Antibiotic susceptibility of coagulase-negative staphylococci (CoNS): emergence of teicoplanin-non-susceptible CoNS strains with inducible resistance to vancomycin

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Coagulase-negative staphylococci (CoNS) have become increasingly recognized as important agents of nosocomial infection. One of the characteristics of CoNS is their resistance to multiple antimicrobial agents commonly used for the treatment of staphylococcal infections. CoNS strains ($n=745$) isolated from a university teaching hospital in China between 2004 and 2009 were tested for antibiotic resistance. The antibiotics were placed into three categories based on resistance levels of the CoNS strains to these antibiotics: high resistance (resistance rate $>70\%$), including penicillin G, oxacillin and erythromycin; medium resistance (resistance rate between 30 and 70%), including tetracycline, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole and chloramphenicol; and low resistance (resistance rate $<30\%$), including rifampicin, ceftizoxime and gentamicin. We also found that the prevalence of strains non-susceptible to teicoplanin increased from 4.5 to 6.7% between 2008 and 2009. A one-step vancomycin agar selection experiment and subsequent population analysis revealed potentially vancomycin-resistant subpopulations that have been selected from the teicoplanin-non-susceptible strains. Vigilant surveillance of nosocomial isolates of CoNS is needed to determine their resistance to glycopeptides.

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INTRODUCTION

Coagulase-negative staphylococci (CoNS) are among the most frequently isolated micro-organisms in clinical microbiology laboratories (Cervera *et al.*, 2009; Hung *et al.*, 2011). Since the 1970s, it has been suggested that CoNS are of great importance as true pathogens, and they have become a common cause of a wide variety of infections (Dubois *et al.*, 2010; Otto, 2009). Infections with CoNS occur mainly in specific groups of patients, including patients with neutropenia, neonates and patients with indwelling foreign devices (Huebner & Goldmann, 1999; Zong *et al.*, 2011). Infections at metastatic sites, such as the central nervous system, heart, bones and joints, are especially common, and such infections in these vulnerable populations are difficult to treat (Casey *et al.*, 2007).

The frequency of oxacillin resistance in CoNS strains has increased substantially over recent decades (Stefani &

Varaldo, 2003). This has led to the frequent use of glycopeptide antibiotics for treatment of CoNS infections (Ferreira *et al.*, 2003; Srinivasan *et al.*, 2002; Tacconelli *et al.*, 2001). As a result, the emergence of strains with decreased levels of susceptibility to vancomycin and teicoplanin has been reported (Cremniter *et al.*, 2010; Trueba *et al.*, 2006). Since the 1990s, a worldwide increase in the number of observations of glycopeptide-resistant CoNS has been described (Bourgeois *et al.*, 2007). Some recent reports have pointed out reduced susceptibility of glycopeptides within *Staphylococcus epidermidis* in various European countries, suggesting that such strains might be highly disseminated in the community and in hospitals (Cremniter *et al.*, 2010; Gazzola & Cocconcelli, 2008; Maniati *et al.*, 2005; Natoli *et al.*, 2009; Trueba *et al.*, 2006).

In this report, we systematically studied the antibiotic resistance of all CoNS strains collected between 2004 and 2009 at the Department of Clinical Microbiology at the Second Affiliated Hospital of China Medical University, which is a 4300-bed university teaching hospital. We also performed an epidemiological study of the teicoplanin-resistant CoNS

Abbreviations: CoNS, coagulase-negative staphylococci; TMP/SMX, trimethoprim/sulfamethoxazole; VRSA, vancomycin-resistant *Staphylococcus aureus*.

strains. The purpose of the study is to provide insight toward our understanding of glycopeptide resistance of CoNS and to evaluate its clinical significance.

METHODS

CoNS strains. In total, 745 CoNS strains were used in this study. The strains were collected between 2004 and 2009 from different clinical specimens, including blood (47.3%), nostrils (15.8%), wound (10.5%), urine (7.9%), catheter tips (2.6%), drainage fluids (5.4%), sputum (4.7%), and secretions in general (5.8%). Only one strain from each patient was analysed. The CoNS strains were collected from the following departments of the Second Affiliated Hospital of China Medical University: internal medicine (including cardiology, $n=49$; neurology, $n=61$; nephrology, $n=39$; haematology, $n=54$; gastroenterology, $n=66$; endocrinology, $n=36$; rheumatology, $n=23$; respiratory medicine, $n=37$), the surgical department ($n=114$), surgery intensive care ($n=78$), neurosurgery ($n=53$), thoracic surgery ($n=61$) and paediatrics ($n=74$). The CoNS strains used in this study were either isolated from clinically significant materials (e.g. blood or normally sterile fluids) in the presence of clinical manifestations not attributable to other causes, or isolated from samples in the presence of clinical evidence of infection not attributable to other micro-organisms. Strains were named in Arabic numerals in accordance with the time collected. The staphylococcal strains were first identified by using Pos Combo type 4I MicroScan panels (Baxter Diagnostics). They were further identified and confirmed by using the API 20 Staph system (bioMérieux). The strains were stored at -20°C in tryptic soy broth containing 20% (v/v) glycerol.

