The prevalence of extended-spectrum $\beta$-lactamase-producing Enterobacteriaceae in urinary isolates from patients visiting a teaching hospital in northern Kerala, India

Version 2

Author:
Joakim Santiago Dahlgren

Supervisors:
Martin Sundqvist, MD, PhD
Sujith Ovallath, MD, Professor

Department of Microbiology, Örebro Universitetssjukhus, Örebro, Sweden
Department of Neurology, Kannur Medical College, Anjarakandy, India
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>4</td>
</tr>
<tr>
<td>Background</td>
<td>5</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td>5</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>5</td>
</tr>
<tr>
<td>ESBL</td>
<td>5</td>
</tr>
<tr>
<td>Carbapenem resistance</td>
<td>6</td>
</tr>
<tr>
<td>Antibiotic resistance in the developing world</td>
<td>6</td>
</tr>
<tr>
<td>Previous studies</td>
<td>7</td>
</tr>
<tr>
<td>Objective</td>
<td>7</td>
</tr>
<tr>
<td>Aim</td>
<td>7</td>
</tr>
<tr>
<td>Method</td>
<td>7</td>
</tr>
<tr>
<td>Setting</td>
<td>7</td>
</tr>
<tr>
<td>Inclusion &amp; exclusion criteria</td>
<td>8</td>
</tr>
<tr>
<td>Collection methods</td>
<td>8</td>
</tr>
<tr>
<td>Bacterial isolates</td>
<td>8</td>
</tr>
<tr>
<td>Specimen handling</td>
<td>8</td>
</tr>
<tr>
<td>Species identification</td>
<td>9</td>
</tr>
<tr>
<td>Antibiotic susceptibility testing</td>
<td>9</td>
</tr>
<tr>
<td>Ethical considerations</td>
<td>11</td>
</tr>
<tr>
<td>Statistics</td>
<td>11</td>
</tr>
<tr>
<td>Result</td>
<td>11</td>
</tr>
<tr>
<td>Study population</td>
<td>11</td>
</tr>
<tr>
<td>Species identification</td>
<td>12</td>
</tr>
<tr>
<td>Antibiotic susceptibility</td>
<td>13</td>
</tr>
<tr>
<td>Discussion</td>
<td>14</td>
</tr>
<tr>
<td>Limitations</td>
<td>15</td>
</tr>
<tr>
<td>Study population</td>
<td>15</td>
</tr>
<tr>
<td>Culture reports</td>
<td>16</td>
</tr>
<tr>
<td>Clinical sources of error</td>
<td>16</td>
</tr>
<tr>
<td>Laboratory sources of error</td>
<td>17</td>
</tr>
<tr>
<td>Conclusion</td>
<td>17</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>17</td>
</tr>
<tr>
<td>References</td>
<td>18</td>
</tr>
</tbody>
</table>
Abstract

Background: Antibiotic resistance is a serious worldwide threat to public health due to the emergence of multidrug resistant bacteria. ESBL producing strains have emerged as a significant challenge to counter with present antibiotics. WHO concludes in its report, Antimicrobial Resistance: Global Report on Surveillance (2014), that there is a lack of data on the global extent of antibiotic resistance.

Objective: This cross sectional study investigated the resistance profiles and prevalence of ESBL-producing *Enterobacteriaceae* in urinary isolates collected in a hospital in northern Kerala, India.

Method: The study was conducted at Kannur Medical College in Kerala, India, during eight consecutive weeks, from April to June 2015. Antibiotic susceptibility data were obtained from clinical urine samples submitted to the Dept. of Clinical Microbiology from patients with suspected UTI.

Result: 216 urine specimens were included in the study. 40 urine cultures displayed positive growth of *Enterobacteriaceae* species. The most frequent uropathogen was *E. coli*. There were high prevalence rates of *E. coli* with resistance to 1<sup>st</sup> line antibiotics including cefuroxime. Only two *E. coli* isolates were however identified as ESBL-producing phenotypes with the methods in use at Kannur Medical College.

