One-Year Surveillance of ESKAPE Pathogens in an Intensive Care Unit of Monterrey, Mexico

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Key Words
Extended spectrum β-lactamases · Multidrug resistance · Intensive care unit · Vancomycin

Abstract
Background: Bacterial species from the ESKAPE group (i.e. Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniea, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species) are frequently resistant to antibiotics. The purpose of this study was to monitor the incidence of ESKAPE pathogens at the intensive care unit (ICU) of a tertiary care hospital in Monterrey, Mexico. Methods: All clinically relevant organisms isolated from June 2011 to June 2012 were included. Identification and susceptibility testing was performed using panels from Sensititre. Resistance to oxacillin, for S. aureus, and the production of extended spectrum β-lactamases (ESBLs), for K. pneumonia, were determined as defined by the Clinical Laboratory Standards Institute. Also, the presence of vanA and vanB genes was determined in E. faecium vancomycin (VAN)-resistant isolates. Results: The majority of pathogens (64.5%) isolated in the ICU unit were from the ESKAPE group. The organisms most frequently isolated were A. baumannii (15.8%) and P. aeruginosa (14.3%). A high resistance to carbapenems was detected for A. baumannii (75.3%) while 62% of S. aureus isolates were confirmed to be methicillin resistant. Of the K. pneumoniae isolates, 36.9% were ESBL producers. We detected three E. faecium VAN-resistant isolates, all of which contained the vanA gene. Conclusion: The presence of the ESKAPE group of pathogens is a major problem in the ICU setting. The results of this study support the implementation of special antimicrobial strategies to specifically target these microorganisms.

Introduction
Antimicrobial resistance among both Gram-positive and Gram-negative bacteria has been on the rise in the past few years [1–3]. The presence of multidrug-resistant (MDR) pathogens has become a cause for serious concern with regard to nosocomial infections. In fact, the World Health Organization has recently recognized antimicrobial resistance as one of the three most important human health concerns [4]. The most common and threatening MDR pathogens have been grouped together under the...
tions, these isolates, thus limiting the number of therapeutic op-

particularly to glycopeptides, is reported for a number of

nosocomial pathogen worldwide. Acquired resistance,

infections is estimated at approximately 20%

continues to increase rapidly in many regions of the

hospital stay

are associated with a high mortality rate and length of

bloodstream infections caused by this bacterial species

is an emerging global problem that deserves special con-

ideration

is an important species of the ESKAPE group is S.

aureus, particularly methicillin-resistant S. aureus

(MRSA), which has an incidence and prevalence that

to increase rapidly in many regions of the

world. The mortality rate associated with invasive MRSA

infections is estimated at approximately 20% [15] and

bloodstream infections caused by this bacterial species

are associated with a high mortality rate and length of

hospital stay [16]. Finally, isolates of Enterococcus faecalis

and E. faecium are the third- to fourth-most prevalent

nosocomial pathogen worldwide. Acquired resistance,

particularly to glycopeptides, is reported for a number of

these isolates, thus limiting the number of therapeutic op-

tions [17, 18].

Global and regional surveillance of ESKAPE patho-
gen is fundamental to control the infections caused by

these bacterial species [19]. The purpose of this study was
to monitor the incidence of ESKAPE pathogens in an

ensive care unit (ICU) of a tertiary care hospital in Mon-
terrey, Mexico.

Methods

Setting and Clinical Isolates

This study was conducted at the Dr. José Eleuterio Gonzalez
University Hospital, a teaching hospital in Monterrey, Nuevo
Leon, Mexico. This hospital provides tertiary medical care in
seven wards, including three ICUs spanning pediatric, medical
and surgical units. This study was performed in the medical and
surgical ICUs with a combined 20-bed area. During the study
period (from 3 June 2011 to 3 June 2012), 1,692 clinical isolates
were obtained from both ICUs. The first isolate of a particular
species per patient, irrespective of the body site, was recorded.
Only patient specimens taken for diagnostic purposes were in-
cluded.

Antimicrobial Susceptibility and Identification Assays

The species identification and susceptibility testing were per-
formed using the broth microdilution method. Panels were ob-
tained from Sensititre (TEK Diagnostic Systems Inc., Cleveland,
Ohio, USA) and were used as described by the manufacturer.
Antimicrobials tested against Gram-negative bacteria included: ami-

kacin (AMK), aztreonam (AZT), cefotaxime (CTX), ceftazide-

(CAZ), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin

(GEN), imipenem (IPN), MEM, ticarcillin (TIC) and tobramycin

(TOB). Antibiotics included against Gram-positive bacteria in-
cluded clindamycin (CLI), erythromycin (ERY), high-concentra-

tion GEN (GEN500), linezolid (LNZ), oxacillin (OXA), penicillin

(PEN), quinupristin/dalfopristin (QUD), teicoplanin (TEI), high-

concentration streptomycin (ST1000), tetracycline (TET) and

vancomycin (VAN). Antibiotics used against both Gram-positive

and Gram-negative organisms included: amoxicillin-clavulanic

acid (AMX), ampicillin (AMP), cefotaxime (CRO), cefuroxime

(CXM), levofloxacin (LVX) and trimethoprim/sulamethoxazole

(SXT).

