ORIGINAL ARTICLE 10.1111/j.1469-0691.2007.01749.x

Distribution and expression of macrolide resistance genes in coagulase-negative staphylococci

S. G. Gatermann, T. Koschinski and S. Friedrich

Abteilung für Medizinische Mikrobiologie, Ruhr-Universität Bochum, Bochum, Germany

ABSTRACT

In total, 494 isolates of coagulase-negative staphylococci (CoNS) were identified to the species level by biochemical tests and *sod*A sequencing. Erythromycin resistance phenotypes were determined and specific resistance genes were identified by PCR. The prevalence of erythromycin resistance varied widely among staphylococcal species, from 0% in *Staphylococcus lugdunensis* to almost 90% in *Staphylococcus haemolyticus*. Most (63%) erythromycin-resistant isolates carried constitutively expressed *erm*(C) as the sole resistance determinant, with the notable exception of *Staphylococcus hominis* subsp. *hominis*, which carried inducible *erm*(C). The *erm*(A) and *erm*(B) determinants were comparatively rare. The *msr*(A) gene was carried by 20–30% of all erythromycin-resistant isolates, with little variation among species, and was combined in 16.7% of isolates with *mph*(C), a resistance gene of unknown clinical relevance found previously in isolates of veterinary origin. No erythromycin resistance that could not be attributed to the genes investigated was detected. It was concluded that the presence of methylases cannot be assumed in CoNS isolates that appear erythromycin-resistant and clindamycin-susceptible; thus, methods that detect the export mechanism should be used with clinically significant isolates to indicate whether use of clindamycin may be effective. In *Staphylococcus epidermidis* and *S. haemolyticus*, 46% and 66%, respectively, of erythromycin-resistant, clindamycin-susceptible isolates were susceptible to clindamycin therapy.

Keywords Clindamycin resistance, coagulase-negative staphylococci, erythromycin resistance, genetic determinants, PCR, staphylococci

Original Submission: 28 September 2006; **Revised Submission:** 14 February 2007; **Accepted:** 12 March 2007

Clin Microbiol Infect 2007; **13**: 777–781

INTRODUCTION

Macrolide–lincosamide–streptogramin B (MLS_B) antibiotics, especially clindamycin, are important therapeutic agents for penicillin-allergic patients suffering from staphylococcal infections. Erythromycin resistance in *Staphylococcus aureus* is associated most often with the presence of an rRNA methylase, whose action also affects resistance to other macrolides, lincosamides and streptogramin B [1]. The structural genes may be expressed either inducibly or constitutively, and mutations occur readily in the regulatory region of these genes to change inducible resistance to constitutive resistance. Consequently, there is a reluctance to use clindamycin against erythromycin-resistant isolates, as resistance may emerge during therapy [2–6]. A second resistance mechanism involves export of the antibiotic, typically mediated by *msr*(A) [7]. This mechanism does not affect the activity of lincosamides. For *S. aureus*, it has been shown that erythromycin resistance is caused almost exclusively by *erm*(A) or *erm*(C), whereas export of macrolide antibiotics by *msr*(A) or inactivation of lincosamides by *lnu*(A) is rare [8–11]. Therefore, erythromycin-resistant isolates of *S. aureus* may also be considered to be clindamycin-resistant in most geographical regions.

Much less is known about the basis of macrolide and lincosamide (ML) resistance in coagulase-negative staphylococci (CoNS), but it has been reported that resistance mechanisms other than

Corresponding author and reprint requests: S. G. Gatermann, Abteilung für Medizinische Mikrobiologie, Ruhr-Universität Bochum, Universitätsstrasse 150, 44800 Bochum, Germany
E-mail: soeren.gatermann@rub.de

methylation are more common [10,12–14]. A macrolide phosphotransferase gene, *mph*(C), has been found in CoNS of veterinary origin [14], but neither its presence nor its relevance in human isolates has yet been studied. In addition, several reports have attempted to associate different species of CoNS with the presence of specific resistance mechanisms. Thus, different strategies to correctly identify ML resistance mechanisms in CoNS might be required, and the assumption that methylases are responsible for erythromycin resistance could lead to an unnecessary avoidance of lincosamides and an increased usage of glycopeptides.

In an era of automated susceptibility testing systems that test for resistance to single antibiotics, but not for induction, it is important to identify isolates that are not predicted correctly by the software algorithms of the automated systems [15]. The present study therefore tested clinical isolates of CoNS by phenotypic and genotypic means to determine the presence and mechanism(s) of ML resistance.