Conclusion: The resistance profiles of *Enterobacteriaceae* species demonstrated alarmingly high prevalence rates of resistance against several 1<sup>st</sup> line antibiotics (including cefuroxime). The reported prevalence of ESBL-producing isolates among *Enterobacteriaceae* of 5, 0 % is intriguing in that perspective and indicates a high prevalence of other enzymes than ESBLs inhibited by clavulanic acid. In conclusion, additional research with larger study population and a revised method for ESBL-detection is needed to further investigate the resistance profiles and the prevalence of ESBL-producing bacteria in Kerala, India.

Key words: ESBL; extended-spectrum β-lactamase, *Enterobacteriaceae*; UTI
**Abbreviations**

ESBL – Extended-spectrum beta-lactamase

WHO – World health organization

UTI – Urinary tract infection

ABR – Antibiotic resistance

KMC – Kannur Medical College

ATCC – American type culture collection

CLSI – Clinical & laboratory standards institute

DDST – Double disc diffusion

MRSA – Methicillin resistant staphylococcus aureus

AMP – Ampicillin

PI – Piperacillin

PIT – Piperacillin/Tazobactam

NA – Nalidixic acid

NX – Norfloxacin

CIP – Ciprofloxacin

AK – Amikacin

NET – Netilmicin sulphate

NIT – Nitrofurantoin

CXM – Cefuroxime

CTX – Cefotaxime

CAZ – Ceftazidime

CFS – Cefoperazone+Sulbactam

CAC – Ceftazidime+Clauvanic acid

IPM – Imipenem

MRP – Meropenem
Background

**Antibiotic resistance**
Antibiotic resistance (ABR) is today a serious worldwide threat to public health due to the emergence of multidrug resistant bacteria, sometimes colloquially referred to as “Superbugs” (1). Antibiotics that were once used to combat bacterial infections are now rendered less effective due to drug resistance, which in turn yields not only therapeutic difficulties, but is also associated with increased societal and medical costs, and a much higher mortality rate for patients suffering with infections of this kind (2). Of further concern, and seemingly paradoxical in contrast to, is the dwindling number of novel antibiotics marketed, with a discovery void yielding no new antibiotic type since 1987 (3).

**Enterobacteriaceae**
A wide range of gram-negative bacteria express extended-spectrum β-lactamase (ESBL), and two of the most common and clinically important pathogens belong to the *Enterobacteriaceae* family (e.g. *Escherichia coli*, *Klebsiella pneumoniae*) (4). *E. coli* and *K. pneumoniae* are part of the normal intestinal flora, albeit they frequently cause common healthcare-associated and community-acquired infections, including urinary tract infection (UTI), pneumonia, and bloodstream infection (5). These bacteria can acquire antibiotic resistance in similar ways: either by spontaneous mutation (6) or, more importantly, by incorporation of DNA from other bacteria via horizontal gene transfer (HGT) (7). Studies have shown that HGT of ESBL-encoding genes does not only occur between bacteria of the same species, but it is also common with interspecies dispersion, such as between *E. coli* and *K. pneumoniae* (8). This feature is important for ESBL spread in community and in health care environment (9).

**ESBL**
Gram-negative bacteria producing extended-spectrum β-lactamase (ESBL) have emerged as a significant challenge to undertake with present antibiotic armamentarium, both in hospital settings and in the community (10). ESBLs are plasmid-mediated enzymes with capacity to hydrolyze and thus inactivate broad spectrum β-lactam antibiotics, which in turn confer a decreased susceptibility against commonly used antibiotic drugs, such as penicillins and extended spectrum cephalosporins (11). The first report of plasmid encoded ESBL was published in Germany 1983 (12); The first outbreak of multidrug resistant bacteria expressing ESBL was reported in France in 1987 (13). Today, outbreaks have been reported all over the developed world, and the amount of identified ESBLs have reached to hundreds (14).
Carbapenem resistance
A high rate of resistance against extended spectrum cephalosporins means that treatment against severe infections, for which *E. coli* and *K. pneumoniae* are likely to cause, has to rely on broader therapy (15). For these strains, the carbapenem class antibiotic is the main remaining treatment option, sometimes ominously referred to as a “drug of last resort” (16). Unfortunately, several recent studies show the emergence of ESBL-producing *Enterobacteriaceae* possessing resistance also to carbapenems throughout the world (17-22).