Multidrug resistance was defined as having a resistance to three

or more classes of antimicrobials. The class definitions used in this

study were: penicillins (PEN), cephalosporins (FEP, CTX, CAZ,

CRO, CXM), carbapenems (IPN, MEM), fluoroquinolones (LVX),
amino glycosides (AMK, GEN, TOB) and tetracyclines (TET). As-

say control organisms and susceptibility breakpoints were those

recommended by the Clinical Laboratory Standards Institute
(CLSI) [20].

ESBL Production and Detection of MRSA

Presumptive ESBL producers were identified by determining
the minimal inhibitory concentrations (MIC) for CAZ and CTX.
The combination disk method, alone and in combination with in-
hibitory clavulanic acid, was used to confirm the expression of ES-
BLs. Both presumptive and confirmatory tests were performed fol-

owing CLSI guidelines [20]. Additionally, the MIC of OXA was
determined as recommended by the CLSI [20].

Detection of the van Genes

Genomic DNA of E. faecium VAN-resistant isolates was ob-
tained by phenol chloroform and ethanol precipitation. PCR as-

says were performed as described previously [21]. Approximately

100 ng of DNA was added to a PCR mixture containing 10× PCR
buffer, 0.2 mM deoxyribonucleotide and 1 U of Taq DNA poly-

merase (Bioline). Two different primer sets (one each for

vanA and vanB) were used in the assay. Amplification of DNA was

formed with denaturation at 94°C for 2 min, followed by 30 cycles

of 1 min at 94°C, 1 min at 54°C and 1 min at 72°C. Amplicons

were analyzed by electrophoresis on 1% agarose gels.

Results

Distribution of Species by Clinical Specimen

A total of 1,692 clinical isolates were recovered from
clinical specimens, which included respiratory, blood,
urine, catheter and other sites (table 1). ESKAPE patho-
gen was identified in 64.5% (1,092/1,692) of isolates.

Overall, the organisms most frequently isolated were
A. baumannii (15.8%), P. aeruginosa (14.3%), S. aureus (14.2) and K. pneumoniae (11.3%). A. baumannii and S. aureus were most frequently isolated from respiratory specimens (18.8 and 18.3%, respectively). From blood specimens, the most frequently isolated species were A. baumannii and S. aureus (13.6 and 12.3%, respectively), while P. aeruginosa was the most common isolate in urine (23.3%). In catheters, K. pneumoniae and P. aeruginosa were the predominant species (15% for both species).

Antibiotic Resistance

Data detailing the MIC50, MIC90 and percentages of antimicrobial resistance for each of the ESKAPE pathogens, as well as for a few other frequent species, are presented in tables 2 and 3. In general, a high prevalence of drug resistance was detected. For A. baumannii, the percentages of resistance were higher than 72% for all antimicrobial agents evaluated, except for FEP (23.2%). For K. pneumoniae, 3 out of the 16 antimicrobial agents evaluated showed resistance percentages higher than 54%

From all isolates, 86.2% (231/268) of A. baumannii isolates were MDR, as were 100% (114/114) of Enterobacter spp., 59.4% (114/192) of K. pneumoniae and 28.9% (70/242) of P. aeruginosa. Furthermore, 20.9% (56/268) of A. baumannii isolates were found to be extensively drug resistant because they showed resistance to all 13 antimicrobial agents evaluated. Among the species isolated that were not part of the ESKAPE group, 76.7% (69/90) of E. coli were MDR.

Prevalence of ESBL-Positive Enterobacteriaceae and MRSA

For K. pneumoniae isolates (n = 192), 89 were determined to be ESBL producers by presumptive assay. Of those, 85.4% (76/89) were further confirmed to be ESBL producers by the double disk method. Given the high level of MDR detected for E. coli, we also screened E. coli isolates for the production of ESBLs. We determined that 62.2% (56/90) of them were ESBL producers by presumptive assay and 75% (42/56) were positive by double disk method.

Among Gram-positive organisms, 62% (150/242) of S. aureus isolates were methicillin resistant and 3 of 35 E. faecium isolates were found to be VAN resistant. All of these isolates also typed positive for the vanA gene. None of the S. aureus isolates were resistant to VAN.