Susceptibility tests. The broth microdilution technique was used for initial antimicrobial susceptibility testing, for which customized broth microdilution panels manufactured by Microscan combo ID/AST (Baxter Diagnostics) were used. A suspension with a density equivalent to that of a 0.5 McFarland standard inoculum was prepared in 0.9% saline to achieve a final density of approximately 5×10^5 c.f.u. ml^{-1} . MICs were read as the lowest concentration at which growth was inhibited. The antimicrobial agents tested included penicillin G, oxacillin, rifampicin, tetracycline, chloramphenicol, ceftizoxime, ciprofloxacin, clindamycin, erythromycin, gentamicin, trimethoprim/sulfamethoxazole (TMP/SMX), teicoplanin and vancomycin. We used the agar dilution method to further confirm the teicoplanin and vancomycin MIC values of the teicoplanin-non-susceptible CoNS isolates. The procedure was performed in duplicate on separate occasions, and the means of the duplicates were used. *Staphylococcus aureus* ATCC 29213 was used as the quality control in each set of tests.

Population analysis of CoNS strains. Analysis of the strain subpopulations resistant to vancomycin and teicoplanin was performed. In brief, strains were first cultured overnight in brain–heart infusion (BHI) broth. Then, 50 μl bacterial suspension was transferred to 4 ml pre-warmed BHI broth and incubated at 37°C until the OD_{578} was approximately 0.7. After the OD_{578} was adjusted to 0.3 (approx. 10^8 c.f.u. ml^{-1}), 10-fold dilutions of cell suspensions were used to inoculate BHI agar plates containing 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 mg vancomycin l^{-1} , or 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256 mg teicoplanin l^{-1} . The plates were incubated at 37°C for 48 h, and then the colonies were counted. Heterogeneous vancomycin-resistant *S. aureus* (hetero-VRSA) Mu3 and intermediate VRSA Mu50 were used as quality controls.

One-step selection of vancomycin-resistant CoNS strains. To screen for potentially vancomycin-resistant strains among teicopla-

nin-non-susceptible CoNS strains, we carried out a one-step resistance selection experiment by using vancomycin agar. Vancomycin agar was prepared by incorporating 4 or 6 μg vancomycin ml^{-1} into BHI agar. Agar was poured into 85 mm Petri dishes, with a 4 mm depth of agar. Overnight cultures in BHI broth were adjusted to an OD_{578} of 0.3 (10^8 c.f.u. ml^{-1}). Aliquots (10 μl) of the cell suspensions were placed evenly onto the vancomycin agar. The plates were then incubated at 37°C for 48 h, after which time cell growth was inspected.

PCR amplification of *van* and *mecA* genes. Chromosomal DNA was prepared by the small-scale phenol extraction method and used as PCR template (Matsushashi *et al.*, 1986). PCRs were performed using a Gene Amp 9600 thermal cycler (Perkin-Elmer Cetus Instruments). All strains were subjected to *mecA*, *vanA*, *vanB* and *vanC* gene PCR with primers described previously (Kristóf *et al.*, 2011; Ma *et al.*, 2011). DNA amplification was performed using the following cycling parameters: denaturation at 94°C for 2 min, followed by 30 amplification cycles of 1 min at 94°C , 1 min at 52°C and 1 min at 72°C , with a final extension step at 72°C for 10 min. The resulting fragments were electrophoresed in 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. Quality-control strains included *S. aureus* N315 (*mecA*), *Enterococcus faecalis* NCTC 12201 (*vanA*), *Enterococcus faecalis* ATCC 51299 (*vanB*), *Enterococcus gallinarum* NCTC 12359^T (*vanC1*) and *Enterococcus casseliflavus* NCTC 12361^T (*vanC2*).

