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# Evaluation of ESBL Positivity Rates for *Escherichia coli* and *Klebsiella pneumoniae* Strains with the Sensititre ESBL Antimicrobic Susceptibility Plates in a Public Hospital, Turkey

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Present study has been performed to evaluate Extended Spectrum Beta-Lactamase (ESBL) positivity rates and antimicrobial susceptibility patterns for *Escherichia coli* and *Klebsiella pneumoniae* strains in order to make some regulations on antimicrobial policy in our medical institute. We retrospectively evaluated 297 strains (204 *E. coli* strains and 93 *Klebsiella pneumoniae*) isolated from inpatient clinics and internal care units of Canakkale State Hospital between November 2007-October 2008 performed by Clinical Microbiology Laboratory Unit. ESBL positivity was found 31.86% (65/204) of *E. coli* strains and 33.33% (31/93) of *Klebsiella pneumoniae* strains. Resistance of *E. coli* strains to amoxicillin clavulanate (AMC), ciprofloxacin (CIP) and trimethoprim-sulfamethoxazole (SXT) was found common (33.8, 33.8 and 36.8%, respectively) on the other hand *K. pneumoniae* strains more resistant to AMC (43%) but less resistant to CIP and SXT (22.6 and 31.2%). It is also found that hospitalization in intensive care units is a risk factor for elevated ESBL production rates.

**Key words:** ESBL, *E. coli*, *K. pneumonia*, inpatient clinics, ICU

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## INTRODUCTION

Extended Spectrum Beta-Lactamases (ESBLs) are beta-lactamases capable of conferring bacterial resistance to the penicillin, first-, second- and third-generation cephalosporins and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics and which are inhibited by beta-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005). ESBLs are plasmid mediated enzymes capable of hydrolyzing penicillin, broad spectrum-cephalosporins and monobactams. Most ESBL are mutants of TEM and SHV β-lactamase types (Bush *et al*., 1995).

Resistance to contemporary broad-spectrum beta-lactams, mediated by Extended-Spectrum Beta-Lactamases (ESBL), is an increasing problem worldwide.

The ESBL-producing Gram-negative bacilli possess genes encoding more than one type of the ESBL and enzymes that are responsible for resistance to other antibiotics such as aminoglycosides and fluoroquinolones that are active against Gram-negative bacilli (Mugnier *et al*., 1998; Paterson *et al*., 2000). The emergence of multidrug resistance in these virulent pathogens has significantly hampered the efforts to devise effective empiric or directed antibiotic treatment regimens (Poutsiaka, 2001).

The development of extended-spectrum cephalosporins in the early 1980s was regarded as a major addition to our therapeutic armamentarium in the fight against beta-lactamase-mediated bacterial resistance (Medeiros, 1997; Bush, 2002). First described in Germany (1983) and France (1985) among *Klebsiella* sp., ESBLs exist in every region of the world and in most genera of enterobacteria (Bradford, 2001).

Laboratory detection of ESBLs is difficult as not all extended spectrum antimicrobial agents will display elevated MIC results. Suspicion of the presence of possible ESBLs in *Escherichia coli, Klebsiella* sp., or *P. mirabilis* occurs when MIC values are elevated ($\geq$2 μg $mL^{-1}$) to either ceftriaxone, cefotaxime, ceftazidime and/or aztreonam as defined by current Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2006, 2007). Because different ESBLs hydrolyze β-lactams at different rates, several different agents must be examined to investigate for their presence. Antibiograms may also be affected by the presence of different resistance mechanisms (porin deletions, efflux or the presence of multiple β-lactamases, including other ESBL enzymes (e.g., TEM, SHV, CTX-M and VEB) or Amp-C type enzymes), further complicating accurate detection (Moland *et al*., 2006).

The present study was taken up to evaluate the ESBL production and *in vitro* susceptibility of *Klebsiella pneumoniae* and *E. coli* isolates from a major public hospital (about 600 bed capacity) in Canakkale Province, Turkey.

Totally, 297 isolates of enterobacteriaceae (204 *Escherichia coli*, 93 *K. pneumoniae* strains) were examined for the presumptive detection of extended-spectrum beta-lactamase production by automated Sensititre system (Trek Diagnostic Systems, Cleveland, OH, USA).

