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Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals

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The polymerase chain reaction (PCR) was used to study the prevalence of the macrolide resistance genes *ermA*, *ermB*, *ermC*, *msrA/msrB*, *ereA* and *ereB*, in 851 clinical isolates of *Staphylococcus aureus* and 75 clinical isolates of *Enterococcus faecium* that were erythromycin resistant. The isolates were from 24 European university hospitals. In *S. aureus*, the *ermA* gene was more common in methicillin-resistant *S. aureus* (MRSA) isolates (88%) than in methicillin-susceptible *S. aureus* (MSSA) isolates (38%), and occurred mainly in strains with constitutive MLS_B expression. In contrast, *ermC* was more common in MSSA (47%) than in MRSA (5%), occurring mainly in strains with inducible expression. The *ereB* gene was only found in MRSA isolates expressing a constitutive MLS_B phenotype (1%). The *ereA* gene was not detected. Macrolide resistance by efflux due to the *msrA*/*msrB* gene was only detected in MSSA isolates (13%). In contrast to *S. aureus*, erythromycin resistance in *E. faecium* was almost exclusively due to the presence of the *ermB* gene (93%).

Introduction

Macrolide resistance can be caused by several mechanisms,[1] the predominant form being target modification mediated by one or more *erm* genes encoding a 23S rRNA methylase. The addition of two methyl residues to a highly conserved adenine residue in domain V, the peptidyl transferase centre of 23S rRNA, leads to a conformational change in the ribosome, rendering the strain resistant to most macrolides, lincosamides and streptogramin B compounds. Phenotypically, this resistance pattern is known as MLS_B resistance.[1] Resistance to MLS antibiotics caused by the presence of macrolide efflux pumps in staphylococci (encoded by *msrA* or *msrB*) has also been documented.[2] Furthermore, inactivation has been described in several organisms.[3] For example, enzymes (EreA and EreB) that hydrolyse the lactone ring of the macrocyclic nucleus and phosphotransferases that inactivate macrolides have been reported in *Staphylococcus aureus*.[4]

In an attempt to update our knowledge of the status of MLS resistance in Europe, we recently investigated the prevalence of resistance to macrolides, clindamycin and quinupristin/dalfopristin.[5] The present investigation was undertaken to study the prevalence of the macrolide resistance genes, *ermA*, *ermB*, *ermC*, *msrA/msrB*, *ereA* and *ereB*, using the polymerase chain reaction (PCR), in the first 851 unrelated clinical isolates of erythromycin-resistant *S. aureus* and the first 75 unrelated clinical isolates of erythromycin-resistant *Enterococcus faecium*. These isolates were sent from 24 different European university hospitals as part of the SENTRY Antimicrobial Surveillance Programme.

Material and methods

Bacterial isolates

The study included clinical isolates collected since the initiation of the European SENTRY programme in April 1997 through to December 1998. The protocol for this study has been described previously.[5]

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PCR for the detection of macrolide resistance genes

Every isolate of *S. aureus* and *E. faecium* demonstrating resistance to erythromycin was screened for the presence of macrolide resistance genes. In total, 851 erythromycin-resistant *S. aureus* (358 methicillin-susceptible *S. aureus* (MSSA) and 493 methicillin-resistant *S. aureus* (MRSA)) and 75 erythromycin-resistant *E. faecium* isolates were analysed. In addition, 50 random isolates that were fully susceptible to macrolides (40 *S. aureus* and 10 *E. faecium*) were screened for the presence of macrolide resistance genes as described below.