Statistical analysis. SPSS for Windows (version 12.0; SPSS) software was used for the analysis. Categorical variables were compared by using χ^2 or Fisher's exact probability tests. *P*-values were calculated and $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Antibiotic-resistance profiles of CoNS strains

Resistance profiles of the CoNS strains against the 13 antibiotics tested are presented in Table 1. In total, 745 CoNS strains were isolated during the study period. Eighty-nine isolates were collected in 2004, 108 in 2005, 129 in 2006, 121 in 2007, 133 in 2008 and 165 in 2009. The mean resistance rates to penicillin G, oxacillin and erythromycin among all tested CoNS strains were high, at 94.2, 79.1 and 89.5%, respectively. In contrast, the mean resistance rates to rifampicin, ceftizoxime and gentamicin were low, at 18.4, 26.7 and 29.5%, respectively. The resistance rates to tetracycline, clindamycin, TMP/SMX, ciprofloxacin and chloramphenicol were medium, at 59.5, 53.7, 58.5, 52.8 and 39.1%, respectively. It was noteworthy that, from 2004 to 2009, all CoNS strains were susceptible to vancomycin. No strains were detected to be non-susceptible to teicoplanin from 2004 to 2007. Six of the 133 isolates (4.5%) collected in 2008 were non-susceptible to teicoplanin (MICs between 16 and 128 mg l^{-1}), whereas in 2009 the number of non-susceptible CoNS strains was 11 out of 165 (6.7%). These results show that resistance to teicoplanin in CoNS appeared in 2008 and increased from 2008 to 2009. It has been reported that the meticillin resistance of *Staphylococcus haemolyticus* is due to the presence of the *mecA* gene (Zong *et al.*, 2011). The *mecA* gene may also spread to other meticillin-susceptible *S.*

Table 1. Resistance rates of CoNS strains to various antibiotics

Resistance level*/ antimicrobial agent	Resistance rate (%)						
	2004 (n=89)	2005 (n=108)	2006 (n=129)	2007 (n=121)	2008 (n=133)	2009 (n=165)	Mean (n=745)
High							
Penicillin G	94.3	94.4	92.2	93.4	94.7	95.8	94.2
Oxacillin	71.9	75	79.8	81.8	81.2	81.8	79.1
Erythromycin	87.6	88.9	86	94.2	87.2	92.1	89.5
Medium							
Tetracycline	71.9	68.5	62	54.5	57.1	50.3	59.5
Clindamycin	52.8	55.6	52.7	47.1	62.4	51.5	53.7
Ciprofloxacin	41.6	47.2	49.6	51.2	66.2	55.2	52.8
TMP/SMX	70.8	75.9	62.8	62	59.4	33.9	58.5
Chloramphenicol	41.6	37.9	41.9	35.5	35.3	41.8	39.1
Low							
Ceftizoxime	16.8	14.8	19.4	23.9	36.8	39.4	26.7
Gentamicin	22.5	24.1	28.7	36.3	44.4	24.9	29.5
Rifampicin	15.7	16.7	17.8	15.7	19.6	22.4	18.4
Teicoplanin	0	0	0	0	4.5	6.7	2.3
Vancomycin	0	0	0	0	0	0	0

*Resistance level was defined as high if >70 %, medium if 30–70 % and low if <30 %.

haemolyticus strains via transposition (Boyce, 2004). *mecA* PCR was performed on all strains to confirm resistance of strains phenotypically resistant to methyl penicillins. The results showed that the *mecA* gene was present in all CoNS strains with oxacillin resistance observed by using phenotypic techniques.

In addition, we analysed the annual change in resistance rate for the CoNS strains to the examined antimicrobial agents over 6 years. As shown in Table 1, we observed that, for penicillin G, oxacillin, erythromycin, clindamycin and chloramphenicol, the annual proportion of resistant CoNS strains did not change significantly from 2004 to 2009. On the other hand, there was a gradual increase in the prevalence of CoNS strains resistant to rifampicin and ciprofloxacin. In contrast, the initial resistance rates of CoNS strains to tetracycline and TMP/SMX in 2004 were high, at 71.9 and 70.8 %, respectively, but decreased to 50.3 and 33.9 %, respectively, in 2009. Although there were no isolates resistant to vancomycin, six isolates in 2008 and 11 isolates in 2009 were resistant to teicoplanin according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2010).

