Molecular typing and characterization of macrolide, lincosamide and streptogramin resistance in *Staphylococcus epidermidis* strains isolated in a Mexican hospital

Natividad Castro-Alarcón,¹ Rosa María Ribas-Aparicio,² Jesús Silva-Sánchez,³ Amparo Calderón-Navarro,⁴ Alejandro Sánchez-Pérez,³ Isela Parra-Rojas¹ and Gerardo Aparicio-Ozores²

Correspondence

Gerardo Aparicio-Ozores
gaparico@ipn.mx

¹Academic Unit of Chemical and Biological Sciences, University of Guerrero, Chilpancingo Guerrero, Mexico

²National School of Biological Sciences, National Polytechnic Institute, Mexico City, Mexico

³Centers for Infectious Disease Research, National Institute of Public Health, Cuernavaca, Morelos, Mexico

⁴General Hospital of Acapulco, Secretary of Health Guerrero, Acapulco Guerrero, Mexico

Staphylococcus epidermidis is a normal commensal of skin that has become a serious clinical problem because of the combination of increased use of intravascular devices and an increasing number of hospitalized immunocompromised patients. In addition, there is a lack of information pertaining to resistance to macrolide, lincosamide and streptogramin type B (MLS_B) in developing countries, including Mexico. The aim of this study was to investigate the incidence of resistance to MLS_B antibiotics in isolates of *S. epidermidis* obtained in the General Hospital of Acapulco in Mexico. Susceptibility to erythromycin, clindamycin and quinupristin–dalfopristin was tested by a diffusion test, and MICs to oxacillin, erythromycin and lincomycin were determined. Differentiation between MLS_B phenotypes was performed by a double disc diffusion test. A total of 38 of the 47 strains of *S. epidermidis* isolated from nosocomial infections were resistant to oxacillin [meticillin-resistant *S. epidermidis* (MRSE)]. The phenotypes obtained were: 18 constitutive MLS_B, 3 inducible MLS_B, 6 macrolide streptogramin and 4 lincosamide; 7 strains were susceptible to MLS_B antibiotics. The genes associated with resistance were detected by PCR. Genotyping showed a predominance of the *ermA* gene followed by genes *ermC* and *msrA*. The frequency of the genes detected varied slightly from results that have been reported in isolates from other countries. Clonal types were identified by PFGE and revealed the dissemination of two major clones of MRSE in the Mexican hospital. This is believed to be the first report in Mexico on the genes associated with the MLS_B resistance phenotype in *S. epidermidis*, in addition to observing a wide distribution of clonal types in the General Hospital of Acapulco, Mexico.

Received 2 November 2010

Accepted 15 February 2011

INTRODUCTION

Staphylococcus epidermidis has emerged as a major pathogen in nosocomial infections. The increasing use of indwelling devices along with changes in patient populations, hospital environments, medical practice and antibiotic policies have turned these micro-organisms into the leading cause of hospital-acquired bacteraemia (Burnie

et al., 1997; Rosenthal *et al.*, 2008; Schoenfelder *et al.*, 2010).

The members of the class of antibiotics comprising macrolides, lincosamides and streptogramin type B (MLS_B) are chemically different, but have similar inhibitory effects on bacterial protein synthesis (Leclercq & Courvalin, 1991). These antibiotics are widely used in the treatment of Gram-positive infections, especially meticillin-resistant staphylococci, and are the best therapeutic alternative for patients who are hypersensitive to penicillin. However, the widespread use of these antibiotics has caused increased resistance among staphylococci (Hamilton-Miller & Shah,

Abbreviations: cMLS_B, constitutive macrolide, lincosamide and streptogramin type B; CNS, coagulase-negative streptococci; iMLS_B, inducible macrolide, lincosamide and streptogramin type B; MLS_B, macrolide, lincosamide and streptogramin type B; MRSE, meticillin-resistant *Staphylococcus epidermidis*; MS, macrolide and streptogramin type B.

2000). Resistance to erythromycin is usually associated with cross-resistance to other MLS_B. The most common resistance mechanisms in staphylococci are target site modification and active drug efflux. Target site modification is mediated by the *ermA*, *ermB* and/or *ermC* genes (Gatermann *et al.*, 2007). In addition, the phenotypic expression of MLS_B resistance can be inducible or constitutive (Jorgensen *et al.*, 2004). In contrast, active efflux of macrolides is affected by membrane proteins encoded by the *msrA* gene, which is specific for 14- and 15-member macrolides and streptogramin type B (MS phenotype); lincosamides and streptogramin A remain unaffected. The *lnuA* gene confers resistance only to lincosamides, whilst the *vga* gene has been characterized as a determinant of streptogramin A resistance (Lina *et al.*, 1999; Roberts *et al.*, 1999).

