBL production and Fosfomycin resistance rates of E. coli and Klebsiella strains that were isolated from urine cultures of patients admitted to various polyclinics and clinics of Afyonkarahisar Health Sciences University Hospital in Turkey between January 2015 and December 2018 have been examined in this study. Mid stream urine specimens were quantitatively cultured on Chromogenic agar media. The media were left to incubate at 37°C for 18-24 hours. Bacterial cultures which have been a uniform bacterial colony and which have been bacterial colony count of 100.000 CFU/mL were considered significant.

Chromogenic agar was used in identification of Klebsiella spp. and E. coli strains. In addition to this, fully automated VITEK 2 identification and antibiogram system (bioMérieux, Inc. Hazelwood, MO, USA) was used for identification of Klebsiella spp. and E. coli strains, and for all antibiogram procedures.6 The urine culture results obtained for the last three years for E. coli and Klebsiella species isolated from urine cultures were evaluated retrospectively. It was confirmed all bacteria by biochemical tests, conventionally.

In order to check the accuracy of the study, external (Oneworld Accuracy Company, Turkey) and internal (E. coli ATCC 25922, E. faecalis ATCC 29212, and S. aureus ATCC 29213 strains were used) quality control studies are carried out regularly for VITEK 2 system. (Ethical approval for this retrospective research was obtained from the local ethics committee of Afyon Kocatepe University).

Statistical Analysis: The relationship between variables was examined by Chi-square statistic test. SPSS Version 18.0 for Windows Software was used to analyze the data (P<0.05). For the sample size estimation, the formula n=P.Q.Z^2/d^2 was used. The P and Q ratios (0.5 (50%)) were determined as Z_0.01 = 2.58 for the significance level of 0.01, and effect size d=0.025 (2.5%). The minimum sample calculated as 2663. According to this study, the total sample size consisted of 2868 bacteria and sufficed.

**Table 1:** ESBL production rates of E. coli and Klebsiella spp. isolated from UTI.

<table>
<thead>
<tr>
<th>Strains</th>
<th>ESBL(+)</th>
<th>ESBL(-)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>844 (34.9%)</td>
<td>1574 (65.1%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>305 (67.8%)</td>
<td>145 (32.2%)</td>
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</tbody>
</table>

*P<0.01.

**Table 2:** The resistance and sensitivity of bacteria to Fosfomycin.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Fosfomycin Sensitivity</th>
<th>Fosfomycin Resistant</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>2369 (98.0%)</td>
<td>49 (2.0%)</td>
<td></td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>282 (65.3%)</td>
<td>130 (34.7%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>13 (72.2%)</td>
<td>5 (27.8%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2664 (92.9%)</td>
<td>204 (7.1%)</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.01.
Production was found statistically significant (P<0.01). All the bacteria were examined according to the distribution of Fosfomycin sensitivity (Table-2). Thus, 2369 (98.0%) of the 2418 E. coli strains were found to be sensitive to Fosfomycin and 49 (2.0%) were resistant. However, 150 (34.7%) of the 432 K. pneumoniae and five (27.8%) of 18 K. oxytoca strains were found to be resistant to Fosfomycin. Relationship between bacteria species and Fosfomycin resistance was found statistically significant (P<0.01).

Similarly, the whole of bacteria were examined according to the relationship between ESBL production and Fosfomycin resistance (Table-3). The sensitivity rate of ESBL producing E. coli strains to Fosfomycin was found to be 96.5%, but the sensitivity rate of ESBL negative K. pneumoniae to Fosfomycin was found to be 53.1%. The none of the 3 ESBL producing K. oxytoca strains were found to be resistant to Fosfomycin, though five of 15 ESBL negative strains were to be resistant to Fosfomycin. Relationship between ESBL production and Fosfomycin resistance was found statistically significant (P<0.01).

**Table 3**: The resistance and sensitivity of ESBL (+) and ESBL (-) bacteria to Fosfomycin.

<table>
<thead>
<tr>
<th>ESBL(+) Strains</th>
<th>Fosfomycin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>814 (96.5%)</td>
<td>30 (3.5%)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>213 (70.5%)</td>
<td>89 (29.5%)</td>
</tr>
<tr>
<td>K. oxytoca**</td>
<td>3 (100.0%)</td>
<td>Nil</td>
</tr>
<tr>
<td>Total</td>
<td>1030 (89.6%)</td>
<td>119 (10.4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ESBL(-) Strains</th>
<th>Sensitivity</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1555 (98.8%)</td>
<td>19 (1.2%)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>69 (53.1%)</td>
<td>61 (46.9%)</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>10 (66.7%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>1634 (95.1%)</td>
<td>85 (4.9%)</td>
</tr>
</tbody>
</table>

*P<0.01, **K. oxytoca was not included in the chi-square statistical test.