## MATERIALS AND METHODS

Totally 297 strains were retrospectively evaluated (204 *E. coli* strains and 93 *Klebsiella pneumoniae*). Strains has been isolated from inpatient clinics and internal care units of Canakkale State Hospital between November 2007-October 2008 performed by Clinical Microbiology Laboratory Unit.

Susceptibility testing was performed by broth microdilution methods in validated microdilution panels manufactured by TREK diagnostics systems (Cleveland, OH, USA). The test medium was Muller-Hinton broth adjusted to McFarland 0.5 for testing susceptibility. The Sensititre is an automatic system that uses a 96-well plate format with a panel of several antimicrobials that are precision dosed at appropriate dilutions.

The Sensititre susceptibility system is a micro version of the classic broth dilution method and can provide both qualitative and quantitative susceptibility results in a dried plate format. Each microdilution plate is dosed with antimicrobial agents at appropriate dilutions and than dried.

After inoculation, the plate is sealed with an adhesive seal, incubated at 34-36°C for 18-24 h and the contents of the wells examined for bacterial growth utilizing the Sensititre automated reading system or read manually.

The Sensititre AutoReader system utilizes fluorescence technology to read 18-24 h test plates. The technology involves the detection of bacterial growth by monitoring the activity of specific surface enzyme produced by the test organism. Growth is determined by generating a fluorescent product from a non-fluorescent (fluorogenic) substrate. The plates can be prepared with the substrate already added to the plate. Enzymatic action of the bacterial surface enzymes on the specific substrates cleaves this bond releasing the fluorophore, which is now capable of fluorescing. The amount of fluorescence detected in directly related to the activity of the bacterial surface enzyme and therefore, to bacterial growth.

Sensititre software interprets the MIC values following CLSI recommendations although manual

interpretations can be performed with novel antimicrobials. The rapid detection of ESBL production with the Sensititre susceptibility system is based on simultaneous assessment of the inhibitory effects of cefepime, cefotaxime and ceftazidime, alone and in the presence of clavulanate (Chapin and Musgnug, 2004).

Data were calculated statistically in order to evaluate their significance using Chi-square test by using SPSS 13.0 statistics software.

## RESULTS AND DISCUSSION

Totally 297 isolates (204 *E. coli* strains and 93 *Klebsiella pneumoniae*) evaluated, retrospectively. Table 1 and 2 represent distribution of isolated strains according to clinics and sample material. ESBL positivity was found 31.86% (65/204) of *E. coli* strains and 33.33% (31/93) of *Klebsiella pneumoniae* strains as seen on Table 1.

Table 1: Distribution of ESBL producing isolates according to clinics

| | *E. coli* strains | | *K. pneumoniae* strains | |
|---|---|---|---|---|
| Clinics | ESBL neg. | ESBL pos. | ESBL neg. | ESBL pos. |
| Emergency med. | 0 | 2 | 0 | 0 |
| Brain surgery | 4 | 1 | 0 | 1 |
| Urology | 70 | 24 | 19 | 8 |
| Gen. surgery | 5 | 0 | 3 | 1 |
| Dermatology | 1 | 0 | 0 | 0 |
| Ped. surgery | 2 | 2 | 0 | 0 |
| Pediatrics | 3 | 1 | 3 | 1 |
| Int. medicine | 17 | 8 | 8 | 1 |
| Physical med. | 4 | 1 | 2 | 1 |
| Thoracic surg. | 0 | 1 | 0 | 0 |
| Pulmonology | 2 | 8 | 7 | 5 |
| Internal care unit | 1 | 3 | 4 | 2 |
| Infect. diseases | 4 | 4 | 3 | 2 |
| Obstetrics | 9 | 1 | 1 | 1 |
| Otolaryngology | 1 | 2 | 3 | 1 |
| Cardiovasc. surg. | 1 | 0 | 0 | 0 |
| Nephrology | 7 | 3 | 1 | 1 |
| Neurology | 4 | 3 | 1 | 2 |
| Orthopedics | 1 | 0 | 1 | 1 |
| Reanimation unit | 3 | 1 | 1 | 1 |
| Neur. intens. care | 0 | 0 | 5 | 2 |
| Total | 139 | 65 | 62 | 31 |

The susceptibilities of those strains to certain antimicrobials are shown on Table 3. In isolated *E. coli* strains resistance to amoxicillin klavunate (AMC), ciprofloxacin (CIP) and trimethoprim-sulfamethoxazole (SXT) is common (33.8, 33.8 and 36.8%, respectively) on the other hand *K. pneumoniae* strains more resistant to AMC (43%) but less resistant to CIP and SXT (22.6 and 31.2%).