Oligonucleotide primers for *ermA*, *ermB*, *ermC*, *msrA/msrB*, *ereA* and *ereB* for use in the PCRs were selected from the DNA sequences published by Sutcliffe *et al.*[3] The specificity of each set of primers was tested using DNA extracts of reference strains containing *ermA*, *ermB*, *ermC*, *msrA/msrB*, *ereA* and *ereB* (*ermA*: *S. aureus* RN 1389; *ermB*: *Streptococcus pyogenes* AC1/pAC1; *ermC*: *S. aureus* RN4220/pE194 and *msrA*: *S. aureus* RN4220/pAT10) all kindly supplied by Joyce Sutcliffe (Central Research Division, Pfizer, Groton, CT, USA). In addition, strains containing *ereA* (*Escherichia coli*/pIP1100) and *ereB* (*E. coli*/pAT72) kindly supplied by Patrice Courvalin (Institute Pasteur, Paris, France) were used. A random sample of PCR products with each set of primers was sequenced. Primers specific for conserved regions of the 16S rRNA gene were used as additional internal controls.[6]

Genomic DNA was isolated and two multiplex PCRs (primer set for *ermA*, *ermB* and *ermC*, together with *msrA/msrB*, as well as a primer set for *ereA* and *ereB* in a second separate PCR) were performed as described by Sutcliffe *et al.*[3] The expected PCR products for *ermA*, *ermB* and *ermC* were between 639 and 645 bp. Therefore, after confirmation of the presence of an *erm* gene, single PCRs were performed in order to verify the class of the *erm* gene, either *ermA*, *ermB* or *ermC*.

Results and discussion

We reported recently that the percentage of MSSA isolates from European university hospitals that showed susceptibility to erythromycin was about 20 times higher than that of MRSA isolates (66.9% versus 3.4%). In 93% of the erythromycin-resistant MRSA and 44% of the erythromycin-resistant MSSA, expression of MLS_B resistance was constitutive.[5] Only 6.6% of the *E. faecium* isolates tested were susceptible to erythromycin, with all erythromycin-resistant isolates displaying the constitutive MLS_B resistance phenotype.[5]

This present survey constitutes the largest collection of clinical isolates of *S. aureus* and *E. faecium* studied for the prevalence of macrolide resistance genes. The multiplex PCRs described by Sutcliffe *et al.*[3] for research purposes can also be routinely applied to survey macrolide resistance mechanisms present in large collections of clinical isolates. Using their method we failed to detect non-specific PCR products. Furthermore, macrolide resistance genes were not detected in any of the 50 erythromycin-sensitive *S. aureus* and *E. faecium* isolates tested. As shown in Table I, the most prevalent resistance gene in *S. aureus* was *ermA* (571/851; 67%), followed by *ermC* (192/851; 23%) and *msrA*/*msrB* (48/851; 6%). Less common were *ermB* and *ereB,* each occurring in 0.6% of the erythromycin-resistant *S. aureus* isolates tested. The *ereA* gene was not detected in any of the isolates.

The *ermA* gene was more common in MRSA isolates (88% in MRSA versus 38% in MSSA), while *ermC* was more common in MSSA (5% in MRSA versus 47% in MSSA). Within the *S. aureus* collection, *ermA* was predominant in strains expressing a constitutive MLS_B phenotype, while *ermC* was predominant in MSSA isolates with an inducible MLS_B phenotype. One *erm* gene was detected in 716 of 851 *S. aureus* isolates (84%), whereas the combination *ermA* and *ermC* was found in only 26 of 851 isolates (3%). In general, our observations are in line with the findings of Lina *et al.*,[7] who studied 144 MLS_B-resistant *S. aureus* strains originating from French hospitals in 1995. They found that the *ermA* gene was more common in MRSA isolates (57.6%), mainly in strains with constitutive MLS_B expression, than in MSSA isolates (5.6%), whereas *ermC* was more common in MSSA isolates (20.1%), mainly in strains with inducible expression, than in MRSA isolates (4.9%).[7] Similar findings to ours were also reported from Denmark, where *ermA* and *ermC* genes were responsible for erythromycin resistance in 98% of the 428 *S. aureus* isolates studied.[8] The *ermA* gene was solely responsible for erythromycin resistance until 1971, while *ermC* became dominant between 1984 and 1988.[8] In accordance with the observations from Denmark, Nicola and colleagues detected the *ermA* gene in 15 of 16 erythromycin-resistant *S. aureus* isolates originating from the USA and isolated between 1958 and 1969.[9] Thus, *ermC* has only recently become prevalent in the *S. aureus* population. Our results on the low prevalence of *ermB* are also in line with earlier studies.[7,9] Although *ermB* was present in only a minority of strains, it was formerly found only in animal strains.[10] In contrast to Lina *et al.*[7] and Nicola *et al.*,[9] we found an association between different *erm* genes, namely *ermA* in combination with *ermC*, in *S. aureus* isolates.