A study from 2009 proposed a classification scheme for ESBL, including: Class A (Ambler) ESBLs, termed ESBL_A, with functional class 2be β-lactamases; Miscellaneous ESBLs, termed ESBL_M, with plasmid mediated AmpC or some OXA-ESBLs; and finally ESBLs with hydrolytic activity against carbapenems, hence referred to as ESBL-CARBA (23). This study will take regard to these definitions accordingly.

Antibiotic resistance in the developing world
The World Health Organization (WHO) concludes in its report, *Antimicrobial Resistance: Global Report on Surveillance (2014)*, that there is a lack of data on the worldwide extent of ABR, making it impossible to estimate the prevalence of this problem in a global scale. This applies especially to the developing world, where surveillance programs are scarce and generally few large scale studies have been performed. The report compiles information on resistance to antibiotic drugs commonly used to treat infections caused by nine pathogenic bacteria of particular international concern, *E. coli* and *K. pneumoniae* with ESBL being two of them. In this report, India is one amongst fifteen of WHO’s 194 member states where currently no national data are available regarding this issue.

India is according to WHO a nation with more than 1.2 billion inhabitants, representing approximately one sixth of the world’s total population. Bacterial infections causes a huge burden of disease in India, and the high prevalence of infectious diseases makes antibiotic drugs commonly prescribed or by other means obtained for self-treatment. A report from the last decade show that the total amount of antibiotics being sold in India increased by 40% between 2005 and 2009, whereas the sales of cephalosporins alone increased by 60% (24). An increasing use of antibiotics in India may not be an issue per se, however this data in conjunction with reports displaying a common non-suitable nor sustainable use of antibiotics are foreboding (25-27).
Previous studies
There have been efforts made to estimate the prevalence for different types of ABR in different regions of India. Many of these studies show evidence of increasing ABR and the emergence of new types of acquired resistance, including ESBL_{CARBA} \(^{2, 24, 28, 29}\). However, it is necessary to approach available data with caution as studies describing limited and skewed patient samples are probably not representative of the population in general. Samples collected from hospitalized patients with severe infections, and whose condition are not responding to first line antibiotic treatment, may in fact display a higher proportion of resistance than would be found in the general population. This is of particular concern when estimating ESBL prevalence, because \textit{Enterobacteriaceae} with ESBL are frequently causing hospital-acquired infections, and previous reports have confirmed that \textit{Enterobacteriaceae} species producing ESBL are demonstrating an augmented survivability in hospital environment, making these strains even more prevalent in hospital settings \(^{30}\). It is important to emphasize this aspect, because exaggerated findings based on limited and skewed samples may ultimately influence the use of antibiotics, which in turn may aggravate the problem of ABR by promoting the use of more broad-spectrum antibiotics. However, WHO acknowledge this kind of studies as valuable for providing information on trends and to alert in case of emerging resistance.

Objective

Aim
The project was designed to assess the resistance profile and prevalence of ESBL producing \textit{Enterobacteriaceae} in clinical urine samples from in- and outpatients visiting a hospital in the Kannur district, one of fourteen districts in Kerala, India.

Method

Setting
This cross-sectional study was conducted from the department of microbiology at Kannur Medical College (KMC) in Kerala, India, during eight consecutive weeks, from April to June 2015. The hospital houses 200 beds distributed over 13 departments.
**Inclusion & exclusion criteria**

All clinical urine samples submitted to the clinical microbiology laboratory from in- and outpatients with suspected UTI were included in the study. Duplicate isolates from the same patient were excluded (in which case only the first isolate was included).

