

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 pneumoniae*, *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 Sensi-titre. Resistance to oxacillin, for *S. aureus*, and the production of extended spectrum β -lactamases (ESBLs), for *K. pneumoniae*, 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. bau-*

mannii (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.

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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

acronym 'ESKAPE,' which stands for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. [5–8].

Three decades ago, *A. baumannii* was sensitive to most antibiotics, but today it is exceptionally resistant to most antibiotics [9], with carbapenem resistance being greater than 50% in some countries [10]. Recently, data from Monterrey, Mexico, reported a meropenem (MEM) resistance of 69% [11]. A major factor contributing to antibiotic resistance is the production of extended spectrum β -lactamases (ESBLs) by the *Enterobacteriaceae* species, particularly *K. pneumoniae* [12]. The dissemination of ESBL-producing Gram-negative pathogens in hospitals is an emerging global problem that deserves special consideration [13, 14].

Another important species of the ESKAPE group is *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), which has an incidence and prevalence that continues 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 options [17, 18].

Global and regional surveillance of ESKAPE pathogens 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 intensive care unit (ICU) of a tertiary care hospital in Monterrey, 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 included.

Antimicrobial Susceptibility and Identification Assays

The species identification and susceptibility testing were performed using the broth microdilution method. Panels were obtained from Sensititre (TEK Diagnostic Systems Inc., Cleveland, Ohio, USA) and were used as described by the manufacturer. Antimicrobials tested against Gram-negative bacteria included: amikacin (AMK), aztreonam (AZT), cefotaxime (CTX), ceftazidime (CAZ), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IPN), MEM, ticarcillin (TIC) and tobramycin (TOB). Antibiotics included against Gram-positive bacteria included clindamycin (CLI), erythromycin (ERY), high-concentration 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), cefepime (FEP), ceftriaxone (CRO), cefuroxime (CXM), levofloxacin (LVX) and trimethoprim/sulfamethoxazole (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), aminoglycosides (AMK, GEN, TOB) and tetracyclines (TET). Assay 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 inhibitory clavulanic acid, was used to confirm the expression of ESBLs. Both presumptive and confirmatory tests were performed following 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 obtained by phenol chloroform and ethanol precipitation. PCR assays were performed as described previously [21]. Approximately 100 ng of DNA was added to a PCR mixture containing 10 \times PCR buffer, 0.2 mM deoxyribonucleotide and 1 U of *Taq* DNA polymerase (Bioline). Two different primer sets (one each for *vanA* and *vanB*) were used in the assay. Amplification of DNA was performed 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 pathogens were identified in 64.5% (1,092/1,692) of isolates. Overall, the organisms most frequently isolated were

Table 1. Distribution of ESKAPE pathogens and other species isolated from clinical specimens collected in the ICU of the José Eleuterio González University Hospital from June 2011 to June 2012

Pathogen	Global	Respiratory	Blood	Urine	Catheter	Other
ESKAPE group						
<i>A. baumannii</i>	268 (15.8)	168 (18.8)	42 (13.6)	10 (5.7)	22 (13.8)	26 (17.2)
<i>P. aeruginosa</i>	242 (14.3)	130 (14.5)	32 (10.3)	41 (23.3)	24 (15)	15 (9.9)
<i>S. aureus</i>	241 (14.2)	163 (18.2)	38 (12.3)	4 (2.3)	16 (10)	20 (13.2)
<i>K. pneumoniae</i>	192 (11.3)	113 (12.6)	23 (7.4)	18 (10.2)	24 (15)	14 (9.3)
<i>E. faecium</i>	35 (2.1)	9 (1)	8 (2.6)	11 (6.3)	1 (0.6)	6 (4)
<i>E. cloacae</i>	69 (4.1)	28 (3.1)	15 (4.8)	7 (4)	12 (7.5)	7 (4.6)
<i>E. aerogenes</i>	28 (1.7)	17 (1.9)	2 (0.6)	4 (2.3)	3 (1.9)	2 (1.3)
Other species	17 (1)	9 (1)	–	6 (3.4)	–	2 (1.3)
Enterobacter						
CoNS	146 (8.6)	37 (4.1)	88 (28.4)	6 (3.4)	10 (6.3)	5 (3.3)
<i>E. faecalis</i>	121 (7.2)	42 (4.7)	13 (4.2)	29 (16.5)	21 (13.1)	16 (10.6)
<i>E. coli</i>	90 (5.3)	36 (4)	9 (2.9)	17 (9.7)	10 (6.3)	18 (11.9)
Other species	243 (14.4)	143 (16)	40 (12.9)	23 (13.1)	17 (10.6)	20 (13.2)
Total	1,692 (100)	895 (100)	310 (100)	176 (100)	160 (100)	151 (100)

Figures in parentheses are percentage. CoNS = Coagulase-negative staphylococci.

