Extended-spectrum β-lactamase (ESBL) in Omani Children

Study of prevalence, risk factors and clinical outcomes at Sultan Qaboos University Hospital, Sultanate of Oman

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ABSTRACT Objectives: Antimicrobial resistance is a growing problem worldwide, which imposes difficulties in the selection of appropriate empirical antimicrobial therapy. This study evaluated extended-spectrum β-lactamase (ESBL) isolates in 2005 in The Department of Child Health at Sultan Qaboos University Hospital (SQUH), Oman. Methods: During the 12 month period from January 2005 to December 2005, ESBL isolates from paediatrics inpatients were identified and analysed. Risk factors for the patients who grew ESBLs were analysed. Results: 13.3% of E. coli and 16.6% of Klebsiella pneumoniae isolated were ESBL producers. Most of the ESBLs were from urine (46.2%) and blood (42.6%). The main risk factors for ESBL in these children were previous exposure to antimicrobials (100%), prolonged hospital stay, severe illness (92.3%) and female gender (84.6%). Sensitivity of 100% was observed to carbapenems whereas 92% of the isolates were susceptible to amikacin. The oximino-cephalosporins were 100% resistant. Klebsiella pneumoniae were 100% resistant to piperacillin-tazobactam and nitrofurantoin. E. coli was 100% resistant to trimethoprim-sulfamethoxazole and ciprofloxacin. No resistance was recorded for the following combinations: amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamicin plus nitrofurantoin. Conclusion: ESBL-producing organisms are becoming a major problem in Omani children. Exposure to antimicrobials and long admissions are modifiable risk factors that should be targeted for better control. Carbapenems are the most sensitive and reliable treatment options for infections caused by ESBLs. Amikacin plus piperacillin-tazobactam or nitrofurantoin are good alternatives.

Keywords: Extended-spectrum β-lactamase; Escherichia coli; Klebsiella pneumonia; Anti-infective agents; Risk factors; Oman.
Antimicrobial resistance is a growing problem worldwide, which imposes difficulties in the selection of appropriate empirical antimicrobial therapy. Since the first extended-spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* were discovered in Western Europe in the mid-1980s, the ESBL producing *Enterobacteriaceae* became the focus of many scientific research studies and investigations. ESBL are enzymes belonging to either class A or class D β-lactamases. Class A ESBLs belong to three types: SHV with more than 50 varieties currently recognized on the basis of unique combinations of aminoacid replacements; TEM with more than 130 TEM enzymes currently recognized; and CTX-M with more than 40 CTX-M enzymes currently known. Other uncommon class A ESBLs are BES-1, GES-1, GES-2, IBC-1, IBC-2, PER-1, SFO-1, TLA-1, VEB-1 and VEB-2. There are also at least twelve ESBLs belonging to the OXA type (class D). ESBLs are plasmid-mediated, and their potential for transfer makes it increasingly difficult to control and treat these organisms effectively. As of 25 January 2005, there were 138 TEM- (TEM-1 to TEM-139) and 62 SHV-types (SHV-1 to SHV-63) of β-lactamases, mostly found in *K. pneumoniae* and *E. coli* strains. These mutant enzymes were termed ‘Extended-Spectrum β-Lactamase’ by Philippon et al in 1989. ESBLs hydrolyse extended spectrum cephalosporins with an oxyimino side chain. These cephalosporins include cefotaxime, ceftriaxone and ceftazidime, as well as the oxyimino-monobactam aztreonam. In addition, ESBL-producing organisms are frequently resistant to many other classes of antibiotics, including fluoroquinolones, the monobactam aztreonam, while resistance to trimethoprim–sulfamethoxazole and aminoglycosides is frequently co-transferred on the same plasmid. ESBLs are sensitive to cephemycins (cefoxitin, cefotetan) and carabapenems. ESBL-producing organisms are poorly responsive to treatment with wide spectrum cephalosporins such as ceftazidime and cefepime. ESBL-producing organisms are difficult to differentiate from AmpC β-lactamase-producing *Enterobacteriaceae*; however, most ESBL producers are generally susceptible to cephemycins (e.g. cefoxitin) in vitro. ESBLs are plasmid-mediated while AmpC β-lactamase enzymes are located on the chromosomes of *Enterobacter sp*, *Citrobacter freundii*, *Morganella morganii*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. The appearance of similar plasmid-mediated β-lactamases in *K. pneumoniae* and *E. coli* raises concerns over the spread of resistance, which will further increase the difficulties of phenotypically identifying β-lactamases.