Production was found statistically significant (P<0.01).

All the bacteria were examined according to the distribution of Fosfomycin sensitivity (Table-2). Thus, 2369 (98.0%) of the 2418 E. coli strains were found to be sensitive to Fosfomycin and 49 (2.0%) were resistant. However, 150 (34.7%) of the 432 K. pneumoniae and five (27.8%) of 18 K. oxytoca strains were found to be resistant to Fosfomycin. Relationship between bacteria species and Fosfomycin resistance was found statistically significant (P<0.01).

Similarly, the whole of bacteria were examined according to the relationship between ESBL production and Fosfomycin resistance (Table-3). The sensitivity rate of ESBL producing E. coli strains to Fosfomycin was found to be 96.5%, but the sensitivity rate of ESBL negative E. coli to Fosfomycin was found to be 98.8%. The sensitivity rate of ESBL producing K. pneumoniae strains to Fosfomycin was found to be 70.5%, but the sensitivity rate of ESBL negative K. pneumoniae to Fosfomycin was found to be 53.1%. The none of the 3 ESBL producing K. oxytoca strains were found to be resistant to Fosfomycin, though five of 15 ESBL negative strains were to be resistant to Fosfomycin. Relationship between ESBL production and Fosfomycin resistance was found statistically significant (P<0.01).

**Discussion**

The last decade an increased number of drug resistant E. coli and Klebsiella spp. cases have been reported from Eastern Mediterranean, Asia and African countries. This indeed causing a serious problem in treating the infected population. In accordance with the CLSI recommendations, presence of ESBL should be routinely examined in E. coli and Klebsiella strains isolated in laboratories. According to the results of our study, 844 of 2418 E. coli strains and 305 of 450 Klebsiella spp. strains produced ESBL. Thus, ESBL producing E. coli and ESBL producing K. pneumoniae rates were respectively determined as 34.90% and 67.8%, between 2015-2018. In a meta-analysis conducted in Turkey, it was reported that ESBL positivity for E. coli was determined as 8.1% between 1996-2001, 10.6% between 2002-2007 and 28.2% between 2008-2012. According to our study, ESBL production rate of E. coli was found to be higher than these earlier reports indicating an emerging threat of drug resistant. In other study, ESBL production rate for K. pneumoniae was determined as 55%. In a study in 2012, ESBL producing in K. pneumoniae strains was
reported as 67%. Results of this study are also consistent with the results of Terzi et al, for K. pneumoniae. According to 2007 data of MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) which is a multicenter study in Turkey, 40.5% of K. pneumoniae strains were found to be ESBL positive. A follow up study in Turkey, spread between 2006 to 2010 revealed that ESBL producing rates of E. coli steadily increased from 4.6 to 10.4%, while Klebsiella climbed from 25.9 to 37.2% for the same period. A slight variation to this order is reported from South America where ESBL producing strains of K. pneumoniae dominated (45%) to E. coli strains (8.5%). In Asia, this rate varies from country to country, ranging from 5% in Korea to 23.3% in Indonesia for E. coli. ESBL positivity in Klebsiella spp. was found to be 48.8% in Korea and 20-40% in Southeast Asia, China and Japan. The present study, found that the rates of ESBL-production of Klebsiella spp. were comparatively higher than that of other countries (Table-1).

Resistance to other commonly used antibiotics in the empirical treatment of UTI is increasing. Therefore, new alternative drugs are needed for effective treatment. Fosfomycin is one of the best options in terms of sensitivity. Fosfomycin effects most of different bacteria isolated from UTI resulting from E. coli and Klebsiella strains, especially. Fosfomycin provides several advantages in treatment. For example, it has low toxic effect and low side effect. In addition, it is present at a higher concentration in urine and does not make cross resistance to different antibiotics. Furthermore, Fosfomycin is found to have a broad spectrum bactericidal activity. Knowledge of national resistance data is crucial in determining empirical treatment and avoiding unnecessary antibiotic use. According to the results obtained in this study, the rate of resistance to Fosfomycin (2%) was found to be quite low for E. coli strains. Fosfomycin can easily be preferred in the treatment of infections associated with E. coli. According to this study, it was found that K. pneumoniae has the highest resistance (34.7%) to Fosfomycin (Table-2).