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 antimi-

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

Antimicrobial Agent	<i>A. baumannii</i> (n = 268)				<i>P. aeruginosa</i> (n = 242)				<i>K. pneumoniae</i> (n = 192)				<i>Enterobacter</i> spp. (n = 114)				<i>E. coli</i> (n = 90)			
	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R
AMK	>32	>32	≤16->32	72.4	>32	>32	≤16->32	18.2	≤16	32	≤16->32	7.3	≤16	>32	≤16->32	9.6	≤16	≤16	≤16->32	3.3
TOB	>8	>8	≤4->8	80.1	>8	>8	≤4->8	22.7	≤4	>8	≤4->8	46.9	≤4	>8	≤4->8	23.7	>8	>8	≤4->8	51.1
GEN	>8	>8	≤2->8	83.2	>8	>8	≤2->8	21.5	≤2	>8	≤2->8	44.8	≤2	>8	≤2->8	14.9	≤2	>8	≤2->8	40
FEP	16	>16	≤8->16	23.2	>16	>16	≤8->16	14.9	≤8	>16	≤8->16	11.5	≤8	>16	≤8->16	9.6	≤8	>16	≤8->16	8.9
CRO	>32	>32	≤8->32	85.8	32	>32	≤8->32	47.1	16	>32	≤8->32	47.4	≥8	>32	≤8->32	26.3	>32	>32	≤8->32	50
CXM	ND	ND	ND	ND	ND	ND	ND	ND	>16	>16	≤2->16	54.7	16	>16	16->16	44.7	>16	>16	≤2->16	61.1
CTX	>32	>32	≤8->32	86.1	16	>32	≤8->32	31.5	≤8	>32	≤8->32	36.5	≤8	>16	≤8->32	27.2	≤8	>32	≤8->32	42.4
CAZ	>16	>16	≤2->16	85	≤2	>16	≤2->16	22.3	≤8	>16	≤2->16	37	≤2	>16	≤2->16	23.7	8	>16	≤2->16	14.4
CIP	>1	>1	≤0.25->2	85.8	≤0.25	>1	≤0.25->1	24.3	1	>1	≤0.25->2	50	≤0.25	>1	<0.25->2	25.4	>1	>1	≤0.25->1	63.3
LVX	>4	>4	≤0.5->4	81.6	≤0.5	>4	≤0.5->4	19.4	≤0.5	>4	≤0.5->4	26.6	≤0.5	>4	≤0.5->4	15.8	>4	>4	≤0.5->4	62.2
IPN	>8	>8	≤4->8	75.7	≤4	>8	≤4->8	28.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEM	>8	>8	≤4->8	75.3	≤4	>8	≤4->8	20.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
AMX	ND	ND	ND	ND	ND	ND	ND	ND	16	>16	≤8->16	19.8	>16	>16	>16->16	100	16	>16	≤8->16	23.3
AMP	ND	ND	ND	ND	ND	ND	ND	ND	>16	>16	>16->16	100	>16	>16	16->16	93	>16	>16	≤4->16	86.6
AZT	ND	ND	ND	ND	≤8	>16	≤8->16	17.8	16	>16	≤8->16	46.9	≤8	>16	>16->16	28.9	≤8	>16	≤8->16	43.3
CHL	ND	ND	ND	ND	>16	>16	≤4->16	95.4	≤4	>16	≤4->16	24.5	8	>16	≤4->16	17.5	8	>16	≤4->16	20
TIC	>64	>64	≤16->64	77.2	32	>64	≤16->64	26	>64	>64	>64->64	100	≤16	>64	≤16->64	23.7	>64	>64	≤16->64	83.3
SXT	>2	>2	≤2->2	86.9	ND	ND	ND	ND	≤2	>2	≤2->2	45.3	≤2	>2	≤2->2	27.2	>2	>2	≤2->2	56.7

icrobial 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.

A. baumannii was the most commonly isolated species in our ICU (15.8%). In recent years, *A. baumannii* has emerged with an extensive antibiotic resistance spectrum [23]. In our study, 20.9% of *A. baumannii* were detected to be pan resistant by the antimicrobial agents evaluated. The alternative therapeutics that could be used include tigecycline or colistin; however, these antibiotics were not evaluated in this study.

A regional difference in *A. baumannii* resistance to carbapenems has been reported with resistance rates ranging from 3 to 59% [24–26]. The surveillance data presented in this study showed that 75.3% of *A. baumannii* clinical isolates were resistant to MEM. This value is greater than that previously reported for this hospital by our group (59%) [11], which suggests a potential selection

Footnote to Table 2

% R = Percent of resistant strains; ND = not determined.