The use of accurate and discriminatory typing methods is critical for epidemiological research and to define the origin of infections. PFGE of *Sma*I chromosomal DNA fragments is a sensitive method available for the discrimination of staphylococci (Tenover *et al.*, 1995). PFGE findings might be useful in staphylococci to estimate local dissemination in an institution, which in turn can be used to improve the accuracy of the clinical diagnosis of bacteraemia (Senger *et al.*, 2007). To determine the status of MLS_B resistance and provide a guideline for antibiotic usage, we investigated the frequency of MLS_B resistance in *S. epidermidis* strains collected from a Mexican hospital.

METHODS

Bacterial isolates. A total of 47 consecutive clinical isolates of *S. epidermidis* were obtained from patients with a nosocomial infection in the General Hospital of Acapulco, Mexico, between January 2002 and December 2004. One bacterial isolate per patient was included. During the study period, 36 % of nosocomial infections were associated with

182 isolates of *Staphylococcus* spp., of which 47 strains were identified as *S. epidermidis*. Isolates were identified by standard procedures: Gram staining, growth on manitol salt agar (BBL; Becton Dickinson), catalase and coagulase tests, and complemented with the API Staph system (bioMérieux). All isolates were stored at -70°C in brain-heart infusion broth with 30 % glycerol until further analysis.

Antimicrobial susceptibility testing. Susceptibility to the antibiotics erythromycin, clindamycin, clarithromycin, azithromycin and quinupristin-dalfopristin was performed by a disc diffusion susceptibility test (CLSI, 2009a). MICs to oxacillin, clindamycin and erythromycin (Sigma) were determined by dilution antimicrobial susceptibility tests for bacteria that grow aerobically (CLSI, 2009b). The results were interpreted according to the Clinical and Laboratory Standards Institute recommendations (CLSI, 2009c). To differentiate MS and MLS_B phenotypes for all isolates, a disc approximation test was performed for detection of inducible clindamycin resistance in *Staphylococcus* spp. (Yilmaz *et al.*, 2007) by placing a 15 µg erythromycin disc (Oxoid) 15 mm away from the edge of a 2 µg clindamycin disc (Oxoid) on Mueller-Hinton agar plates (Oxoid) and incubating for 24 h at 35°C . Following incubation, organisms that showed flattening of the clindamycin zone (called a 'D' zone) were considered to have inducible clindamycin resistance [inducible MLS_B (iMLS_B)], whereas isolates that did not show flattening of the clindamycin zone were reported as clindamycin sensitive. *S. epidermidis* isolates resistant to both erythromycin and clindamycin were defined as having constitutive MLS_B resistance (cMLS_B). *Staphylococcus aureus* ATCC 25923 was used as a negative control.

PCRs. Chromosomal DNA from all isolates was extracted as described by Vannuffel *et al.* (1995). The DNA was used as a template for amplification of seven resistance genes: *mecA*, *ermA*, *ermB*, *ermC*, *msrA*, *lnuA* and *vga*. The primer pairs and amplification conditions used for PCR are listed in Table 1. Each 50 µl reaction mixture contained 0.25 mM each dNTP, 50 pmol primers, 10 mM Tris/HCl (pH 8.8), 1.5 mM MgCl₂, 50 mM KCl, 0.1 % Triton X-100, 1.25 U DNA polymerase (Applied Biosystems) and 10 µl DNA sample. Amplification was carried out in a GeneAmp PCR System 2400 thermocycler (Applied Biosystems) with an initial 5 min denaturation at 94°C and 10 min final extension at 72°C . DNA was detected by gel electrophoresis on 1.5 % agarose gels run for 60 min at 95 V and stained with ethidium bromide (1 µg ml⁻¹). *S.*