There are many precipitating factors for selection of ESBL producing organisms. These include the increasing use of oxyimino-β-lactams such as ceftazidime, cefotaxime and ceftriaxone. Other risk factors for the acquisition of ESBLs include presence of intravascular catheters; emergency intra-abdominal surgery; a gastrostomy or jejunostomy tube; gastrointestinal colonisation; length of hospital or intensive care unit stay; prior antibiotics (including third-generation cephalosporins); prior nursing home stay; severity of illness; presence of a urinary catheter and ventilator assistance.

The problem of ESBL production is still relatively unappreciated by most clinicians. This may be due in part to difficulty in laboratory identification of ESBLs and misreporting them as sensitive organisms. Many ESBL-producing isolates are not always phenotypically resistant to all oximino-cephalosporins; however, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if treated with one of the oximino-cephalosporins. Therefore, it is imperative for the clinical microbiology laboratory to identify isolates that possess increased minimum inhibitory concentrations (MICs) (≥ 2 µg/mL) to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC ≤ 8 µg/mL).

The rate of ESBL varies from country to country. The prevalence of ESBLs in the UK in 2002 was 7.4%. In Europe, the prevalence of ESBL producing *E.coli* is 10.8% while *K. pneumoniae* is 13.6%. In the USA, the prevalence of ESBL producing *E.coli* is 1.4% while *K. pneumoniae* is 4.4%. The prevalence of ESBL at Sultan Qaboos University Hospital, (SQUH), Oman is not yet known. We have analysed the sensitivity and distribution of some ESBL isolates in SQUH previously without studying the prevalence rate of ESBL in SQUH as a whole, or in individual departments. In this article, we are reporting the prevalence of ESBL isolates in paediatric patients admitted to SQUH with an analysis of the risk factors and clinical outcomes of ESBLs infections.
METHODS

SQUH is a 500-bed tertiary and teaching hospital covering all major medical specialties. It is located on the campus of Sultan Qaboos University in Muscat, Oman. The Department of Child Health occupies three different wards. Each ward accommodates 24 beds of which 4 beds are for isolation.

All specimens received from Department of Child Health from January-December 2005 were properly processed to identify ESBLs. Initially, the isolates were screened by a commercial system (Phoenix Identification and Susceptibility System from Becton Dickinson) for ESBL production. The positive results were further confirmed using the Clinical and Laboratory Standards Institute (CLSI) approved double-disk diffusion method, which is based on a synergistic increase of inhibition zone of ceftazidime and cefotaxime when they are combined with clavulanate. The test is considered positive when the increase of the inhibition zone is (≥ 5 mm).

Susceptibility results were recorded for the following antimicrobials using the Phoenix Identification and Susceptibility System: gentamicin, amikacin, imipenem, meropenem, cefotaxime, ceftazidime, cefepime, ciprofloxacin, piperacillin-tazobactam, trimethoprim/sulfamethoxazole and nitrofurantoin.

There was no resistance recorded for the following combinations: amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamicin plus nitrofurantoin [Table 5].

RESULTS

A total of 87 isolates of E. coli and Klebsiella pneumoniae were isolated from patients admitted to pediatric wards in SQUH in 2005. Out of these 13 (14.9 %) were ESBL producers, out of which 6 (46.2%) were E. coli and 7 (53.8%) were Klebsiella pneumoniae [Table 1]. The percentage of E. coli producing ESBL from the total number of E. coli isolated in 2005 was 13.3% [Table 1]. While the percentage of Klebsiella pneumoniae producing ESBL from the total number of Klebsiella pneumoniae isolated in 2005 was 16.6% [Table 1].

Most of the ESBLs isolates were from urine (46.2%) and blood (42.6%) [Table 2]. A total of 85.7% of ESBL producing Klebsiella pneumoniae were isolated from urine samples, while 83.3% of ESBL producing E. coli were from blood [Table 1]. No ESBL were isolated from wound and pus swabs.

The main risk factors for ESBL in these children were the previous exposure to antimicrobials (100%) [Table 3], hospital stays of more than 5 days (92.3%) and female sex (84.6%). Malignancies, admission to the Intensive Care Unit and the use of a urinary catheter were each (38.5%) associated with ESBL. Only one patient (1/13) was ventilated. Abdominal surgery and obstructive disease of the urinary tract were not found to be risk factors in our patients.