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

Antimicrobial agent	<i>S. aureus</i> (n = 242)				<i>Enterococcus</i> spp. (n = 156)			
	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	range	%R
AMX	>16	>16	≤4->16	62.4	ND	ND	ND	ND
AMP	≥0.25	>0.25	≤0.25->0.25	90	ND	ND	ND	ND
OXA	>4	>4	≤0.5->4	61.9	ND	ND	ND	ND
PEN	>0.25	>0.25	≤0.25->0.25	89.3	ND	ND	ND	ND
ST1000	ND	ND	ND	ND	≤1,000	>1,000	≤1,000->1,000	49.3
FEP	>16	>16	≤8->16	63.6	ND	ND	ND	ND
CRO	>64	>64	≤8->64	62.0	ND	ND	ND	ND
CXM	>16	>16	≤4->16	50.0	ND	ND	ND	ND
CLI	>2	>2	≤0.5->2	74.8	ND	ND	ND	ND
ERY	>4	>4	≤0.5->4	76.9	>4	>4	≤0.25->4	75.6
GEN500	ND	ND	ND	ND	>500	>500	≤500->500	51.9
LVX	>2	>2	≤1->2	53.3	ND	ND	ND	ND
LNZ	≤2	≤2	≤2->4	0.8	≤2	≤2	≤2->4	3.8
QUD	≤1	2	≤1->2	4.9	>2	>2	≤1->2	75
TEI	≤8	≤8	≤8->16	4.9	≤8	>16	≤8->16	10.2
TET	≤2	8	≤2->16	9.5	>8	>8	≤2->8	56.4
SXT	≤2	>2	≤2->2	12.4	ND	ND	ND	ND
VAN	2	2	≤1->16	4.5	2	>16	≤1->16	10.2

% 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

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

- Gould I: The epidemiology of antibiotic resistance. *Int J Antimicrob Agents* 2008; 32(suppl 1):S2–S9.
- Wienke M, Pfeifer Y, Weissgerber P, Marschal M, Autenrieth IB, Grobner S: In vitro activity of tigecycline and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from a university hospital in South-Western Germany. *Chemotherapy* 2012;58:241–248.
- Koksai F, Ak K, Kucukbasmaci O, Samasti M: Prevalence and antimicrobial resistance patterns of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from blood cultures in an Istanbul university hospital. *Chemotherapy* 2009;55:293–297.
- Bassetti M, Ginocchio F, Mikulska M: New treatment options against Gram-negative organisms. *Crit Care* 2011;15:215.
- Rice LB: Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *J Infect Dis* 2008;197:1079–1081.
- Rice LB: Progress and challenges in implementing the research on ESKAPE pathogens. *Infect Control Hosp Epidemiol* 2010; 31(suppl 1):S7–S10.
- Slama T: Gram-negative antibiotic resistance: there is a price to pay. *Crit Care* 2008; 12(suppl 4):S4.
- Kunz AN, Brook I: Emerging resistant Gram-negative aerobic bacilli in hospital-acquired infections. *Chemotherapy* 2010;56:492–500.
- Peleg A, Seifert H, Paterson D: *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008;21:538–582.
- Rhomberg P, Jones R, Sader H, Fritsche T: Antimicrobial resistance rates and clonality results from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programme: report of year five (2003). *Diagn Microbiol Infect Dis* 2004;49:273–281.
- Garza-Gonzalez E, Llaca-Diaz JM, Bosques-Padilla FJ, Gonzalez GM: Prevalence of multidrug-resistant bacteria at a tertiary-care teaching hospital in Mexico: special focus on *Acinetobacter baumannii*. *Chemotherapy* 2010;56:275–279.
- Paterson D, Bonomo R: Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005;18:657–686.
- Hawkey P: Molecular epidemiology of clinically significant antibiotic resistance genes. *Br J Pharmacol* 2008;153(suppl 1):S406–S413.
- Schiappa DA, Hayden MK, Matushek MG, Hashemi FN, Sullivan J, Smith KY, Miyashiro D, Quinn JP, Weinstein RA, Trenholme GM: Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: a case-control and molecular epidemiologic investigation. *J Infect Dis* 1996;174:529–536.
- Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK, Investigators ABCsAM: Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007;298:1763–1771.
- de Kraker ME, Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, Icket C, Kalenic S, Horvatic J, Seifert H, Kaasch AJ, Paniara O, Argypoulou A, Bompola M, Smyth E, Skally M, Raglio A, Dumpis U, Kelmere AM, Borg M, Xuereb D, Ghita MC, Noble M, Kolman J, Grabljevec S, Turner D, Lansbury L, Grundmann H, Group BS: Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant *Staphylococcus aureus* bloodstream infections. *Antimicrob Agents Chemother* 2011;55:1598–1605.
- Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, Klare I, Kristinsson KG, Leclercq R, Lester CH, Lillie M, Novais C, Olsson-Liljequist B, Peixe LV, Sadowy E, Simonsen GS, Top J, Vuopio-Varkila J, Willems RJ, Witte W, Woodford N: Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill* 2008;13:19046.
- Deshpande LM, Fritsche TR, Moet GJ, Biedenbach DJ, Jones RN: Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the sentry antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 2007;58:163–170.
- Stamm W, Grayson ML, Nicolle L, Powell M: WHO Global Strategy for Containment of Antimicrobial Resistance. Geneva, World Health Organization, 2001.
- Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing: 20th Informational Supplement, M100-S20. Wayne, Clinical and Laboratory Standards Institute, 2010.
- Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC: Characterization of glycopeptide-resistant enterococci from U.S. Hospitals. *Antimicrob Agents Chemother* 1993;37: 2311–2317.
- Ramphal R: Importance of adequate initial antimicrobial therapy. *Chemotherapy* 2005; 51:171–176.
- Cerqueira GM, Peleg AY: Insights into *Acinetobacter baumannii* pathogenicity. *IUBMB Life* 2011;63:1055–1060.
- Al-Tawfiq J, Mohandhas T: Prevalence of antimicrobial resistance in *Acinetobacter calcoaceticus-baumannii* complex in a Saudi Arabian hospital. *Infect Control Hosp Epidemiol* 2007;28:870–872.
- Gales A, Jones R, Forward K, Liñares J, Sader H, Verhoef J: Emerging importance of multi-drug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997–1999). *Clin Infect Dis* 2001;32(suppl 2):S104–S113.
- Seifert H, Dowzicky M: A longitudinal analysis of antimicrobial susceptibility in clinical institutions in Germany as part of the tigecycline evaluation and surveillance trial (2004–2007). *Chemotherapy* 2009;55:241–252.
- Diekema DJ, Pfaller MA, Jones RN, Doern GV, Kugler KC, Beach ML, Sader HS: Trends in antimicrobial susceptibility of bacterial pathogens isolated from patients with bloodstream infections in the USA, Canada and Latin America. SENTRY participants group. *Int J Antimicrob Agents* 2000;13:257–271.
- Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, Oh MD, Choe KW: Bloodstream infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. *Antimicrob Agents Chemother* 2004;48:4574–4581.