Table 1. Primer sequences and amplification conditions used in this study

Gene	Primer sequence (5'→3')	Product (bp)	Cycles (and conditions)	Reference
<i>ermA</i>	GTTCAGAACAATCAATACAGAG GGATCAGGAAAAGGACATTTTAC	421	30 (30 s, 94°C ; 30 s, 52°C ; 1 min 72°C)	Lina <i>et al.</i> (1999)
<i>ermB</i>	CCGTTTACGAAATTTGAACAGGTAAAGGGC GAATCGAGACTTGAGTGTGC	359	30 (30 s, 94°C ; 30 s, 55°C ; 1 min, 72°C)	Lina <i>et al.</i> (1999)
<i>ermC</i>	GCTAATATTGTTTAAATCGTCAATTCC GGATCAGGAAAAGGACATTTTAC	572	30 (30 s, 94°C ; 30 s, 52°C ; 1 min, 72°C)	Lina <i>et al.</i> (1999)
<i>msrA</i>	GGCACAATAAGAGTGTTTAAAGG AAGTTATATCATGAATAGATTGTCCTGTT	940	25 (1 min, 94°C ; 1 min, 50°C ; 90 s, 72°C)	Lina <i>et al.</i> (1999)
<i>lnuA</i>	GGTGGCTGGGGGGTAGATGTATTAAGTGG GCTTCTTTTGAAATACATGGTATTTTCGATC	323	30 (30 s, 94°C ; 30 s, 57°C ; 1 min, 72°C)	Lina <i>et al.</i> (1999)
<i>vga</i>	CCAGAACTGCTATTAGCAGATGAA AAGTTCGTTTCTCTTTTCGACG	470	30 (30 s, 94°C ; 30 s, 54°C ; 1 min, 72°C)	Lina <i>et al.</i> (1999)
<i>mecA</i>	TGGCTATCGTGTCAATCG CTGGAACCTGTTGAGCAGAG	310	30 (1 min, 94°C ; 1 min, 56°C ; 3 min, 72°C)	Vannuffel <i>et al.</i> (1995)

aureus NCTC 8325 was used as a control for the detection of *mecA*. DNA products from *ermA*, *ermB*, *ermC* and *msrA* were purified and sequenced on an ABI Prism 3100 DNA sequencer with a BigDye terminator cycle sequencing ready reaction kit. BLAST (Altschul *et al.*, 1997) was used to determine sequence similarity.

Molecular typing. PFGE analysis of *Sma*I-digested chromosomal DNA was performed using a CHEF Mapper II (Bio-Rad) as described by Chung *et al.* (2000), and separated on a 2% agarose gel (pulsed field-certified; Pronadisa) using a GenePath system (Bio-Rad). Gels were stained with ethidium bromide (1 µg ml⁻¹) and analysed according to Tenover *et al.* (1995). The percentage relatedness was calculated with the Dice coefficient using NTSYSpc version 2.0 software (Applied Biostatistics).

RESULTS

Antimicrobial susceptibility and the *mecA* gene

A total of 38 out of 47 *S. epidermidis* isolates (80.8%) from nosocomial infections were resistant to oxacillin [meticillin-resistant *S. epidermidis* (MRSE)] and also were positive for the *mecA* gene (Table 2). Of the 9 isolates susceptible to oxacillin, only 2 had a macrolide and lincosamide phenotype; thus, only the 38 resistant isolates were included for further characterization. The antibiotic profiles for resistance to erythromycin, clindamycin and quinupristin-dalfopristin are shown in Table 2. Eighteen (47%)

Table 2. Molecular and general characteristics of *mecA* *S. epidermidis* strains