The carbapenems (imipenem and meropenem) were the most active antibiotics against the ESBLs tested, with no resistance recorded [Table 4], followed by amikacin with 8% resistance. All the ESBLs were resistant to oximino-cephalosporins.

All ESBL producing Klebsiella pneumoniae were sensitive to gentamycin and amikacin [Table 4], whereas all E. coli were resistant to gentamycin and but only 18% were resistant to amikacin.

All Klebsiella pneumoniae were resistant to piperacillin-tazobactam and nitrofurantoin, whereas no resistance was seen in E. coli to nitrofurantoin and only 16% were resistant to piperacillin-tazobactam. All E. coli were resistant to ciprofloxacin and trimethoprim/sulfamethoxazole, while 14% of Klebsiella pneumoniae isolates were resistant to these antibiotics.

Table 1: Percentage of ESBLs among E. coli and K. pneumoniae in Sultan Qaboos University Hospital, Oman, paediatrics wards in 2005

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Total</th>
<th>ESBLs</th>
<th>ESBL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>45</td>
<td>6</td>
<td>13.3</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>42</td>
<td>7</td>
<td>16.6</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>13</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Table 2: Source of ESBL isolates from paediatric wards

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Blood</th>
<th>Respiratory</th>
<th>Swabs</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>
were resistant to ciprofloxacin and 28% were resistant to trimethoprim/sulfamethoxazole [Table 4].

No resistance was recorded for the following combinations: amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamicin plus nitrofurantoin [Table 5]. Klebsiella pneumoniae isolates were sensitive to all combinations containing gentamicin and amikacin [Table 5]. E. coli isolates were sensitive to all combination containing nitrofurantoin [Table 5].

All patients (100%) were isolated in a single room and nursed using gloves. They were all treated with a carbapenem (imipenem or meropenem). All patients (100%) cleared the infection.

**DISCUSSION**

The percentage of ESBL producing E. coli and Klebsiella pneumoniae in children admitted at SQUH was low compared to other SQUH wards. The prevalence of ESBLs in medical wards was 28.3% (unpublished data); however, the prevalence rate of ESBLs among E. coli and Klebsiella pneumoniae isolated from paediatrics patients was significantly high (13.3% and 16.6% respectively) compared to the prevalence of ESBLs in the USA in 2004 (1.4% for E. coli and 4.4% Klebsiella pneumoniae) \(^{31}\) and Europe (10.8% for E. coli and 13.6% for Klebsiella pneumoniae). \(^{31}\) The rate of ESBL among E. coli (13.3%) was lower than that for Klebsiella pneumoniae (16.6%). This was the same as the prevalence in USA (1.4% for E. coli versus 4.4% for Klebsiella pneumoniae) and Europe (10.8% for E. coli versus 13.6% for Klebsiella pneumoniae). \(^{31}\)

Urine (70.8%) was the main source of ESBLs from all patients, followed by blood (15%). The high rate of ESBLs in urine samples is not striking if we consider the high prevalence of ESBLs in the gut as shown in Hong Kong where the faecal carriage rates of ESBL is 19% in general outpatients, 19.3% in hospitalized patients, 22.5% in healthy inmates, and 33.3% in convalescent patients. \(^{35}\) In one Middle East country, 1.25% of all gram-negative organisms causing community acquired urinary tract infections during 1999 were reported as ESBL producers. \(^{32, 36}\)

Previous exposure to antimicrobials, female sex,
prolonged hospital stays of more than 5 days, malignancies, admission to the Intensive Care Unit and the use of a urinary catheter were all found to be risk factors for ESBL acquisition. Exposure to antimicrobials was present in 100% of cases, which supports the need for antimicrobial control. 84.6% of cases were female, this might be explained by the increased frequency of urinary tract infections (UTI) in females.