- 29 Tumbarello M, Sanguinetti M, Montuori E, Trecarichi EM, Posteraro B, Fiori B, Citton R, D'Inzeo T, Fadda G, Cauda R, Spanu T: Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*: importance of inadequate initial antimicrobial treatment. *Antimicrob Agents Chemother* 2007;51:1987–1994.
- 30 Muro S, Garza-González E, Camacho-Ortiz A, González GM, Llaca-Díaz JM, Bosques F, Rositas F: Risk factors associated with extended-spectrum β -lactamase-producing *Enterobacteriaceae* nosocomial bloodstream infections in a tertiary care hospital: a clinical and molecular analysis. *Chemotherapy* 2012;58:217–224.
- 31 Garza-Gonzalez E, Mendoza Ibarra SI, Llaca-Diaz JM, Gonzalez GM: Molecular characterization and antimicrobial susceptibility of extended-spectrum β -lactamase-producing *Enterobacteriaceae* isolates at a tertiary-care centre in Monterrey, Mexico. *J Med Microbiol* 2011;60:84–90.
- 32 Stefani S, Varaldo P: Epidemiology of methicillin-resistant staphylococci in Europe. *Clin Microbiol Infect* 2003;9:1179–1186.
- 33 Alp E, Klaassen CH, Doganay M, Altöparlak U, Aydın K, Engin A, Kuzucu C, Ozakin C, Ozinel MA, Turhan O, Voss A: MRSA genotypes in Turkey: persistence over 10 years of a single clone of ST239. *J Infect* 2009;58:433–438.
- 34 Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A: Mobile genetic elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. *Clin Microbiol Infect* 2010;16:541–554.
- 35 Cuellar-Rodríguez J, Galindo-Fraga A, Guevara V, Pérez-Jiménez C, Espinosa-Aguilar L, Rolón AL, Hernández-Cruz A, López-Jácome E, Bobadilla-del-Valle M, Martínez-Gamboa A, Ponce-de-León A, Sifuentes-Osornio J: Vancomycin-resistant enterococci, Mexico City. *Emerg Infect Dis* 2007;13:798–799.