MATERIALS AND METHODS

Bacteria

In total, 494 bacterial isolates identified presumptively as CoNS were collected during 2004–2006. Samples came from tertiary-care hospitals in Bochum, Germany, and from other hospitals in the same region. Most isolates were considered to be clinically relevant and originated from blood cultures or venous catheter infections. The isolates were identified to the species level using biochemical techniques [16], with unclear or equivocal results being resolved by sequencing of the *sod*A gene [17].

Characterisation of resistance mechanisms

Phenotypic characterisation of MLS_B resistance was performed using the disk approximation test with erythromycin, clindamycin and lincomycin disks. The D phenomenon was considered to be indicative of the presence of an inducible *erm* gene [18]. Oxacillin resistance was tested as described by Ferreira *et al*. [19], and all isolates were investigated by PCR for the presence of *mecA* [20]. Multiplex PCR for the *erm* genes [21] and PCRs for the presence of *msr*(A), *lnu*(A) and *mph*(C) were performed as described previously [10,22].

RESULTS

Of the 494 isolates, the majority were *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* subsp. *hominis* and *Staphylococcus lugdunensis* (Table 1). Sequencing of *sod*A was performed for 44 isolates because biochemical identification was equivocal. Erythromycin resistance was found in 305 (62%) of the isolates; three (0.6%) were resistant to lincomycin only and harboured *lnu*(A).

Of the 305 erythromycin-resistant isolates, 155 (51%) expressed constitutive clindamycin resistance, 78 (25.6%) were inducibly resistant, and 72 (23.6%) were non-inducible (Table 1). *S. haemolyticus* was mostly (89.8%) erythromycin-resistant; resistance was less common but still prevalent in *S. epidermidis* (62.5%) and *S. hominis* subsp. *hominis* (51.4%); no erythromycin resistance was found in

Table 1. Macrolide–lincosamide–streptogramin B (MLS_B) phenotypes and single resistance genes in coagulase-negative staphylococci

	Total		Erythromycin-resistant		Phenotype						*erm*(A)				*erm*(B)	*erm*(C)						*msr*(A)	
Species	*n*	%	*n*	%	ind	%	const	%	n.i.	%	*n*	%	ind	const	*n*	*n*	%	ind	%	const	%	*n*	%
S. epidermidis	333	67.4	208	62.5	54	26.0	108	51.9	46	22.1	12	5.8	3	9	1	134	64.4	47	35.1	87	64.9	14	6.7
S. haemolyticus	59	11.9	53	89.8	8	15.1	29	54.7	16	30.2	2	3.8	1	1	3	15	28.3	5	33.3	10	66.7	5	9.4
S. hominis hominis	37	7.5	19	51.4	10	52.6	3	15.8	6	31.6	2	10.5		2		11	57.9	10	90.9	1	9.1	1	5.3
S. lugdunensis	15	3.0																					
S. warneri	9	1.8	5	55.6	1	20.0	2	40.0	2	40.0						3	60.0	1	33.3	2	66.7	1	20.0
S. caprae	7	1.4	3	42.9	2	66.7	1	33.3								2	66.7	1	50.0	1	50.0		0.0
S. simulans	6	1.2	4	66.7	1	25.0	2	50.0	1	25.0						3	75.0	1	33.3	2	66.7	1	25.0
S. capitis	5	1.0	1	20.0			1	100.0								1	100.0		0.0	1	100.0		0.0
S. cohnii	5	1.0	4	80.0	1	25.0	3	75.0							1	3	75.0	1	33.3	2	66.7		0.0
S. hominis novobiosepticus	5	1.0	4	80.0			4	100.0							1	2	50.0		0.0	2	100.0		0.0
S. xylosus	4	0.8	2	50.0			2	100.0							1	1	50.0		0.0	1	100.0		0.0
S. saprophyticus	2	0.4	1	50.0	1	100.0				0.0						1	100.0	1	100.0		0.0		0.0
S. sciuri	3	0.6																					
S. schleiferi	3	0.6	1	33.3					1	100.0													
S. chromogenes	1	0.2																					
Total	494	100.0	305	61.7	78	25.6	155	50.8	72	23.6	16	5.2	4	12	7	200	65.6	74	37.0	126	63.0	72	23.6

ind., inducible expression of clindamycin resistance; const., constitutive expression of clindamycin resistance; n.i., erythromycin-resistant, clindamycin-susceptible, no induction.