PFGE profile	Isolation date	Sample	Department	MIC oxacillin (µg ml ⁻¹)	MLS _B phenotype	MLS _B genotype
A4	02/12/2002	Catheter	Paediatrics	2	cMLS _B	<i>msrA</i>
A5	30/12/2003	Blood	Paediatrics	4	cMLS _B	<i>msrA</i> + <i>vga</i>
A7	15/12/2003	Blood	Paediatrics	2	cMLS _B	<i>ermA</i> + <i>ermC</i> + <i>msrA</i>
B	27/08/2004	Blood	Paediatrics	4	cMLS _B	<i>ermA</i> + <i>vga</i>
B	01/09/2004	Pleura liquid	Intensive care	4	cMLS _B	<i>ermA</i> + <i>vga</i>
B1	17/08/2004	Ocular secretion	Paediatrics	4	cMLS _B	<i>ermA</i> + <i>vga</i>
B3	21/10/2002	Surgical wound	Surgery	256	cMLS _B	<i>ermC</i>
B4	07/10/2002	Peritoneal liquid	Surgery	32	cMLS _B	<i>ermA</i>
B5	12/04/2004	Catheter	Emergency	64	cMLS _B	<i>ermA</i> + <i>vga</i>
B6	06/02/2004	Liquid testicular	Surgery	8	cMLS _B	<i>ermA</i> + <i>vga</i>
B7	16/01/2004	Blood	Paediatrics	2	cMLS _B	<i>ermA</i> + <i>vga</i>
NR	14/04/2004	Blood	Paediatrics	4	cMLS _B	<i>ermA</i> + <i>vga</i>
NR	16/02/2004	Blood	Paediatrics	2	cMLS _B	<i>ermA</i> + <i>ermC</i> + <i>vga</i>
NR	23/05/2002	Surgical wound	Paediatrics	8	cMLS _B	<i>ermB</i>
NR	18/03/2004	Blood	Paediatrics	256	cMLS _B	<i>ermC</i>
NR	08/08/2002	LCR	Paediatrics	4	cMLS _B	<i>msrA</i>
NR	03/03/2004	Urine	Emergency	8	cMLS _B	None
NR	24/09/2002	Peritoneal liquid	Internal medicine	1	cMLS _B	<i>ermA</i> + <i>ermC</i>
A3	10/03/2004	Catheter	Paediatrics	4	iMLS _B	<i>ermC</i> + <i>lnuA</i>
NR	15/12/2003	Blood	Paediatrics	2	iMLS _B	<i>ermC</i> + <i>lnuA</i>
NR	08/06/2004	Blood	Paediatrics	2	iMLS _B	<i>ermC</i> + <i>msrA</i>
A1	17/08/2004	Ocular secretion	Paediatrics	4	MS	<i>msrA</i>
A9	06/09/2004	Blood	Gynaecology	4	MS	<i>msrA</i>
A10	05/09/2004	Ocular secretion	Emergency	4	MS	<i>msrA</i>
NR	23/01/2004	Ocular secretion	Paediatrics	4	MS	<i>ermC</i> + <i>lnuA</i>
NR	27/08/2004	Blood	Paediatrics	1	MS	<i>msrA</i>
NR	03/02/2004	Blood	Paediatrics	32	MS	<i>ermC</i>
A	03/07/2003	Blood	Intensive care	2	L	<i>msrA</i> + <i>lnuA</i>
A	29/09/2004	Catheter	Surgery	2	L	<i>lnuA</i>
A	29/09/2004	Blood	Paediatrics	2	L	<i>lnuA</i>
A6	29/07/2004	Surgical wound	Traumatology	4	L	<i>lnuA</i>
A	28/05/2003	Catheter	Surgery	2	Susceptible	<i>ermA</i> + <i>lnuA</i>
A	29/09/2004	Catheter	Intensive care	2	Susceptible	<i>lnuA</i>
A8	27/06/2003	Abscess	Intensive care	2	Susceptible	<i>msrA</i>
A2	15/06/2004	Ocular secretion	Emergency	4	Susceptible	None
B2	22/05/2003	Surgical wound	Intensive care	4	Susceptible	None
NR	03/01/2002	Ocular secretion	Paediatrics	8	Susceptible	None
NR	25/02/2002	Surgical wound	Surgery	>256	Susceptible	None

NR, Not related.

presented the cMLS_B phenotype, three (8 %) the iMLS_B phenotype, six (16 %) the MS phenotype, four (11 %) the L phenotype and seven (18 %) were susceptible to all antibiotics. The MIC₅₀ value to erythromycin was high (64 µg ml⁻¹) and the MIC₅₀ level to clindamycin was low (1 µg ml⁻¹). However, the MIC₉₀ level of both was high (>256 µg ml⁻¹).

MLS_B resistance genotypes

A total of 33 out of 38 MRSE had the genes *ermA*, *ermB*, *ermC*, *msrA*, *lnuA* and/or *vga*, which have been associated with resistance to MLS_B antibiotics. The major determinant in MRSE was *ermA*, which was detected in 12 (32 %) isolates and was frequently associated with *vga*, but it was not present in iMLS_B isolates. The gene *ermC* was observed in ten strains, whereas *ermB* was located in a single strain of *S. epidermidis*. Finally, five clinical isolates were negative for PCR, and three susceptible strains had the resistance genes *ermA* + *lnuA*, *lnuA* and *msrA*, respectively (Table 2).

