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ORIGINAL ARTICLE

## Etiology and antimicrobial resistance patterns of Extended Spectrum Beta Lactamases (ESBL) producing *E. coli* from urinary tract infections in Tehsil Head Quarter (THQ) Hospital, Burewala, Pakistan

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### ABSTRACT

**Objectives:** The present study was conducted to isolate Extended Spectrum Beta-Lactamases (ESBL) producing strain of *E. coli* causing UTIs in hospitalized patients and to evaluate antimicrobial resistance pattern of most commonly used antibiotics in treatment therapy, in Tehsil headquarter hospital in Burewala.

**Methods:** A total of 150 samples were collected from Patients diagnosed with urinary tract infections (UTIs), from Tehsil Head Quarter (THQ) Hospital, Burewala, Pakistan. All samples were streaked on specific media, MacConkey agar where positive isolates of *E. coli* showed pink colored colonies, further confirmation was done by performing some biochemical tests. ESBL detection of *E. coli* isolates was confirmed by performing Double Disc Synergy Test (DDST). In the end antimicrobial resistance pattern was checked by using some commonly used antibiotics such as ampicillin, amoxi-clavulanic acid combination, ciprofloxacin, norfloxacin, TMP-SMX, ceftriaxone and nitrofurantoin and results were observed.

**Results:** A total of positive 58 samples for *E. coli* were detected and out of 58 samples, 43 samples were positive for ESBL-*E. coli* strain and rests were non-ESBL producers. The results showed higher prevalence of positive ESBL producer strain of *E. coli* in urinary tract infections and this in turn indicates higher positive ratio of 74.1% in UTIs among patients admitted in Tehsil Head Quarter (THQ) Hospital, Burewala.

**Conclusion:** The outcome of our study was that higher prevalence of ESBL-*E. coli* (74.1%) was present among patients having UTIs, and maximum resistance was observed against ampicillin (89%) and ciprofloxacin (83.7%), however minimum resistance was observed against ceftriaxone (16%) and nitrofurantoin (14%), means that these drugs are effective in treatment therapy for UTIs, among patients in THQ, Burewala, Pakistan.

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## INTRODUCTION

Urinary tract infections account for most common bacterial infections and are second common cause of bacteremia in nosocomial infections<sup>1</sup>. There are approximately 150 million urinary tract infections all around the world<sup>2,3</sup>. A predictable amount of microbes cause urinary tract infections (UTIs) which account for significant rate of morbidity and mortality especially in hospitalized patients<sup>4</sup>. Among Enterobacteriaceae, *E. coli* is most prevalent Gram negative urinary tract pathogen, accounting for 75-90% UTIs, especially extended spectrum beta lactamase (ESBL) enzyme producing strains of *E. coli* are more aggressive to cause complicated urinary tract infections (UTIs)<sup>5,6</sup>. However, other members of Gram negative bacteria such as *Klebsiella* spp., *Pseudomonas* spp. and *Enterobacter* spp. are also contributing for worsening the urinary tract infections (UTIs) in immuno-compromised patients<sup>7</sup>.

Urinary tract infections are becoming much challenging in these days not only because of increasing burden of UTIs each year but also because of inappropriate diagnosis of these infections<sup>8,9</sup>. Sometimes UTIs are asymptomatic or less distinguished from other diseases by presenting similar clinical signs and symptoms those results in recommending false antibiotic therapy making these infections much complicated by raising antibiotic resistance and making empirical treatment less worthy<sup>10,11</sup>. However, the level of resistance varies regarding to geographical ranges and due to some other external and internal factors<sup>12</sup>. So physicians must order for a perfect clinical laboratory analysis of urine samples to find out the cause of infections in order to suggest the proper antibiotic therapy<sup>13</sup>.

UTIs are most commonly treated with broad spectrum antibiotics<sup>14</sup>. The Fluroquinolones are mostly used first treatment options for regular UTIs because they have higher bacteriological actions and show better sensitivity and maximum cure rates in normal treatment regimen of most common uropathogens<sup>15</sup>. However, in many parts of world usage of quinolones is no more effective because of emergence of resistance against these drugs had made them ineffective<sup>16</sup>. In many UTIs infections higher level of resistance has been observed against Ciprofloxacin but minimal resistance has shown against ceftriaxone and nitrofurantoin<sup>17</sup>. But unwise use of antibiotics has been raised the resistance to most commonly used antimicrobial agents, making treatment less effective, which in turn has become a significant issue all over the world. the present study was conducted to isolate ESBL producing strain of *E. coli* causing UTIs in hospitalized patients and to evaluate antimicrobial efficacy of most commonly used antibiotics in treatment therapy, in Tehsil headquarter hospital in Burewala.