Table 2. Combinations of macrolide–lincosamide–streptogramin B (MLS_B) resistance genes in coagulase-negative staphylococci

	Total		Erythromycin-resistant		*mph*(C) +*msr*(A)		*mph*(C)+*erm*(C)			*msr*(A)+*erm*(C)			*erm*(A)+*erm*(C)			*erm*(C) + *msr*(A) + *mph*(C)		
Species	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**ind**	**const**	***n***	**ind**	**const**	***n***	**ind**	**const**	***n***	**ind**	**const**
S. epidermidis	333	67.4	208	62.5	32	15.4	9	4	5	6		6						
S. haemolyticus	59	11.9	53	89.8	12	22.6	15	2	13							3		3
S. hominis hominis	37	7.5	19	51.4	5													
S. lugdunensis	15	3.0																
S. warneri	9	1.8	5	55.6	1													
S. caprae	7	1.4	3	42.9			1	1										
S. simulans	6	1.2	4	66.7														
S. capitis	5	1.0	1	20.0														
S. cohnii	5	1.0	4	80.0														
S. hominis novobiosepticus	5	1.0	4	80.0									1		1			
S. xylosus	4	0.8	2	50.0														
S. saprophyticus	2	0.4	1	50.0														
S. sciuri	3	0.6																
S. schleiferi	3	0.6	1	33.3	1	100.0												
S. chromogenes	1	0.2																
Total	494	100.0	305	61.7	51	16.7	25	7	18	9		9	1		1	3		3

ind, inducible; const, constitutive.

S. lugdunensis, *Staphylococcus sciuri* and *Staphylococcus chromogenes*. The *erm*(C) gene was most common (200 isolates, 65.6%), followed by *msr*(A) (72 isolates, 23.6%), *erm*(A) (16 isolates, 5.3%) and *erm*(B) (seven isolates, 2.3%). The *mph*(C) gene was found in 79 (25.9%) isolates, and was always detected in combination with *msr*(A) or *erm*(C) (Table 2).

When different species were analysed, the only significant variation in the above distribution was a higher prevalence of *msr*(A) in *S. haemolyticus* (30.2%) and *S. hominis* subsp. *hominis* (31.6%), compared with *S. epidermidis* (22.1%) (Table 1). Interestingly, *msr*(A) was most often combined in *S. haemolyticus* and *S. epidermidis* with *mph*(C) (14/18, 77.8%, and 32/46, 69.6%, respectively) (Table 2); *erm*(C) was most often inducibly expressed in *S. hominis* subsp. *hominis*, whereas expression was mostly constitutive in other prevalent species.

Isolates that were erythromycin-susceptible in the phenotypic test were also tested for the presence of *mph*(C), but no isolate with this genotype was detected. Combinations of methylases and the exporter were detected only rarely, and occurred mostly in oxacillin-resistant isolates. Oxacillin resistance was common (272/494, 55.1%), but its prevalence differed significantly among species, being greatest in *S. haemolyticus* (51/59, 86.4%) and *S. hominis* subsp. *novobiosepticus* (4/5, 80%), followed by *S. epidermidis* (186/333, 55.9%) and *Staphylococcus warneri* (5/9, 55.6%). No oxacillin resistance was found in *S. lugdunensis*, *Staphylococcus schleiferi* or *Staphylococcus saprophyticus*. The phenotypic oxacillin screening test and *mecA* PCR always yielded concordant results. As expected, erythromycin resistance was more prevalent in oxacillin-resistant isolates (83.5%).

DISCUSSION

Erythromycin resistance was more common (62%) in the present study than was described in 2002 by John *et al*. [23] (35%), but was similar to the level (56%) reported by Hamilton-Miller and Shah [9]. However, most erythromycin-resistant isolates in the present study showed constitutive expression of clindamycin resistance (51%), in contrast to previous studies that reported constitutive resistance in 25%, 39% and 26–37%, respectively, of erythromycin-resistant CoNS isolates [9,10,24]. As reported previously [10,12,13,18], MLS_B resistance was caused most often by *erm*(C), in contrast to the situation in *S. aureus*, in which constitutive resistance tends to be caused by *erm*(A) and the inducible phenotype is caused by *erm*(C) [10,13,18,25]. Export of macrolides is rarely seen in *S. aureus*, but seems to be more frequent in CoNS [9,10]. However, one study [18] reported that the *msr*(A) exporter was present in a high proportion of *S. aureus* isolates, indicating that geographical differences may exist.

The present study extends the data from previous studies in which only unspeciated CoNS

were analysed. The overall species distribution of CoNS was similar to that in previous reports [26–28]. Erythromycin resistance was most prevalent in *S. haemolyticus* (86.4%), where it was usually constitutive and caused by *erm*(C). Erythromycin resistance was less prevalent in *S. epidermidis* and *S. hominis* subsp. *hominis*, but was still caused by constitutively expressed *erm*(C). In these three species, *msr*(A), alone or in combination with *mph*(C), was the only detectable resistance mechanism in *c.* 20–30% of isolates. A similar prevalence of the exporter gene was seen in *S. warneri* and *Staphylococcus simulans*.