Identification of CoNS species and their multiple-resistance profiles

Using several different techniques, we identified and confirmed that the 745 CoNS strains belong to 11 species (Table 2). To test whether the antibiotic resistance was correlated with the CoNS species, we compared the resistance profile to multiple antimicrobial agents among the CoNS species between 2004 and 2009. From all assays carried out,

both *S. haemolyticus* and *S. epidermidis* had high-resistance profiles, as shown in Fig. 1. The percentage of multiple-resistant strains, however, did not change significantly in *S. epidermidis* between 2004 and 2009; it did change significantly in *S. haemolyticus* ($P=0.04$), and *Staphylococcus capitis* ($P=0.047$). Furthermore, we found no multiple-resistance profiles in strains of *Staphylococcus auricularis*, *Staphylococcus sciuri*, *Staphylococcus simulans* or *Staphylococcus xylosus* in 2004. However, multiple-resistance profiles were identified in 2009 with rates of 4.7, 2.8, 2.3 and 1.3 %, respectively, among the four species. It was noted that the multiple-resistance profile of *Staphylococcus saprophyticus* decreased significantly during the study period ($P=0.034$). Resistance

Table 2. CoNS species identified

We identified and confirmed that the 745 CoNS strains belonged to 11 species, using several different techniques as described in Methods.

Species	No. of strains (%)
<i>S. haemolyticus</i>	254 (34.1)
<i>S. epidermidis</i>	204 (27.4)
<i>S. simulans</i>	66 (8.9)
<i>S. hominis</i> subsp. <i>hominis</i>	53 (7.1)
<i>S. warneri</i>	43 (5.7)
<i>S. capitis</i>	31 (4.1)
<i>S. sciuri</i>	28 (3.8)
<i>S. auricularis</i>	24 (3.2)
<i>S. saprophyticus</i>	16 (2.1)
<i>S. caprae</i>	15 (2.0)
<i>S. xylosus</i>	11 (1.5)

Fig. 1. Comparison of multiple-resistance profiles among different CoNS species between 2004 and 2009. * $P \leq 0.05$.

profiles per major species of the CoNS strains against 13 antimicrobial agents were assayed. Resistance to erythromycin was found in

79 % of *S. simulans*, 88 % of *S. epidermidis* and 89 % of *S. haemolyticus* isolates. Resistance to tetracycline was observed in 64 % of *S. epidermidis* and 57 % of *S. haemolyticus* isolates; 44 % of the latter were resistant to ciprofloxacin, whereas *S. simulans* showed low resistance i.e. <14 % of strains were resistant. *S. hominis* showed high susceptibility to tetracycline, clindamycin, ciprofloxacin, TMP/SMX, chloramphenicol and rifampicin.

Screening and characterization of teicoplanin-non-susceptible CoNS strains

As shown in Table 3, among the six CoNS strains isolated in 2008, the teicoplanin MICs were $64 \mu\text{g ml}^{-1}$ (*S. epidermidis*, $n=1$; *S. haemolyticus*, $n=1$; *S. hominis*, $n=1$) and $16 \mu\text{g ml}^{-1}$ (*S. epidermidis*, $n=2$; *S. haemolyticus*, $n=1$). Among the 11 CoNS strains isolated in 2009, the teicoplanin MICs were $128 \mu\text{g ml}^{-1}$ (*S. epidermidis*, $n=2$; *S. haemolyticus*, $n=2$; *S. caprae*, $n=1$; *S. warneri*, $n=1$; *S. hominis*, $n=1$), $64 \mu\text{g ml}^{-1}$ (*S. epidermidis*, $n=1$; *S. capitis*, $n=1$; *S. hominis*, $n=1$) and $32 \mu\text{g ml}^{-1}$ (*S. haemolyticus*, $n=1$). The result indicated that seven of the 11 CoNS strains (63.6 %) isolated in 2009 showed a higher level of teicoplanin resistance (MIC = $128 \mu\text{g ml}^{-1}$). MICs for vancomycin of all the isolates were $\leq 2 \mu\text{g ml}^{-1}$ ($2 \mu\text{g ml}^{-1}$ for nine strains, $1 \mu\text{g ml}^{-1}$ for five strains and $0.5 \mu\text{g ml}^{-1}$ for three strains).