Patient data was collected from culture reports following routine analysis in the microbiology laboratory. All culture reports were saved in registers written by hand at the laboratory following a standard protocol. Data collected included gender, age, culture growth, bacterial species, resistance profile, and information regarding the patient as being an in- or outpatient.

**Collection methods**

All clinical urine samples were collected by midstream clean catch – Patient voided first portion of urine, then collected the urine specimen midstream and discarded the latter portion. Specimens were collected from both uncomplicated and complicated UTI.

**Bacterial isolates**

The clinical relevance of the urine-culture isolates was determined by UTI symptoms described on the referral (dysuria, pelvic pain, with or without fever), presence of pyuria (white blood cell count greater than 1 per high-power field upon light microscopic examination), and/or bacteriuria (single species growth and/or clinical evidence of UTI).

**Specimen handling**

Clinical urine samples submitted to the microbiology laboratory for routine analysis were investigated using standard methods in the KMC Dept. of Clinical Microbiology. Clinical urine samples were collected in clean specimen containers and processed within 2 hours of collection, or kept refrigerated at 4°C and processed within 24 hours. The clinical urine samples were inoculated on MacConkey’s agar (HIMEDIA, Mumbai), using a sterile 1 μl Nichrome loop-D-1 (HIMEDIA, Mumbai). Inoculation was performed using semi quantitative method of urine culture. After inoculation the culture plates were incubated at 37°C aerobically for 18 hours before evaluated for growth.

Positive urine cultures were defined as those with presence of a single type of bacteria with significant growth. Cultures with more than 100,000 colony forming units (10^5 CFU)/mL were always considered as significant growth. However, growth of 1,000-100,000 CFU/mL was considered suggestive of UTI, if a specimen was taken at cystoscopy or by other invasive procedures, or in conjunction with clinical evidence for UTI.
Negative urine cultures included those with no growth, with an insignificant quantity of growth (<100,000 CFU/mL) and no symptoms, those with mixed growth of 2 or more different bacterial species or polymicrobial growth due to probable contaminants. If one type of bacteria was present in significantly higher colony counts than the others in a mixed growth, for example 100,000 CFU/mL versus 1,000 CFU/mL, then additional testing was performed in order to isolate and identify the predominant strain.

A sterile Nichrome straight wire (HIMEDIA, Mumbai) was used to pick up colonies. The colony was placed in a tube and suspended in peptone water broth (HIMEDIA, Mumbai), and was left for incubation in 37°C aerobically for 1 hour. After incubation, the inoculum turbidity was determined using McFarland standard. Peptone water was used to dilute the inoculum until the turbidity reached a standardized level of 0, 5 McFarland. The suspension was then used for species identification and susceptibility testing.

**Species identification**
Identification of the isolates was performed using growth based conventional methods. Colonies on the culture plate were investigated for morphology traits and ability to ferment lactose in MacConkey's agar. Gram staining was used to identify the isolate as Gram positive or negative. Oxidase test, catalase test, sugar fermenting test, and nitrate reduction test were used to determine gram negative isolates as *Enterobacteriaceae* genus. Indole test, citrate test, urease test, methyl red & Voges-Proskauer (MR/VP) test, amino acid decarboxylation test, hydrogen sulfide test, and motility test were used to differentiate between the different *Enterobacteriaceae* genera.

**Antibiotic susceptibility testing**
Bacteria considered significant were tested for antibiotic susceptibility using Kirby-Bauer disc diffusion method according to CLSI guidelines (M100-S23, 2013). A sterile cotton swab was dipped in the peptone broth (see above) and to the suspension distributed evenly on a Mueller Hinton 2 (HIMEDIA, Mumbai) agar plate. A panel of antibiotic discs (HIMEDIA, Mumbai) was placed on the agar; the discs were placed 20 mm from each other, center to center, and the culture plate was incubated in 37°C aerobically for 18 hours.
The 1\textsuperscript{st} line panel of antibiotics tested included: Extended spectrum penicillins: Ampicillin (10 µg), Piperacillin (100 µg), Piperacillin/Tazobactam (100/10 µg); Quinolones/Fluoroquinolones: Nalidixic acid (30 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg); Aminoglycosides: Amikacin (30 µg), Netilmicin sulphate (30 µg); Nitrofurantoin (300 µg); Cephalosporins: Cefuroxime (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Cefoperazone+Sulbactam (50/50 µg) CAC Ceftazidime+Clavulanic acid (30/10 µg). The 2\textsuperscript{nd} line panel tested included: Carbapenems: Imipenem (10 µg), Meropenem (10 µg).