Discussion

In the hospital setting, different bacterial species may be the causative agents of infectious diseases. Due to their high level of pervasiveness and association with antimicrobial resistance and other features, it is important to monitor and understand the epidemiology and resistance patterns of these pathogens. The data provided in this study highlight the prevalence and resistance patterns of the ESKAPE pathogens and other common species in an ICU setting, which can be used to guide future infection control and antimicrobial stewardship strategies.
crobial resistance, the ESKAPE group of pathogens deserves particular attention. To control the incidence of infections due to ESKAPE pathogens, site-by-site surveillance studies are necessary to establish hospital-specific guidelines for effective empirical therapy [22]. In this study, we report a 1-year surveillance of ESKAPE pathogens and describe the incidence of ESBL-producing *K. pneumoniae*, of MRSA and of the presence of VAN-resistant *E. faecium* isolates in an ICU of a Mexican tertiary care teaching hospital.

The organisms most frequently recovered from our ICU were ESKAPE pathogens (64.5%, 1,092/1,692), with a predominance of Gram-negative bacteria. The most common ESKAPE organisms were *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*. These common bacterial pathogens were found to be similar to other prevalent pathogens reported in other countries [1, 8]. In general, a high MDR was observed for *A. baumannii* and *P. aeruginosa*. *E. coli* deserves special attention because it also showed a high level of MDR (76.7%), even though this bacterial species is not included within the ESKAPE group. It seems that, for our hospital, surveillance monitoring should include *E. coli*. As such, it could be added to the ESKAPE group, thus forming a new acronym: ESKAPEE. In this hospital, the MDR ESKAPE pathogens were responsible for a considerable number of infections and represented the majority of isolates for which resistance to multiple antimicrobial agents reduces therapeutic alternatives for physicians.

### Table 2. MIC and percent of resistant strains data for Gram-negative species of the ESKAPE group plus *E. coli*

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th><em>A. baumannii</em> (n = 268)</th>
<th><em>P. aeruginosa</em> (n = 242)</th>
<th><em>K. pneumoniae</em> (n = 192)</th>
<th>Enterobacter spp. (n = 114)</th>
<th><em>E. coli</em> (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; μg/ml</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; μg/ml</td>
<td>range %R MIC&lt;sub&gt;50&lt;/sub&gt; μg/ml</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; μg/ml</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; μg/ml</td>
</tr>
<tr>
<td>AMC</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>≤16–&gt;32 72.4</td>
<td>≤16</td>
<td>≤16</td>
</tr>
<tr>
<td>TOB</td>
<td>≥8</td>
<td>≤4–&gt;8</td>
<td>≥8 22.7</td>
<td>≤4</td>
<td>≤4</td>
</tr>
<tr>
<td>GEN</td>
<td>≥8</td>
<td>≤4–&gt;8</td>
<td>≥8 21.5</td>
<td>≤8</td>
<td>≤8</td>
</tr>
<tr>
<td>FEP</td>
<td>≥16</td>
<td>≤8–&gt;16</td>
<td>≤8 14.9</td>
<td>≤8</td>
<td>≤8</td>
</tr>
<tr>
<td>CRO</td>
<td>≥32</td>
<td>≤8–&gt;32</td>
<td>≥32 71.4</td>
<td>≥8</td>
<td>≥8</td>
</tr>
<tr>
<td>CXM</td>
<td>ND</td>
<td>ND</td>
<td>ND 16.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CTX</td>
<td>≥32</td>
<td>≤8–&gt;32</td>
<td>≥32 65.6</td>
<td>≥8</td>
<td>≥8</td>
</tr>
<tr>
<td>CAZ</td>
<td>≥16</td>
<td>≤8–&gt;16</td>
<td>≥16 36.5</td>
<td>≥8</td>
<td>≥8</td>
</tr>
<tr>
<td>CIP</td>
<td>≥1</td>
<td>≤0.25–&gt;5.2</td>
<td>≤0.25 72.3</td>
<td>≤0.25–&gt;5.2</td>
<td>≤0.25–&gt;5.2</td>
</tr>
<tr>
<td>LVX</td>
<td>≥4</td>
<td>≤0.5–&gt;4</td>
<td>≤0.5 23.4</td>
<td>≤0.5–&gt;4</td>
<td>≤0.5–&gt;4</td>
</tr>
<tr>
<td>IPN</td>
<td>≥8</td>
<td>≤0.5–&gt;4</td>
<td>≤0.5 23.4</td>
<td>≤0.5–&gt;4</td>
<td>≤0.5–&gt;4</td>
</tr>
<tr>
<td>MEM</td>
<td>≥8</td>
<td>≤0.5–&gt;4</td>
<td>≤0.5 23.4</td>
<td>≤0.5–&gt;4</td>
<td>≤0.5–&gt;4</td>
</tr>
<tr>
<td>AMX</td>
<td>ND</td>
<td>ND</td>
<td>ND 16.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AMP</td>
<td>ND</td>
<td>ND</td>
<td>ND 16.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AZT</td>
<td>ND</td>
<td>ND</td>
<td>ND 16.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CHL</td>
<td>ND</td>
<td>ND</td>
<td>ND 16.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TIC</td>
<td>≥64</td>
<td>≤16–&gt;64</td>
<td>≤16 77.2</td>
<td>≤16</td>
<td>≤16</td>
</tr>
<tr>
<td>SXT</td>
<td>≥2</td>
<td>≤2–&gt;2</td>
<td>≤2 45.3</td>
<td>≤2</td>
<td>≤2</td>
</tr>
</tbody>
</table>