All the ESBL isolates in SQUH were susceptible to carbapenems (100%). The susceptibility rate is similar to ESBLs in the USA where they were 100% susceptible to meropenem and imipenem.31 Meropenem and imipenem activity against ESBL producing E. coli and Klebsiella spp. collected in Europe during 1997–2004 was between 96.9–100.0%, which was lower than the susceptibility of ESBLs in SQUH and the USA.31 Susceptibility of ESBLs from SQUH to amikacin was very high (95.9% for E. coli and 90% for Klebsiella pneumoniae). Kizirgila et al have shown similar susceptibility patterns to amikacin for ESBLs in Turkey (94.5 for E. coli and 83.3% for Klebsiella pneumoniae),37 which makes amikacin a good antibiotic in treatment of ESBLs especially in combination therapy. On the contrary, gentamicin had very low activity against ESBLs at SQUH. Gentamicin had only (28.8%) activity against E. coli compared to Europe and USA where the E. coli susceptibility to gentamicin was 66.7% and 80% respectively in 2004.31 Gentamicin had only 25% activity against Klebsiella pneumoniae which is similar to the USA (26.3%), which are lower rates than those (47.5%) reported in Europe in 2004.31

ESBLs at SQUH had low susceptibility against piperacillin/tazobactam (50.7% E. coli and 32.5% Klebsiella pneumoniae). This level was lower than that reported in Europe (72.5% E. coli and 38.6% Klebsiella pneumoniae)31 and the USA (80.0% for E. coli and 42.1% for Klebsiella pneumoniae)35, which does not make piperacillin/tazobactam a good empirical choice if suspicion of ESBL is high.

Ciprofloxacin had very low activity against ESBLs in SQUH. It was only 16.4% active against E. coli which is similar to Europe (20.2%) and the USA (20%) in 2004,31 whereas higher activity against Klebsiella pneumoniae (32.5%) was recorded. This higher activity compared to E. coli has also been demonstrated in Europe (57.5%) and the USA (36.8%) in 2004.31 The opposite situation has been detected in Turkey where ciprofloxacin was more active against E. coli (33.3%) compared to Klebsiella pneumoniae (25.9%).37

The best non-carbapenem containing combinations were amikacin plus piperacillin-tazobactam, amikacin plus nitrofurantoin and gentamycin plus nitrofurantoin. So if ESBL is expected in a severely ill patient the best empirical combination therapy would be amikacin plus piperacillin-tazobactam. If Klebsiella pneumoniae were cultured and a suspension of ESBL was present, the empirical combination therapy should include either gentamycin or amikacin. If E. coli was isolated from a urine culture of a stable patient nitrofurantoin would be the drug of choice.

All patients underwent a good infection control procedure of isolation and barrier nursing according to accepted standards. All patients made a full clinical recovery with microbiologic eradication of ESBLs on carbapenem.

Overall prevalence of ESBL-producing isolates in Omani children was high compared to other countries. Prevention and good infection control practices should be our priority because these organisms have very limited treatment options. Modification of risk factors and control of antimicrobials should be considered. Carbapenem should be the drug of choice in treatment of ESBLs, which theoretically may lead to increase in carbapenem-resistant Acinetobacter sp and carbapenem-resistant P. aeruginosa. However, Robert G et al have not seen any increase in carbapenem
resistance despite continued use of meropenem and imipenem. Other options would be amikacin plus piperacillin-tazobactam or nitrofurantoin. Wong-Berginger suggested the use of piperacillin–tazobactam in the case of a non-outbreak situation, to preserve the therapeutic value of carbapenem. 

**CONCLUSION**

ESBL-producing organisms are becoming a major problem in Omani children. Exposure to antimicrobials and long admissions are modifiable risk factors that should be targeted for better control. Carbapenems are the most sensitive and reliable treatment options for infections caused by ESBLs. Amikacin plus piperacillin-tazobactam or nitrofurantoin are good alternatives.

**REFERENCES**


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**Table 5: ESBL Resistance to combination therapy of Sultan Qaboos University Hospital paediatric patients**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>CN + CEP</th>
<th>CN + TAZ</th>
<th>CN + CIP</th>
<th>CN + F</th>
<th>AK + CEP</th>
<th>AK + TAZ</th>
<th>AK + CIP</th>
<th>AK + F</th>
<th>CIP + CEP</th>
<th>CIP + TAZ</th>
<th>CIP + F</th>
<th>F + TAZ</th>
<th>F + CEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>E. Coli</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>7</td>
<td>54</td>
<td>1 (8)</td>
<td>7 (54)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (46)</td>
<td>1 (8)</td>
<td>6 (46)</td>
<td>0</td>
<td>1 (8)</td>
<td>0</td>
<td>1 (8)</td>
<td>2 (16)</td>
<td>7 (54)</td>
<td>1 (8)</td>
<td>7 (54)</td>
<td>0</td>
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</tbody>
</table>
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