## MATERIALS AND METHODS

### Specimen collection and data analysis

About 150 (n=150) urine samples were collected from patients diagnosed with urinary tract infections, admitted in Tehsil headquarter hospital Burewala, with the consent of patients and by following all ethical rules. All

samples were transported to microbiology laboratory of the hospital for processing and all specimens were first inoculated on primary media and plated on specific media with overnight incubation at 35-37°C, here MacConkey agar media was employed because it is selective and differential media for *E. coli*, here this microbe shows pink color colonies on this media because of fermentation of lactose and bile salts prevent growth of Gram positive bacteria on this media.

### Biochemical profiling

Only positive cultures were selected for performing routine biochemical tests for further confirmation. In which different biochemical tests such as motility test, lysine fermentation test, and glucose production test were performed. These all tests showed positive result for *E. coli*.

### ESBL detection by CLSI phenotypic method

The presence of extended spectrum beta-lactamases (ESBL) was confirmed according to CLSI (Clinical and Laboratory Standard Institute) criteria by phenotypic screening, Double Disc Synergy test (DDST). Here all positive isolates were streaked on Muller Hinton agar and after discs plating on agar, overnight incubation was done and diameter of inhibition was measured. Here Cefotaxime disc was used alone CTX-30 and in combination with clavulanic acid CTC-40. As the diameter of inhibition zone of CTX-30 alone should be within criteria of its resistance, however the diameter of inhibition zone of combined discs, Cefotaxime+clavulanic acid CTC-40 should have larger diameter of at least difference of  $\geq 5$ mm in diameter. Here in our results, CTX-30 had 6mm diameter and CTC-40 showed diameter of 16mm. so difference between both zones is more than 4mm, means that this isolate was positive for ESBL production.

### Antimicrobial susceptibility testing

Antimicrobial activity of different antibiotics such as ciprofloxacin, norfloxacin, ampicillin, ceftriaxone, TMP-SMX, amoxicillin+clavulanic acid combination and nitrofurantoin, was evaluated by using disc diffusion method by streaking samples on Muller Hinton agar, according to clinical laboratory standard institute (CLSI). After overnight incubation at 37°C the zone diameters were measured for all discs and results were collected. The antibiotic activity of all discs was divided into resistance, intermediate and sensitive scale.

## RESULTS

A total of 150 urine samples were collected from patients having urinary tract infections admitted in Tehsil Headquarter (THQ) hospital Burewala, out of which 90 samples were positive for Gram negative bacteria and rest samples were of other microbes. Out of 90 positive samples isolated, 58 were positive for *E. coli* by morphological, microscopic and biochemical profiling and rests were other gram negative microbes such as *Klebsiella* spp. *Pseudomonas* spp. *Proteus* spp. and other *Enterobacter* spp. also observed as shown in Table 1.

Out of 58 *E. coli* isolates, 43 samples showed ESBL production through double disc synergy testing and rest 15 were non- ESBL producers as shown in Figure 1 and Figure 2. After performing antimicrobial profile on ESBL producer *E. coli*, maximum resistance was observed against most commonly used beta lactam antibiotics such as ampicillin (n= 38/43, 89%), amoxicillin-clavulanic acid (n=31/43, 72.6%) and also against quinolones such as Ciprofloxacin (n=37/43, 85.6%), norfloxacin (n=36/43, 83.7% ). However, resistance was observed against Trimethoprim-sulfamethoxazole (TMP-SMX) (n=34/43, 78.9%). But minimum level of resistance and maximum sensitivity was detected against nitrofurantoin (n=6/43, 14%) and ceftriaxone (n=7/43, 16%) as shown in Figure 3, means that these drugs are effective in current treatment therapy.



Figure 1: Zone diameter of both discs CTC-40 and CTX-30 showing ESBL producing strain of *E. coli*.