The 1\textsuperscript{st} line antibiotics were tested against all isolates; the 2\textsuperscript{nd} line antibiotics were tested if the clinical microbiologist deemed it necessary, e.g. in case two or more of the 1\textsuperscript{st} line of antibiotics failed to demonstrate activity against the isolate. Importantly, for both 1\textsuperscript{st} line and 2\textsuperscript{nd} line testing the set of antibiotic discs was not always the same as some antibiotic discs were lacking at times.

Quality control strain \textit{E. coli} ATCC No. 25922 was used as control strains for antibiotic susceptibility tests. The results were saved as SIR (i.e. Susceptible; Intermediate; Resistant), and interpreted according to CLSI guidelines (M100-S23, 2013).

ESBL\textsubscript{A}-production was verified using Double Disc Synergy Test (DDST) on Mueller-Hinton 2 agar (HIMEDIA, Mumbai) using third generation cephalosporins together with a beta-lactamase inhibitor. The third generation cephalosporins used were Ceftazidime (10 µg) and Cefotaxime (30 µg). The beta-lactamase inhibitors used were Clavulanic acid (10 µg) and

\textbf{Figure 1. Example of antibiotic susceptibility test of a clinical in \textit{E. coli} isolate on Mueller-Hinton agar. In this example several of the tested antibiotics shows no activity (no zone around the disc) to the bacterial isolate tested.}
Tazobactam (10 µg). A clear-cut enhancement, or synergy, of the inhibition between the 3rd generation cephalosporin disc and the beta-lactamase inhibitor disc was interpreted as positive for ESBL-production.

All strains with positive ESBL-production were cryopreserved at -20 ºC for potential further analysis.

**Ethical considerations**

The project was laboratory based, meaning there was no direct involvement with any of the patients concerned. All patient data such as gender, age and setting was carefully recorded from laboratory routine forms but the person identity was not recorded. All results were presented so the individual patient cannot be traced or identified.

Ethical approval for this study was granted by the KMC Hospital Ethical Committee.

**Statistics**

The project was designed as a descriptive study and data does not allow statistical analysis.

**Result**

**Study population**

During eight consecutive weeks, a total of 228 urine specimens were submitted to the microbiology laboratory for culture. Out of these, 12 were duplicate samples and therefore excluded leaving 216 urine specimens included in the study.

100 samples (46, 3 %) were collected from inpatients; 99 (45, 8 %) were from outpatients. There were 17 samples (7, 9 %) from patients with incomplete patient status (i.e. missing information regarding age, gender or whether the patient was as in- or outpatient). There were a total of 49 (22, 7 %) urine cultures that met the criteria for positive growth, including 29 (13, 4 %) from inpatients and 20 (9, 3 %) from outpatients. Inpatients were slightly more likely to display a positive culture (59, 2 %), than outpatients (40, 8 %) (Figure 2).

There were 50 (23, 1 %) male and 159 (73, 6 %) female patients included. Male patients displayed almost the same rate of positive urine culture (11 out of 50 [22, 0 %]), as female patients (38 out of 159 [23, 9 %]) (Figure 2).

A total of 167 (77, 3 %) clinical urine samples displayed negative growth.
The median age of the patients with positive urine culture was 54, range 1 to 90 years. The frequency of positive urine cultures increased with patient age. Most of the positive cultures were isolated from patients of age group 61-80 years (Table 1).