When we evaluate ESBL positivity rates according to internal medicine specialties, surgery specialties and intensive care units (Internal Medicine Care Unit, Reanimation Unit and Neurology Intensive Care Unit) shown on Table 4, hospitalization in intensive care units was found as a risk factor for increasing ESBL production in *E. coli* strains ($p = 0.029$). On the other hand no association was found with hospitalization clinic and ESBL producer *Klebsiella* strains ($p = 0.984$).

We also examined about antimicrobial co-resistance of isolated strains (Table 5).

Overall resistance was found 31.6% for ciprofloxacin (CIP) and 3.4% for imipenem (IMP). Resistance rates was found greater in ESBL producing strains than non-producers (71.1 vs. 12.5% for CIP and 10.3 vs. 0% for IMP; $p = 0.001$).

In a study from Hacettepe University Medical Faculty Hospital the ESBL production were found as 33% of *E. coli* and 31.4% of *Klebsiella* sp. in strains isolated from nosocomial blood borne infections by E-test method (Zarakoglu *et al*., 2007).

Table 2: ESBL producing isolate numbers according to sample

| | *E. coli* strains | | *K. pneumoniae* strains | |
|---|---|---|---|---|
| Specimen | ESBL neg. | ESBL pos. | ESBL neg. | ESBL pos. |
| Ascite fluid | 0 | 0 | 0 | 1 |
| Sputum | 4 | 10 | 14 | 11 |
| Urine | 122 | 44 | 34 | 10 |
| Blood | 1 | 0 | 3 | 0 |
| Ear | 1 | 4 | 1 | 2 |
| Seminal fluid | 4 | 2 | 3 | 0 |
| Wound | 7 | 5 | 7 | 7 |
| Total | 139 | 65 | 62 | 31 |

Table 3: Antimicrobial susceptibility patterns of isolated strains

| | *K. pneumoniae* (n-%) | | | *E. coli* (n-%) | | |
|---|---|---|---|---|---|---|
| Antimicrobial* ($MIC_{50/90}$) | Intermediate | Resistant | Susceptible | Intermediate | Resistant | Susceptible |
| AMC (<16 μg $mL^{-1}$) | 9 (9.7) | 40 (43.0) | 44 (47.0) | 42 (20.6) | 69 (33.8) | 93 (45.6) |
| CIP (2 μg $mL^{-1}$) | 3 (3.2) | 21 (22.6) | 69 (74.2) | 2 (1.0) | 69(33.8) | 133 (65.2) |
| SXT (>4 μg $mL^{-1}$) | 1 (1.1) | 29 (31.2) | 63 (67.7) | 1 (0.5) | 75 (36.8) | 128 (62.7) |
| AK (16 μg $mL^{-1}$) | 2 (2.2) | 6 (6.5) | 85 (91.4) | 3 (1.5) | 4 (2.0) | 197 (96.5) |
| CN (>16 μg $mL^{-1}$) | 2 (2.2) | 21 (22.6) | 70 (75.3) | 1 (0.5) | 30 (14.7) | 173 (84.8) |
| CEFX (32 μg $mL^{-1}$) | 7 (7.5) | 28 (30.1) | 58 (62.4) | 8 (3.9) | 53 (26.0) | 143 (70.1) |
| IMP (>8 μg $mL^{-1}$) | 0 (0.0) | 6 (6.5) | 87 (93.5) | 0 (0.0) | 4 (2.0) | 200 (98.0) |
| PIP/TZP (>128 μg $mL^{-1}$) | 5 (5.4) | 16 (17.2) | 72 (77.4) | 20 (9.8) | 30 (14.7) | 154 (5.5) |

*AMC: Amoxicillin clavulanate, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole, AK: Amikacin, CN: Gentamicin, CEFX: Cefoxitin, IMP: Imipenem, PIP/TZP: Piperacillin-tazobactam