One report from Turkey, found Fosfomycin resistance to be 0.4% in ESBL producing E. coli strains. ESBL positivity in E. coli strains was determined to be 19.5%. In our study, these rates are quite high (Tables-1,3). In the study conducted by Hosbul and colleagues, Fosfomycin resistance was not found in ESBL negative isolates, whereas in our study Fosfomycin resistance was determined as 1.2% in ESBL negative E. coli isolates.

Other studies from Turkey, reported low resistance rates of Fosfomycin. In this study, the Fosfomycin resistance of K. pneumoniae was found to be quite high (34.7%). Similar studies conducted in Korea, Japan and China, the rate of Fosfomycin resistance in ESBL-producing E. coli strains was 7.1%, 1%, and 4.3%, respectively. In a study by Fagalas et al in Greece, a total of 152 MDR Enterobacteriaceae isolates were studied. Of the examined 152 isolates, 85 (55.9%) were extensively drug-resistant (XDR), of which 78 (91.8%) remained susceptible to Fosfomycin. Sensitivity to Fosfomycin of the 34 extended-spectrum-lactamase-producing isolates was determined to be 94.1%. Thus, 105 (90.5%) of 116 K. pneumoniae strains were determined to be susceptible to Fosfomycin, and 26 (100%) of the E. coli strains were determined to be susceptible to Fosfomycin. In other words, eight (9.6%) of 116 K. pneumoniae strains were determined to be resistant to Fosfomycin. According to the results in the Netherlands, sensitivity rates to Fosfomycin were determined to be 95.9% for E. coli and 87.6% for K. pneumoniae. In a study in Taiwan, sensitivity rates of Fosfomycin were 94% for ESBL producing E. coli. According to a study in India, 99.6% of 384 ESBL producing E. coli strains were found susceptible to Fosfomycin, while 87.7% of 80 ESBL producing K. pneumoniae strains were found to be susceptible to Fosfomycin.

A study in Thailand, 359 ESBL producing K. pneumoniae and 394 ESBL producing E.coli strains were susceptible to Fosfomycin at rates of 88.4% and 97.3%, respectively. According to a study by Cueto et al, in Spain, Fosfomycin has exhibited an excellent activity against ESBL producing 290 E. coli and 138 K. pneumoniae isolates.

When the results of study conducted in the Netherlands, Taiwan, India and Thailand were compared with the results of our study, it was found which the susceptibility rates of Fosfomycin for E. coli were similar. However, it was reported the sensitivity rates of Fosfomycin for K. pneumoniae were higher than those of our studies. In addition, the results of the study conducted in Spain compared to our study has high sensitivity rates.

In a previous study in Northern Taiwan, sensitivity rate to Fosfomycin was determined to be 56.7% for 66 ESBL producing K. pneumoniae strains isolated from patients with UTI. Our study results were generally found to be compatible with the results of many studies conducted...
in different countries with some exceptions. In Czech Republic, Fajfr et al have demonstrated that there were no significant differences in Fosfomycin susceptibilities among the E. coli and K. pneumoniae isolates regardless of their β-lactamase-producing status. They have profounded to be indicating that lactamase production is not a mechanism for Fosfomycin resistance. However, according to the results of our study, the relationship between ESBL production and Fosfomycin resistance was found statistically significant. Our data exhibited that the Fosfomycin resistance rates of ESBL producing strains were higher than all ESBL non-producing strains except K. oxytoca.

Conclusion
Properties of antibiotic resistance of bacteria isolated from UTI may vary regionally. Therefore, the results of this study and other similar studies will help in determining of the regional distribution of the antibiotic resistance profiles of ESBL producing E. coli and Klebsiella spp., thereby assist in the treatment success and in the selection of antibiotics to be used in treatment. Based on the results of this study, Fosfomycin displayed very high activity against all ESBL producing strains. For this reason, it has been concluded that Fosfomycin, use could be of significant advantage, and is a good option for first-line empirical treatment of UTI caused by community-acquired E. coli. The increase in Fosfomycin resistance was observed in direct proportion to the presence of ESBL in general. Therefore, this and similar studies indicated that the problem of resistance in Fosfomycin treatment has started and, this problem may increase with higher resistance rates observed in Klebsiella strains. In line of these information, it would be useful to demonstrate the clinical success of Fosfomycin treatment in vivo and in vitro experiments by carrying out studies by geographic region, periodically.

Disclaimer: The part of data summary of this research submitted to 1st International Eurasian Conference on Biological and Chemical Sciences on April 26-27, 2018. (EurasianBioChem 2018-Turkey)

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References