The *ereB* gene, coding for a macrolide-inactivating enzyme, was only found in MRSA isolates expressing the constitutive MLS_B phenotype (5/458; 1%) (Table I). Neither *ereA* nor *ereB* in combination with other macrolide resistance determinants was found. We are not aware of any other surveillance study describing the prevalence of *ereA* and *ereB* in erythromycin-resistant *S. aureus* isolates.

Macrolide resistance by efflux due to the *msrA*/*msrB* gene was only found in MSSA isolates (14/358; 13%). This is in contrast to the results of Lina *et al.*,[7] who detected the *msrA/msrB* gene in both MSSA and MRSA isolates. Moreover, they found the gene in only 2.1% of the 144 *S. aureus*

Table I. Prevalence of macrolide resistance genes in erythromycin-resistant *Staphylococcus aureus* and *Enterococcus faecium* isolates

Genes present	MRSA (n = 493)		MSSA (n = 358)		*E. faecium* (n = 75)
	constitutive phenotype (n = 458; 93%)	inducible phenotype (n = 35; 7%)	constitutive phenotype (n = 158; 44%)	inducible phenotype (n = 200; 56%)	constitutive phenotype (n = 75; 100%)
ermA	397 (86.7%)	26 (74.2%)	92 (58.2%)	30 (15%)	1 (1.3%)
ermB	2 (0.4%)	0	0	3 (1.5%)	68 (90.7%)
ermC	9 (2.0%)	3 (8.6%)	34 (21.5%)	120 (60%)	0
ermA and *ermB*	0	0	0	0	2 (2.6%)
ermA and *ermC*	9 (2.0%)	3 (8.6%)	11 (7.0%)	3 (1.5%)	0
ereB	5 (1.1%)	0	0	0	0
msrA/*msrB*	0	0	13 (8.2%)	35 (17.5%)	0
No known gene detectable	36 (7.9%)	3 (8.6%)	8 (5.1%)	9 (4.5%)	4 (5.3%)

strains tested. In line with their observations, however, we found no combination of *msrA*/*msrB* with other macrolide resistance determinants. To date, three *S. aureus* isolates have been found to harbour esterase activity-hydrolysing macrolides and a macrolide efflux system.[4]

The *ermB* gene was the most prevalent resistance determinant found in erythromycin-resistant *E. faecium* isolates, followed by *ermA* (93% versus 4%). The combination of *ermA* and *ermB* was detected in two of 75 isolates (3%). Jensen and colleagues recently analysed 113 erythromycin-resistant enterococcal isolates of human and animal origin and found the *ermB* gene to be present in 88%.[11]

The frequency of isolates that displayed erythromycin resistance in the absence of one of the six resistance genes tested for ranged between 4.5% and 8.6% in the five groups of isolates analysed. This implies that other mechanisms contribute to macrolide resistance in *S. aureus* and *E. faecium*.

In summary, resistance to erythromycin in *S. aureus* isolates from French hospitals was due mainly to the presence of *ermA* and *ermC* genes. The *ermA* gene was more common in MRSA isolates, mainly in strains with a constitutive MLS_B expression, than in MSSA isolates, whereas *ermC* was more common in MSSA isolates, mainly in strains with inducible expression. Only a few strains had the *ereB* or *ermB* gene, while macrolide resistance by efflux due to the *msrA* gene was more common, but only detectable in MSSA. In contrast to *S. aureus*, erythromycin resistance in *E. faecium* was almost exclusively due to the presence of the *ermB* gene.

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