Table 4: The ESBL positivity rates of specialty units

| | *E. coli* (n-%) | | *K. pneumoniae* (n-%) | |
|---|---|---|---|---|
| Clinics | ESBL neg. | ESBL pos. | ESBL neg. | ESBL pos. |
| Internal med. clinics | 42 (58.3) | 30 (41.7) | 25 (65.8) | 13 (34.2) |
| Surgery clinics | 93 (75.0) | 31 (25.0) | 27 (69.2) | 12 (30.8) |
| Intensive care units | 4 (50.0) | 4 (50.0) | 9 (69.2) | 4 (30.8) |
| Total | 139 (68.1) | 65 (31.9) | 61 (67.8) | 29 (32.2) |

Table 5: Antimicrobial co-resistance of isolated strains

| | Ciprofloxacin (n-%) | | Imipenem (n-%) | |
|---|---|---|---|---|
| Strain | Sensitive | Resistant | Sensitive | Resistant |
| ESBL non-producer | 175 (87.5) | 25 (12.5) | 200 (100.0) | 0 (0.0) |
| ESBL producer | 28 (28.9) | 69 (71.1) | 87 (89.7) | 10 (10.3) |
| Total | 203 (68.4) | 94 (31.6) | 287 (96.6) | 10 (3.4) |

Between 2000-2002 years, in a large retrospective study performed by Istanbul University Medical Faculty Hospital, the frequency of ESBL producers were found 14% of *E. coli* and 48% of *K. pneumoniae* by disk diffusion method. Ciprofloxacin resistance was found 77% of *E. coli* and 27% of *K. pneumoniae* strains, respectively (Buluc *et al*., 2003).

A multi-center surveillance study from tertiary care hospitals from Turkey has been determined the ESBL producers as 58% for *Klebsiella* sp. by E-test method. This strains also found resistant for ciprofloxacin and imipenem too (46.6 and 9.8%, respectively) (Gunseren *et al*., 1999).

Ozakin *et al*. (2003) determined that 11.9% of *E. coli* and 66.7% of *K. pneumoniae* as ESBL producers by using an automated bacterial identification and susceptibility detection system, Scepter.

We could not find any other antimicrobial resistance study with Sensititre automated bacterial identification and susceptibility system in Turkish literature. So, comparison with Turkish studies mainly based on E-test results.

SENTRY study was the first study mentions about increase in putative ESBL producers *E. coli* and *K. pneumoniae* and non-homogeneous distributions of worldwide (Western Pacific region (25%), Europe (23%), the United States (8%) and Canada (5%)) (Jones, 2001). Many studies has stated that a new resistance enzyme CTX-M is the reason of problem (Chanawong *et al*., 2002; Woodford *et al*., 2004; Rodriquez *et al*., 2006).

MYSTIC program is an international, multicenter, longitudinal surveillance study of antimicrobial activity. This study revealed that ESBL phenotype rates in *Klebsiella* sp. (32.8%) and *E. coli* (14.4%) were generally stable, but extensive hospital-to-hospital and unit-to-unit variations were noted. The highest ESBL rates were found in Eastern Europe (including Turkey) and in intensive care unit patient populations (Jones *et al*., 2003).

ESBL producer *Klebsiella* spp. was found as 22.8% (220/966) in a multicenter study from Europe (Livermore and Yuan, 1996). Another international multicenter study stated that overall 30.8% (78/253) episodes of nosocomial bacteriemia and 43.5% (30/69) episodes acquired in intensive care units were due to ESBL-producing *K. pneumoniae* (Paterson *et al*., 2004).

When compared with SENTRY and MYSTIC studies and other studies performed in European region our data is over the European average for ESBL producer *E. coli* and *Klebsiella* sp., but similar with ESBL producer rates of *Klebsiella* in Turkish hospitals. On the other hand, our ESBL producer rates of *E. coli* are higher than Turkish average rates. This finding is coherent with extremely high usage of third generation cephalosporins in clinical care in our hospital. Hospital Infection Control Unit is performing active surveillance for only three year and informing clinics about resistance rates so antibiotics policy is newly organized with the findings of these studies. By the help of performed and future studies on resistance and antimicrobial restriction policies we hope to decrease resistance rates below Turkish averages in our medical institute.

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