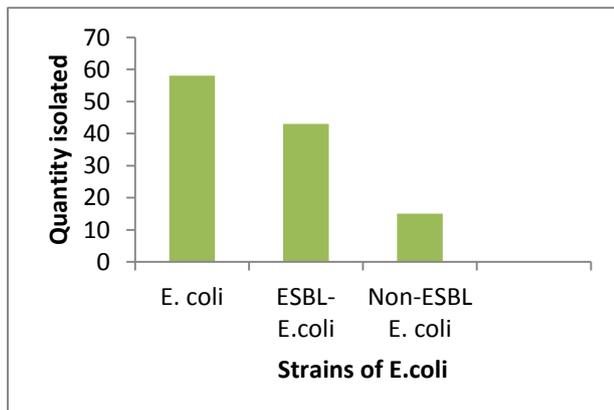


Figure 2: Frequency of isolation of ESBL-*E. coli* from urinary tract infections.

Table 1: Distribution of different microorganisms isolated from urinary tract infections.

Microorganisms Isolated	No. of isolates	Percent occurrence(%)
<i>E. coli</i>	58	64.44
<i>Klebsiella</i> spp.	16	17.77
<i>Pseudomonas</i> spp.	07	7.77
<i>Proteus</i> spp.	05	5.55
<i>Enterobacter</i> spp.	04	4.44
<b>Total</b>	<b>90</b>	<b>100</b>

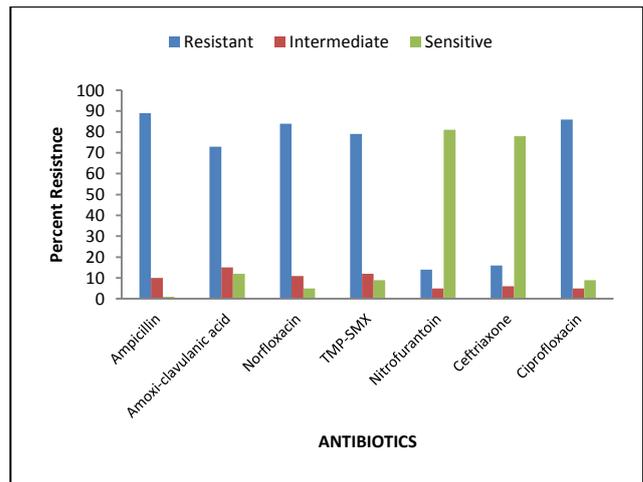


Figure 3: Percentage pattern of resistance, intermediate and sensitivity of different antibiotics used.

Blue: Resistant, Red: Intermediate, Green= Sensitive.

## Discussion

Now day's urinary tract infections are emerging rapidly in a large number of populations. Urinary tract infections are infections caused by bacteria or yeast in normal sterile urine or underlying genitourinary tract<sup>18</sup>. Among bacterial infections, Gram negative bacteria especially *E. coli*, that is Gram negative, rod shaped facultative anaerobe. *E. coli* is the predominant pathogen in causing urinary tract infections as UTIs are most common infections caused by extra intestinal strains of *E. coli* that acquires some special virulence factors in the form of bacterial products (hemolysin), structures such as P fimbriae, type 1fimbriae, K capsules and other properties that enable it to evade human defense system and colonizes the urinary tract area to cause infections because more virulent properties a strain expresses, more severe infections it causes<sup>19,20</sup>. So at least during the life span almost 12% men and 10-20% women experience symptomatic UTIs and much less asymptomatic bacteriurea<sup>21</sup>. So, resistance of urinary isolates of *E. coli* to most commonly used antibiotics such trimethoprim-sulfamethoxazole is an indicator of either using this drug in regular treatment effective or not . A trend of antimicrobial resistance has set which not only increase morbidity and mortality rates among the patients but also health related risk factors and high treatment costs, loner hospitalization and usage of broad spectrum antibiotics<sup>22</sup>.