Clonal distribution of MRSE

From the 38 isolates, we obtained two major patterns by PFGE analysis: pattern A (including ten subtypes, A1–A10), and pattern B (with seven subtypes, B1–B7) (Fig. 1). Pattern A included 15 isolates and pattern B 9 isolates, whilst 14 isolates were not related (Table 2). In addition, the dendrogram showed similar rates of 58–100 %, where the two major clusters appeared to be associated with identity percentages of >70 % (Fig. 2). Although there was no relationship between phenotype and genotype assays, pulsotype A predominantly expressed the MLS_B susceptible and the MS resistance phenotypes. In contrast, pulsotype B mainly expressed the cMLS_B phenotype. Three isolates had the *ermC* gene and expressed the iMLS_B phenotype but had no epidemiological relationship. A total of 13 of the 17 MRSE isolates with the cMLS_B phenotype carried at least

one member of the *erm* gene family. Independent of the phenotype, the *Sma*I genomic fingerprints of the MRSE isolates from the General Hospital of Acapulco were distributed among different wards, mainly in the paediatric ward, and were unrelated from 2002 to 2004 (Table 2).

DISCUSSION

S. epidermidis is a leading cause of infectious disease in hospital settings, and when found in the blood has been associated with central venous catheters (Burnie *et al.*, 1997; Brito *et al.*, 2009). Multiresistant strains to different classes of antibiotic have been reported to acquire resistance to macrolides and related antibiotics. Therefore, the treatment of infections due to MRSE and their eradication are very difficult. Constant monitoring of these strains is essential to control their spread (Miragaia *et al.*, 2002). The displayed frequency between the phenotypes of resistance to macrolides, lincosamides and streptogramins varies among different hospitals and geographical areas. For coagulase-negative staphylococci (CNS) in London (Hamilton-Miller & Shah, 2000), a resistance of 55 % [macrolide (M)], 12 % [lincosamide (L)] and 0 % [streptogramin type B (S)] has been reported, whilst for other European countries (Schmitz *et al.*, 1999) results were 75 % (M), 54 % (L) and 0 % (S). The results obtained in this study showed a resistance of 64 % (M), 42 % (L) and 32 % (S). Thus, the resistance range obtained for macrolides and lincosamides was similar to that reported, but was higher for the streptogramins, which was surprising as these antibiotics are not used in Mexico. The iMLS_B phenotype was determined in only three isolates (7.8 %), representing a low frequency compared with 21.9 % (Yilmaz *et al.*, 2007) and 35 % (Schreckenberger *et al.*, 2004) reported previously.

The CNS isolates were analysed for the presence of *erm* methylase and the *msrA* efflux pump by PCR. The *ermA* gene was the resistance determinant detected most frequently in *S. epidermidis* isolates, and similar results have been reported by other authors (Ardic *et al.*, 2005).

Fig. 1. Representative gel from *Sma*I macro-restriction fragments of *S. epidermidis* clinical clones identified in this work. This analysis was performed on two gels; one is shown here containing 27 samples, representing the subtypes obtained. Lanes 1 and 29, molecular size standard (λ oligomers). Letters at the bottom indicate the PFG pattern. NR, Not related.

Fig. 2. Dendrogram of PFGE patterns obtained after *Sma*I digestion of total DNA from 27 *S. epidermidis* isolates obtained at the General Hospital of Acapulco, Mexico, between January 2002 and December 2004. Taxa are designated type A or B; subtypes are indicated with numbers. NR, Not related.

However, this result is in contrast to other findings, where the *ermC* gene was the main resistance determinant observed in CNS, including *S. epidermidis* (Lim *et al.*, 2002; Cetin *et al.*, 2010). In this study, only one isolate had the *ermB* gene, which has been detected by other groups in a low percentage of staphylococci (Hamilton-Miller & Shah, 2000; Chaieb *et al.*, 2007). In contrast to Novotna *et al.* (2005) who confirmed a high occurrence (26 %) of the *msrA* gene for *S. epidermidis*, we found a frequency of 12.5 %. In addition, the methylase genes *ermA*, *ermB* and *ermC*, as well as the *msrA* gene, were detected alone or in different combinations in this study and in another (Lüthje & Schwarz, 2007). Two of the susceptible strains had the *lnuA* gene, which was similar to the study of Lüthje & Schwarz (2006) who observed the presence of this gene in *S. epidermidis* isolates susceptible to erythromycin; however, they showed that the presence of the *lnuA* gene was associated with a specific resistance to pirlimycin, a lincosamide of veterinary use, which was not used in this study. Two strains with the genes *ermA* and *msrA* showed a susceptible phenotype, which may be due to a defect in the expression of the resistance mechanisms involved. Finding such evidence in the literature is complicated because studies that address the detection of genes are exclusively for strains that are resistant to MLS_B antibiotics.