Combinations of resistance mechanisms were seen only rarely, and occurred mostly in oxacillin-resistant isolates. Interestingly, the *mph*(C) macrolide-modifying enzyme was found only in combination with other resistance genes, a situation that has been described previously [29], although *mph*(C) has also been reported to be the only resistance mechanism in *Staphylococcus xylosus* and *Staphylococcus equorum* (veterinary isolates) with low-level erythromycin resistance [14]. The clinical implications of *mph*(C) in human and animal infections are presently unknown. However, since *mph*(C) occurred in the present study only in combination with resistance mechanisms of known clinical significance, the presence of *mph*(C) may be of minor importance, so that microbiological interpretations can be guided by the appearance of the phenotype.

No isolates that were erythromycin-resistant but did not harbour any of the tested resistance mechanisms were encountered. This is in contrast to previous reports that have found unidentified resistance mechanisms in a considerable proportion of CoNS [18,25]. In addition, isolates that were phenotypically erythromycin-susceptible never harboured any of the tested genes. These observations validate the results and interpretations of the phenotypic tests. No erythromycin resistance caused by *msr*(A) was found in *Staphylococcus caprae*, *Staphylococcus capitis*, *Staphylococcus cohnii*, *S. hominis* subsp. *novobiosepticus*, *S. xylosus* or *S. saprophyticus*.

Thus, in conjunction with previous reports, the results of the present study corroborate the assumption that there are geographical differences in the prevalence of erythromycin resistance mechanisms among staphylococci [13,24]. This distribution should be investigated in order to determine the most appropriate testing strategy. In the Bochum region, it would be ineffective to test all erythromycin-resistant *S. aureus* isolates for the presence of inducible resistance, as the export mechanism is rare. In CoNS, especially in *S. epidermidis*, *S. hominis* subsp. *hominis* and *S. haemolyticus*, the export mechanism is found in 20–30% of erythromycin-resistant isolates. Clindamycin cannot be considered to be ineffective in these species without testing for inducible resistance. The present data indicate that this test would reveal 50% of *S. epidermidis* and *S. hominis* subsp. *hominis* isolates to be clindamycin-susceptible; in *S. haemolyticus*, a species with limited therapeutic options because of its multiple antibiotic resistance, up to 65% of all erythromycin-resistant, clindamycin-susceptible isolates would be susceptible to clindamycin therapy. The results therefore suggest that the D-test should be used to investigate clinically relevant, erythromycin-resistant, clindamycin-susceptible CoNS. It can be expected that at least 50% of isolates tested with this method will be susceptible to clindamycin therapy. It should be noted that this test is necessary even for isolates that have been tested using automated commercial susceptibility testing systems, as these machines do not test for inducible resistance genes.

REFERENCES

1. Roberts MC, Sutcliffe J, Courvalin P *et al.* Nomenclature for macrolide and macrolide–lincosamide–streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999; **43**: 2823–2830.
2. Schmitz FJ, Petridou J, Jagusch H *et al.* Molecular characterization of ketolide-resistant *erm*(A)-carrying *Staphylococcus aureus* isolates selected in vitro by telithromycin, ABT-773, quinupristin and clindamycin. *J Antimicrob Chemother* 2002; **49**: 611–617.
3. Werckenthin C, Schwarz S, Westh H. Structural alterations in the translational attenuator of constitutively expressed *erm*C genes. *Antimicrob Agents Chemother* 1999; **43**: 1681–1685.
4. Werckenthin C, Schwarz S. Molecular analysis of the translational attenuator of a constitutively expressed *erm*(A) gene from *Staphylococcus intermedius*. *J Antimicrob Chemother* 2002; **46**: 785–788.
5. Siberry GK, Tekle T, Carroll K *et al.* Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. *Clin Infect Dis* 2003; **37**: 1257–1260.
6. Drinkovic D, Fuller ER, Shore KP *et al.* Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J Antimicrob Chemother* 2001; **48**: 315–316.