**Table 1. Proportion of positive cultures according to age**

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Number of isolates (n)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>8</td>
<td>16, 3 %</td>
</tr>
<tr>
<td>21-40</td>
<td>10</td>
<td>20, 4 %</td>
</tr>
<tr>
<td>41-60</td>
<td>10</td>
<td>20, 4 %</td>
</tr>
<tr>
<td>61-80</td>
<td>20</td>
<td>40, 8 %</td>
</tr>
<tr>
<td>&gt;80</td>
<td>1</td>
<td>2, 0 %</td>
</tr>
</tbody>
</table>

**Species identification**

A total of 40 urinary isolates were identified as belonging to the *Enterobacteriaceae*. The most frequent uropathogen was *E. coli* (27), followed by *K. pneumoniae* (6). Rare *Enterobacteriaceae* uropathogens included *Proteus mirabilis* (3), *Citrobacter* spp (1), *Enterobacter* spp (1), *Klebsiella* spp (1), and *M. morgani* (1).
Antibiotic susceptibility

There were very high prevalence rates of E. coli isolates with resistance to first line antibiotics (Table 2a). All isolates displayed resistance to ampicillin. Second line antibiotics demonstrated varying activity against the E. coli isolates. However Imipenem was deemed susceptible to all tested isolates (Table 2b).

Table 2a. Antibiotic susceptibility patterns to “1st line antibiotics” in E. coli (n=27) collected from urinary samples at Kannur Medical College. The variation in isolates tested was due to lack of discs at different times of the study.

<table>
<thead>
<tr>
<th>Antibiotic drug</th>
<th>Susceptible, n (%)</th>
<th>Intermediate, n (%)</th>
<th>Resistant, n (%)</th>
<th>Total tested, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>19 (100)</td>
<td>19</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>2 (11, 8)</td>
<td>1 (5, 9)</td>
<td>14 (82, 4)</td>
<td>17</td>
</tr>
<tr>
<td>Piperacillin+Tazobactam</td>
<td>6 (28, 6)</td>
<td>3 (14, 3)</td>
<td>12 (57, 1)</td>
<td>21</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>0 (0, 0)</td>
<td>4 (18, 2)</td>
<td>18 (81, 8)</td>
<td>22</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>6 (50, 0)</td>
<td>0 (0, 0)</td>
<td>6 (50, 0)</td>
<td>12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10 (38, 5)</td>
<td>2 (7, 7)</td>
<td>14 (53, 8)</td>
<td>26</td>
</tr>
<tr>
<td>Amikacin</td>
<td>5 (83, 3)</td>
<td>1 (16, 7)</td>
<td>0 (0, 0)</td>
<td>6</td>
</tr>
<tr>
<td>Netillin</td>
<td>15 (65, 2)</td>
<td>3 (13, 0)</td>
<td>5 (21, 7)</td>
<td>23</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>22 (81, 5)</td>
<td>0 (0, 0)</td>
<td>5 (18, 5)</td>
<td>27</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>2 (8, 3)</td>
<td>0 (0, 0)</td>
<td>22 (91, 7)</td>
<td>24</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>2 (22, 2)</td>
<td>0 (0, 0)</td>
<td>7 (77, 8)</td>
<td>9</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>3 (13, 0)</td>
<td>1 (4, 3)</td>
<td>19 (82, 6)</td>
<td>23</td>
</tr>
<tr>
<td>Cefoperazone+Sulbactam</td>
<td>1 (33, 3)</td>
<td>0 (0, 0)</td>
<td>2 (66, 6)</td>
<td>3</td>
</tr>
<tr>
<td>Ceftazidime+Clavanic acid</td>
<td>2 (33, 3)</td>
<td>0 (0, 0)</td>
<td>4 (66, 6)</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 2b. *E. coli* resistance patterns against 2\textsuperscript{nd} line antibiotics

<table>
<thead>
<tr>
<th>Antibiotic drug</th>
<th>Susceptible, n (%)</th>
<th>Intermediate, n (%)</th>
<th>Resistant, n (%)</th>
<th>Total tested, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>17 (100, 0)</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>17</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>3 (100, 0)</td>
<td>3</td>
</tr>
</tbody>
</table>

Antibiotic susceptibility rates for other *Enterobacteriaceae* are not presented, given the low number of isolates.