The persistence and dissemination of *S. epidermidis* clones within hospitals have been documented as having their origin in the community or being endemic in the hospital (Widerström *et al.*, 2006; Brito *et al.*, 2009). Also, it has been suggested that antibiotic use is a factor in the selection of CNS clones (Krediet *et al.*, 2004). The results presented here demonstrated that isolates corresponded to clone B with a cMLS_B phenotype as well the *ermA* gene, whereas strains corresponding to clone A presented the MS and susceptibility phenotypes. To our knowledge, this is the first study oriented towards characterizing *S. epidermidis* strains collected in a Mexican hospital. In conclusion, the isolates used in this study showed a relatively high rate of resistance to MLS_B antibiotics, presenting a constitutive behaviour. The *ermA* gene was the most frequently detected among all the isolates. Infections by *S. epidermidis* isolates from the General Hospital of Acapulco, Mexico, formed two clusters of CNS molecular types that were able to persist in different nosocomial areas at least for 3 years.

ACKNOWLEDGEMENTS

We are grateful to Etzel Carreon-Valle for technical assistance. This work was partially supported by Fomix-CONACYT (Mexico) grants GUE-2002-C01-5586 and SALUD-2003-C01-009. N. C.-A. was a fellow

of CONACYT. G.A.-O. is grateful for support to this work from SIP-IPN-Mexico (grant nos 20091358 and 20101151). G.A.-O. and R.M.R.-A. are recipients of COFAA-IPN and EDD-IPN fellowships.