7. Ross JI, Eady EA, Cove JH *et al.* Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. *Mol Microbiol* 1990; **4**: 1207–1214.
8. Azap ÖK, Arslan H, Timurkaynak F, Yapar G, Oruç E, Gağir Ü. Incidence of inducible clindamycin resistance in staphylococci: first results from Turkey. *Clin Microbiol Infect* 2005; **11**: 582–584.
9. Hamilton-Miller JM, Shah S. Patterns of phenotypic resistance to the macrolide–lincosamide–ketolide–streptogramin group of antibiotics in staphylococci. *J Antimicrob Chemother* 2000; **46**: 941–949.
10. Lina G, Quaglia A, Reverdy ME *et al.* Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother* 1999; **43**: 1062–1066.
11. Lim JA, Kwon AR, Kim SK *et al.* Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. *J Antimicrob Chemother* 2002; **49**: 489–495.
12. Westh H, Hougaard DM, Vuust J *et al. erm* genes in erythromycin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. *APMIS* 1995; **103**: 225–232.
13. Jenssen WD, Thakker-Varia S, Dubin DT *et al.* Prevalence of macrolides–lincosamides–streptogramin B resistance and *erm* gene classes among clinical strains of staphylococci and streptococci. *Antimicrob Agents Chemother* 1987; **31**: 883–888.
14. Lüthje P, Schwarz S. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide–lincosamide resistance phenotypes and genotypes. *J Antimicrob Chemother* 2006; **57**: 966–969.
15. Jorgensen JH, Crawford SA, McElmeel ML, Fiebelkorn KR. Detection of inducible clindamycin resistance of staphylocccci in conjunction with performance of automated broth susceptibility testing. *J Clin Microbiol* 2004; **42**: 1800–1802.
16. De Paulis AN, Predari SC, Chazarreta CD *et al.* Five-test simple scheme for species-level identification of clinically significant coagulase-negative staphylococci. *J Clin Microbiol* 2003; **41**: 1219–1224.
17. Poyart C, Quesne G, Boumaila C *et al.* Rapid and accurate species-level identification of coagulase-negative staphylococci by using the *sodA* gene as a target. *J Clin Microbiol* 2001; **39**: 4296–4301.
18. Fiebelkorn KR, Crawford SA, McElmeel ML *et al.* Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol* 2003; **41**: 4740–4744.
19. Ferreira RB, Iorio NL, Malvar KL *et al.* Coagulase-negative staphylococci: comparison of phenotypic and genotypic oxacillin susceptibility tests and evaluation of the agar screening test by using different concentrations of oxacillin. *J Clin Microbiol* 2003; **41**: 3609–3614.
20. Kobayashi N, Wu H, Kojima K *et al.* Detection of *mec*A, *fem*A, and *fem*B genes in clinical strains of staphylococci using polymerase chain reaction. *Epidemiol Infect* 1994; **113**: 259–266.
21. Khan SA, Nawaz MS, Khan AA *et al.* Simultaneous detection of erythromycin-resistant methylase genes *erm*A and *erm*C from *Staphylococcus* spp. by multiplex-PCR. *Mol Cell Probes* 1999; **13**: 381–387.
22. Schnellmann C, Gerber V, Rossano A *et al.* Presence of new *mecA* and *mph*(C) variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. *J Clin Microbiol* 2006; **44**: 4444–4454.
23. John MA, Pletch C, Hussain Z. In vitro activity of quinupristin/dalfopristin, linezolid, telithromycin and comparator antimicrobial agents against 13 species of coagulase-negative staphylococci. *J Antimicrob Chemother* 2002; **50**: 933–938.
24. Schreckenberger PC, Ilendo E, Ristow KL. 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* 2004; **42**: 2777–2779.
25. Schmitz FJ, Petridou J, Fluit AC *et al.* Distribution of macrolide-resistance genes in *Staphylococcus aureus* blood-culture isolates from fifteen German university hospitals. MARS Study Group. Multicentre Study on Antibiotic Resistance in Staphylococci. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 385–387.
26. Boisson K, Thouverez M, Talon D *et al.* Characterisation of coagulase-negative staphylococci isolated from blood infections: incidence, susceptibility to glycopeptides, and molecular epidemiology. *Eur J Clin Microbiol Infect Dis* 2002; **21**: 660–665.
27. Jarløv JO, Højbjerg T, Busch-Sørensen C *et al.* Coagulase-negative staphylococci in Danish blood cultures: species distribution and antibiotic susceptibility. *J Hosp Infect* 1996; **32**: 217–227.
28. Kleeman KT, Bannerman TL, Kloos WE. Species distribution of coagulase-negative staphylococcal isolates at a community hospital and implications for selection of staphylococcal identification procedures. *J Clin Microbiol* 1993; **31**: 1318–1321.
29. Matsuoka M, Inoue M, Endo Y, Nakajima Y. Characteristic expression of three genes, *msr*(A), *mph*(C) and *erm*(Y), that confer resistance to macrolide antibiotics on *Staphylococcus aureus*. *FEMS Microbiol Lett* 2003; **220**: 287–293.