Out of the *Enterobacteriaceae* isolates tested for ESBL-production, 38 (95, 0 %) resulted in negative DDST on the Mueller-Hinton agar 2 plates. Thus, those cultures were not identified as ESBL-producing bacteria and no further investigation was performed. The remaining two (5, 0 %) displayed synergy between 3\textsuperscript{rd} generation cephalosporins and clavulanic acid, thus suggesting the presence of ESBL\textsubscript{A} production. According to this analysis, *E. coli* was the only producer of ESBL.

Discussion

The main objective of this study was to assess the resistance profiles and prevalence of extended-spectrum beta-lactamase of *Enterobacteriaceae* species in clinical urine samples collected from in- and outpatients visiting a hospital in northern Kerala, India.

In this study, *E. coli* was the most common etiological agent of urinary tract infection, as in many parts of the world. The data collected demonstrates very high rates of resistance. To some antibiotics almost all tested isolates were resistant: ampicillin (100%), Cefuroxime 91.9%) and Nalidixic acid (81.8%) suggesting high levels of both extended spectrum Beta-lactamases and fluoroquinolones (as almost 54% of the isolates were resistant to ciprofloxacin. On the isolates tested (17/27) Imipenem demonstrated activity against all tested isolates but all three isolates tested for meropenem resistance was deemed resistant. The study included more samples from female patients, elderly, and inpatients. These finding are in line with other studies on UTI (31) and probably due to the known risk factors for acquiring an UTI, age-related comorbidities, decreased immunity and prolonged hospitalization.

Given the high resistance rates against 2\textsuperscript{nd} and 3\textsuperscript{rd} generation cephalosporins the amount of isolates with verified ESBL production was surprisingly low. These findings might suggest a higher prevalence rate for β-lactamases not inhibited by clavulanic acid such as plasmid
mediated AmpC-, OXA-type ESBLs or Carbapenemases like NDM and VIM. Although left undefined due to limitations of laboratory resources in this study. Additionally, it is possible that ESBLs with hydrolytic activity against carbapenems (ESBL\textsubscript{CARBA}) were left undetected as carbapenems were not routinely tested in the first line panel of antibiotics. The three isolates displaying meropenem resistance can be anticipated to have carbapenemase activity not detected by the present methodology. Thus, the results presented in this study should be interpreted with caution since it may highly underestimate the prevalence of isolates with other ESBL enzymes than those inhibited by clavulanic acid (i.e. ESBL\textsubscript{A}, ESBL\textsubscript{CARBA}).

In this study, the prevalence rate for ESBL was estimated to 5.0%; whereas the resistance rates for 3\textsuperscript{rd} generation cephalosporins were high, ranging from 66.6% to 91.7%, and carbapenem resistance was present. These data may be compared with a routine survey of urinary cultures from Örebro University Hospital in Sweden, where 4106 samples were collected for six consecutive weeks between April and May in year 2015. The prevalence rate for ESBL for this period of time was 4.6%, the resistance rate for Cefotaxime (3\textsuperscript{rd} generation cephalosporin) was 4.8% and none of the isolates were resistant to carbapenems (unpublished data).