REFERENCES

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Ardic, N., Ozyurt, M., Sareyyupoglu, B. & Haznedaroglu, T. (2005). Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. *Int J Antimicrob Agents* **26**, 213–218.
- Brito, D. V., von Dolinger, E. J., Abdallah, V. O., Darini, A. L. & Gontijo Filho, P. P. (2009). Two outbreaks of mixed etiology associated with central venous catheters inserted by phlebotomy in critical neonates. *Braz J Infect Dis* **13**, 177–182.
- Burnie, J. P., Naderi-Nasab, M., Loudon, K. W. & Matthews, R. C. (1997). An epidemiological study of blood culture isolates of coagulase-negative staphylococci demonstrating hospital-acquired infection. *J Clin Microbiol* **35**, 1746–1750.
- Cetin, E. S., Gunes, H., Kaya, S., Aridogan, B. C. & Demirci, M. (2010). Distribution of genes encoding resistance to macrolides, lincosamides and streptogramins among clinical staphylococcal isolates in a Turkish university hospital. *J Microbiol Immunol Infect* **43**, 524–529.
- Chaieb, K., Zmantar, T., Chehab, O., Bouchami, O., Ben Hasen, A., Mahdouani, K. & Bakhrouf, A. (2007). Antibiotic resistance genes detected by multiplex PCR assays in *Staphylococcus epidermidis* strains isolated from dialysis fluid and needles in a dialysis service. *Jpn J Infect Dis* **60**, 183–187.
- Chung, M., de Lencastre, H., Matthews, P., Tomasz, A., Adamsson, I., Aires de Sousa, M., Camou, T., Cocuzza, C., Corso, A. & other authors (2000). Molecular typing of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis: comparison of results obtained in a multilaboratory effort using identical protocols and MRSA strains. *Microb Drug Resist* **6**, 189–198.
- CLSI (2009a). *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 10th edn, approved standard M02–A10. Wayne, PA: Clinical and Laboratory Standards Institute.
- CLSI (2009b). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 8th edn, approved standard M07–A7. Wayne, PA: Clinical and Laboratory Standards Institute.
- CLSI (2009c). *Performance Standards for Antimicrobial Susceptibility Testing*, 19th informational supplement M02–A10. Wayne, PA: Clinical and Laboratory Standards Institute.
- Gatermann, S. G., Koschinski, T. & Friedrich, S. (2007). Distribution and expression of macrolide resistance genes in coagulase-negative staphylococci. *Clin Microbiol Infect* **13**, 777–781.
- Hamilton-Miller, J. M. & Shah, S. (2000). Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci. *J Antimicrob Chemother* **46**, 941–949.
- Jorgensen, J. H., Crawford, S. A., McElmeel, M. L. & Fiebelkorn, K. R. (2004). Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. *J Clin Microbiol* **42**, 1800–1802.
- Krediet, T. G., Mascini, E. M., van Rooij, E., Vlooswijk, J., Paauw, A., Gerards, L. J. & Fleer, A. (2004). Molecular epidemiology of coagulase-negative staphylococci causing sepsis in a neonatal intensive care unit over an 11-year period. *J Clin Microbiol* **42**, 992–995.
- Leclercq, R. & Courvalin, P. (1991). Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob Agents Chemother* **35**, 1267–1272.
- Lim, J.-A., Kwon, A.-R., Kim, S.-K., Chong, Y., Lee, K. & Choi, E.-C. (2002). Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. *J Antimicrob Chemother* **49**, 489–495.
- Lina, G., Quaglia, A., Reverdy, M. E., Leclercq, R., Vandenesch, F. & Etienne, J. (1999). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother* **43**, 1062–1066.
- Lüthje, P. & Schwarz, S. (2006). Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. *J Antimicrob Chemother* **57**, 966–969.
- Lüthje, P. & Schwarz, S. (2007). Molecular basis of resistance to macrolides and lincosamides among staphylococci and streptococci from various animal sources collected in the resistance monitoring program BfT-GermVet. *Int J Antimicrob Agents* **29**, 528–535.
- Miragaia, M., Couto, I., Pereira, S. F., Kristinsson, K. G., Westh, H., Jarlöv, J. O., Carriço, J., Almeida, J., Santos-Sanches, I. & de Lencastre, H. (2002). Molecular characterization of methicillin-resistant *Staphylococcus epidermidis* clones: evidence of geographic dissemination. *J Clin Microbiol* **40**, 430–438.
- Novotna, G., Adamkova, V., Janata, J., Melter, O. & Spizek, J. (2005). Prevalence of resistance mechanisms against macrolides and lincosamides in methicillin-resistant coagulase-negative staphylococci in the Czech Republic and occurrence of an undefined mechanism of resistance to lincosamides. *Antimicrob Agents Chemother* **49**, 3586–3589.
- Roberts, M. C., Sutcliffe, J., Courvalin, P., Jensen, L. B., Rood, J. & Seppala, H. (1999). Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* **43**, 2823–2830.
- Rosenthal, V. D., Maki, D. G. & Graves, N. (2008). The International Nosocomial Infection Control Consortium (INICC): goals and objectives, description of surveillance methods, and operational activities. *Am J Infect Control* **36**, e1–e12.
- Schmitz, F. J., Verhoef, J., Fluit, A. C. & SENTRY Participants Group (1999). Prevalence of resistance to MLS antibiotics in 20 European university hospitals participating in the European SENTRY surveillance programme. *J Antimicrob Chemother* **43**, 783–792.
- Schoenfelder, S. M., Lange, C., Eckart, M., Hennig, S., Kozytska, S. & Ziebuhr, W. (2010). Success through diversity – how *Staphylococcus epidermidis* establishes as a nosocomial pathogen. *Int J Med Microbiol* **300**, 380–386.
- Schreckenberger, P. C., Ilendo, E. & Ristow, K. L. (2004). Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J Clin Microbiol* **42**, 2777–2779.
- Senger, S. S., Saccozza, M. E. & Yuce, A. (2007). Compatibility of pulsed-field gel electrophoresis findings and clinical criteria commonly used to distinguish between true coagulase-negative staphylococcal bacteremia and contamination. *Infect Control Hosp Epidemiol* **28**, 992–996.
- Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233–2239.

Vannuffel, P., Gigi, J., Ezzedine, H., Vandercam, B., Delmee, M., Wauters, G. & Gala, J. L. (1995). Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR. *J Clin Microbiol* **33**, 2864–2867.

Widerström, M., Monsen, T., Karlsson, C. & Wiström, J. (2006). Molecular epidemiology of methicillin-resistant coagulase-negative

staphylococci in a Swedish county hospital: evidence of intra- and interhospital clonal spread. *J Hosp Infect* **64**, 177–183.

Yilmaz, G., Aydin, K., Iskender, S., Caylan, R. & Koksai, I. (2007). Detection and prevalence of inducible clindamycin resistance in staphylococci. *J Med Microbiol* **56**, 342–345.