The objective of this study was to find out the ESBL- *E. coli* resistance pattern for most commonly used antibiotics used in treatment of urinary tract infections. The higher rate of resistance was observed for trimethoprim-sulfamethoxazole, ampicillin, ciprofloxacin and norfloxacin. Conversely the drugs like nitrofurantoin, ceftriaxone exhibited small changes in resistance or maximum sensitivity. However emergence of *E. coli* resistance to fluoroquinolone family is due to most common use in early 2001, prescribed by clinicians for treatment of normal urinary tract infections but with over use of these drugs produced a rapid rate of *E. coli* resistance to Fluroquinolones, higher resistance rate to

this drug is the reflection of its overuse in regular urinary tract treatments that must be prohibited and occasionally used only when lower rate of local antimicrobial resistance are exhibited.

Urinary isolates of ESBL-*E. coli* to TMP/SMX started to increase from year 2000-2010 that remained continued for decades. TMP/SFX is prescribed as second lone drug for the treatment of uncomplicated urinary tract infections in women according to guidelines of IDSA in 2010. Our data is coherent with the previous studies showing the urinary *E. coli* resistance to TMP/SMX is increasing that decreasing its efficacy as empirical therapy among patients<sup>23</sup>.

It is noteworthy that a small amount of resistance of *E. coli* was observed against ceftriaxone and a negligible amount of resistance was observed against nitrofurantoin, so our finding is coherent to the previous case of resistance prevalence that was reported in the NAUTICA study<sup>24</sup>. Most antimicrobial agents in urinary tract infections achieve a significant urinary concentration including TMP-SMX but only patients are infected with resistant strains to TMP-SMX. It has been considered that fluoroquinolone are empirical treatment options for UTIs but our data is totally opposing this statement because in our findings a higher level of resistance against this drug is observed<sup>25</sup>, it might be because of overuse of the Fluoroquinolones may accelerate the development of resistance towards these drugs as same observed in our study.

Antimicrobial resistance is a significant problem in treating clinical infections caused by Gram negative microbes, as the resistance level has risen with the passage of time and shows country wise variations<sup>26</sup>. Such as Latin American isolates exhibit the minimum level of susceptibility followed by isolates collected from Asian-Pacific and European countries<sup>27</sup>. However, the isolates from Canada show the maximum results for susceptibility testing according to SENTRY Antimicrobial Surveillance Program, SASP<sup>28,29</sup>. In our study out of 90 isolates of Gram negative family, maximum isolates were of *E. coli*, so it is coherent with results of pervious study in which *E. coli* was the most prevalent microbe isolated from urinary tract infections. However *Klebsiella* are not much encountered in regular UTIs, but in our study 9 isolates of *Klebsiella* out of all uropathogens were detected. These isolates showed maximum resistance against macrolides, aminoglycosides and against first generation Cephalosproins that is consistent with other studies did at that time against UTI pathogens. In our study *E. coli* showed maximum resistance against ampicillin and against TMP-SMX that is consistent with the results of Indian uropathogens isolates (76% for ampicillin and 75% for TMP-SMX) but controversial to results collected from isolates in USA UTIs (39.1%, 18.6%) and to Europe isolates (29.8% and 14.1%)<sup>30</sup>. On the other way, antimicrobial resistance rate against these drugs in countries such as Senegal (77%, and 55%), Taiwan (80% and 56%), Israel (66% and 26%) and Spain (65% and 33%) is comparable with our isolates

results<sup>31,32</sup>. In this study, *E. coli* showed minimum level of resistance against nitrofurantoin(%) that is controversial to the results of study conducted in Aligarh hospital India (80%), however this drug has encountered a low level of resistance in many parts of the world (0-5.4%) that are consistent with the results of our study, despite of its usage for many years<sup>33,34</sup>. Although maximum resistance to most consumed drugs in this area reflects their unwise and uncontrolled uses during the past decades that is totally alarming to combat this enemy properly in future and a serious concern for all clinicians to provide an empirical treatment therapy to overcome all these threats.

## Conclusions

UTIs due to ESBL-*E. coli* are emerging and also increasing antibiotic resistance. In the present study ESBL-*E. coli* strain involving in UTIs, showed maximum resistance against Fluoroquinolones, ampicillin, and amoxi-clavulanic acid and against TMP-SMX but minimum level of resistance was observed against nitrofurantoin and ceftriaxone drugs in the treatment of UTIs, means that these drugs are effective in the current treatment therapy. Furthermore proper guidelines for the treatment therapy must be revised by clinicians to overcome this serious issue at maximum level.

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