**Limitations**

**Study population**

The study population was relatively small and limited to individuals seeking medical attention. The Kannur Medical college serves a population of approximately 2 000 000 people and received 228 urinary cultures during the study. The corresponding figures for the Örebro laboratory is (285 000 people and 4106 cultures). Also, patients admitted to the hospital with symptoms for urinary tract infection were the main study population, so positive results from clinical urine samples were expected at a higher rate than in the community. As these factors can exaggerate resistance in the material the result must be approached with caution as it may not be applicable to the general population in the area. This is of particular concern regarding the prevalence of ESBL-producing pathogens, since they are shown to demonstrate a higher rate of survivability in hospital setting, making these bacteria even more prevalent in hospital environment (29).
Culture reports

The culture reports used in the study provided limited amount of information to work with. The data included were patient number, age, gender, whether the sample was from an in- or outpatient, and the species grown together with its antibiotic resistance profile. Thus, it was not possible to differentiate between positive urine cultures coming from patients with uncomplicated and complicated urinary tract infection, or displaying risk factors for complicated urinary tract infection, such as prostatic enlargement or diabetes. Nor was it possible to determine how the clinical urine sample was collected, i.e. by midstream clean catch, catheterization, suprapubic aspiration, cystoscopy or other invasive procedures. Since the data acquired did not provide information of how long inpatients had been admitted to the hospital, it was not possible to differentiate between nosocomial and community acquired infections. To better represent the population, it would be preferable to limit such study to clinical urine samples collected from midstream clean catch, coming from outpatients with suspected uncomplicated, community acquired UTI and displaying no risk factors for complicated UTI.

There was no possibility to determine if the patients included in the study were using any antibiotics by the time the clinical urine samples were collected. The use of antibiotics is likely to have an impact on the culture report and resistance profiles of positive culture isolates.

Also, there was no additional information of patient origin available on the culture report other than whether the patient was an in- or outpatient. For study purpose, it would have been interesting to analyze the amount of urine samples coming from each and every ward of the hospital, and also to determine the rate of positive versus negative culture growth of the clinical urine samples collected from the different wards.

Clinical sources of error

Referrals of clinical urine samples to the department of microbiology were performed with the sample together with a hand-written note containing information of the patient concerned, i.e. patient number, age, gender and whether the sample was from an in- or outpatient. Out of the 216 included culture reports received at the laboratory between 6\textsuperscript{th} of April and 1\textsuperscript{st} of June, a total of 22 culture reports were missing at least one of these variables. The clinical samples missing patient information were examined upon retrieval regardless.
Laboratory sources of error

Of both first line and second line panels of antibiotic discs predetermined to be tested against cultured *Enterobacteriaceae* species, only nitrofurantoin was used consistently to test susceptibility in *E. coli*. The other most tested drugs were Ciprofloxacin (96, 3 %). The least tested drugs were Cefoperazone+Sulbactam (11, 1 %), followed by Meropenem (11, 1 %). The discrepancy between protocol and procedure was due to supply shortage of antibiotic discs. The decision of which antibiotics to test in each individual case was carefully made by the clinicians in the Dept. of Microbiology.

Conclusion

The prevalence of ESBL-producing isolates among *Enterobacteriaceae* species was estimated to 5, 0 % in the study population according to the tests performed – However the resistance profiles of *E. coli* demonstrated alarmingly high resistance rates against 1st line antibiotics, including 3rd generation cephalosporins. These findings may suggest a much higher prevalence rate of Extended Spectrum β-lactamases, such as plasmid mediated AmpC- or ESBL\_CARBA. In conclusion, additional research with larger study population, a consistent testing of the same antibiotics and an extended method of ESBL-detection is needed to further investigate the resistance profiles and prevalence of ESBL-producing bacteria at Kannur Medical College Hospital in Kerala, India.

Acknowledgement

I would like to express my sincere gratitude to my supervisor Martin Sundqvist for shown great amount of support, enthusiasm and patience, making this project not only possible but also very inspiring. I would also like to thank Professor Sujith Ovallath and Professor Gufran Ahmed Bijapur for welcoming me to Kannur Medical College and whose support was indispensable for realizing this project. Additionally, I would like to acknowledge Umeå University and SIDA for granting this project the Minor Field Studies scholarship.

Finally, I wish to extend my heartfelt thanks to Annika Wilbe, my best friend and colleague, for igniting the first spark of inspiration for this journey.
References

2. Data WLC-i-P. Antimicrobial resistance: global report on surveillance. 2014;Section 03: The health and economic burden due to antibacterial resistance;36-7.