**Footnote to Table 2**

% R = Percent of resistant strains; ND = not determined.
for MEM-resistant strains. Of note, the previous study we refer to was performed in all hospital wards, while this study was restricted to only two ICUs.

ESBL-producing strains have been reported around the world in different genera of the Enterobacteriaceae family and have been isolated from different clinical specimens [27–30]. In this study, 39.6% of K. pneumoniae isolates were found to be ESBL producers. Higher values were detected for E. coli, as 46.7% of isolates were ESBL positive. This finding supports the importance of monitoring E. coli strains at this particular site. Of interest, the ESBL type has been previously studied within our hospital. For E. coli the dominant type was CTX-M-15 (66.7%), and for K. pneumoniae the dominant type was SHV-12 (51.5%) [31]. There was also an important clonal relatedness among K. pneumoniae ESBL isolates [30]. The spread of ESBLs in hospitals is an important challenge for clinicians, as the therapeutic options for these organisms are limited. Additionally, infections attributed to ESBL-producing E. coli and K. pneumoniae are associated with increased mortality, length of hospital stay and increased cost [29].

The most common Gram-positive organism recovered from our ICUs was S. aureus. Among these isolates, MRSA made up 62% of all S. aureus isolates. This rate is higher than that reported in the USA (55%), Canada (22.3%) and Europe (ranging between >1 and 24%), but lower than values observed in Turkey (75%) [15, 16, 32, 33].

Different types of potentially influential genes have been reported in VAN-resistant isolates. In a wide variety of enterococcal species, the vanA genotype is associated with a high level of resistance, the vanB, vanB2 and vanD genotype with a moderate to high level of resistance, and the vanC (C1, C2, C3) with an intrinsically low level of resistance [17, 34]. Accordingly, in this study, three E. faecium isolates were VAN-resistant and were also typed for the vanA gene. There is only one previous report of the presence of VAN-resistant E. faecium isolates in Mexico that were also typed for the vanA gene [35]. Our results highlight the presence of a high-level resistance to VAN in Mexico. Over the past few years, our ICUs have predominately used IPN and VAN for the treatment of many complicated infections, including ventilator-associated pneumonia, central line-associated bloodstream infections and many complicated intra-abdominal infections. This fact may explain, at least in part, the high level of drug resistance observed.

One weakness of this study is that we used the commercial methodology of Sensititre, which includes a range

### Table 3. MIC and percent of resistant strains data for Gram-positive species of the ESKAPE group

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>S. aureus (n = 242)</th>
<th>Enterococcus spp. (n = 156)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; µg/ml</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; µg/ml</td>
</tr>
<tr>
<td>AMX</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>AMP</td>
<td>≥0.25</td>
<td>&gt;0.25</td>
</tr>
<tr>
<td>OXA</td>
<td>&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>PEN</td>
<td>&gt;0.25</td>
<td>&gt;0.25</td>
</tr>
<tr>
<td>ST1000</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FEP</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>CRO</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>CXM</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>CLI</td>
<td>≥2</td>
<td>≥2</td>
</tr>
<tr>
<td>ERY</td>
<td>&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>GEN500</td>
<td>ND</td>
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</tr>
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<td>LVX</td>
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<td>LINZ</td>
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<tr>
<td>QUD</td>
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</tr>
<tr>
<td>TET</td>
<td>≥8</td>
<td>≥8</td>
</tr>
<tr>
<td>TET</td>
<td>≤2</td>
<td>8</td>
</tr>
<tr>
<td>SXT</td>
<td>≤2</td>
<td>2</td>
</tr>
<tr>
<td>VAN</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

% R = Percent of resistant strains; ND = not determined.
of concentrations that sometimes does not allow one to detect the exact MIC. Nonetheless, as the purpose of this study is to assess resistant strains, the testing of concentrations around each breakpoint was deemed acceptable and the data were analyzed with the knowledge of this bias.

The high rate of antibiotic resistance in our ICU underlines the urgent need for strategies for the prevention and control of infections caused by the ESKAPE pathogens. The results of this study will help us to implement an appropriate infection control of these highly resistant species.

References


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480