To screen for potentially vancomycin-resistant strains among the 17 teicoplanin-non-susceptible CoNS strains, we carried out a one-step resistance selection experiment using vancomycin-containing agar. We found that all CoNS strains could grow on selection agar with $4 \mu\text{g vancomycin ml}^{-1}$. However, only strains CoNS25 (*S. haemolyticus*), CoNS06 (*S. epidermidis*), CoNS29 (*S. epidermidis*), CoNS74 (*S. haemolyticus*), CoNS102 (*S. warneri*), CoNS104 (*S. hominis*) and CoNS144 (*S. hominis*) could grow on selection

agar containing $6 \mu\text{g vancomycin ml}^{-1}$. The MIC values of the subpopulation of CoNS strains that could grow on the $6 \mu\text{g vancomycin ml}^{-1}$ selection agar were determined by the agar dilution method. The results indicated that the MIC values for teicoplanin in these strains did not change significantly. However, the MIC values for vancomycin in six of the seven (85.7 %) strains growing on the $6 \mu\text{g vancomycin ml}^{-1}$ agar were $\geq 4 \mu\text{g ml}^{-1}$.

It has been reported that the *vanA*, *vanB* and *vanC* genes are related to acquired glycopeptide resistance in enterococci. *vanA* can induce resistance to high levels of both vancomycin and teicoplanin (Lorenzo-Díaz *et al.*, 2004; Périchon & Courvalin, 2009), while *vanB* induces resistance to various levels of vancomycin only (Lorenzo-Díaz *et al.*, 2004). *vanC* was characterized by its low-level resistance to vancomycin (Tschudin Sutter *et al.*, 2010). Our PCR results showed that none of the 17 teicoplanin-non-susceptible strains carried the *vanA*, *vanB* or *vanC* genes.

The CLSI (2010) recommended that all staphylococcal isolates should have their MICs tested, and CoNS strains should be evaluated further by a reference centre if the vancomycin MIC is $\geq 32 \mu\text{g ml}^{-1}$. The term 'heteroresistance' to vancomycin has been used for staphylococcal isolates that contain a cell population with different levels of vancomycin susceptibility, including vancomycin-resistant cells (Palazzo *et al.*, 2005). Population analysis of the representative strains CoNS25 (*S. haemolyticus*), CoNS06 (*S. epidermidis*), CoNS29 (*S. epidermidis*), CoNS102 (*S. warneri*), CoNS104 (*S. hominis*) and CoNS144 (*S. hominis*) in the presence of vancomycin or teicoplanin was performed.

Fig. 2 illustrates the population curves of the six CoNS strains that could grow on agar containing $6 \mu\text{g vancomycin ml}^{-1}$. Their vancomycin MICs were $\geq 4 \mu\text{g ml}^{-1}$, similar to the control strains VRSA Mu50 and hetero-VRSA Mu3. CoNS25, CoNS06, CoNS29 and CoNS144 showed a similar type of heteroresistance to vancomycin as Mu3. CoNS102, with a vancomycin MIC of $6 \mu\text{g ml}^{-1}$, and CoNS104, with a vancomycin MIC of $8 \mu\text{g ml}^{-1}$, harboured a subpopulation of cells that could grow on agar plates containing $8 \mu\text{g vancomycin ml}^{-1}$ at a frequency of >0.1 and 0.01 %, respectively, suggesting a similar type of intermediate vancomycin resistance to Mu50.

Demographic and clinical characteristics of the patients in which the CoNS strains were non-susceptible to teicoplanin are summarized in Table 3. Among the 17 patients, ten were male and seven were female. Their ages ranged from 12 days to 82 years, and 12 (70.6 %) of the infected patients had been treated previously with vancomycin. After adequate treatment, five patients recovered from the infections, seven became afebrile and five died. None of the patients had been treated previously with teicoplanin. It is notable that eight of the 12 patients received vancomycin and β -lactam antibiotics concomitantly at the time of the underlying disease. It has been speculated that the emergence of staphylococci with heteroresistance to vancomycin could be related to the common practice of

Table 3. Characteristics of 17 teicoplanin-non-susceptible CoNS strains and clinical characteristics of the patients from whom CoNS strains were isolated

Abbreviations: Amik, amikacin; Cefti, ceftizoxime; Ceftri, ceftriaxone; Cilas, cilastatin; Clox, cloxacillin; CVC, central venous catheter; Czid, ceftazidime; Fus, fusidic acid; Gen, gentamicin; Imi, imipenem; Mero, meropenem; Mtz, metronidazole; Pip, piperacillin; Pipe, piperacillin; Rif, rifampicin; SSI, surgical-site infection; Taz, tazobactam; Tei, teicomycin; Van, vancomycin.

Strain information							Patient information						
Strain no.	Year of isolation	Species	MIC		Growth on agar containing vancomycin at:		MIC from subpopulation on vancomycin agar		Sex	Age (years unless specified)	Antimicrobial agent(s) administered	Length of vancomycin treatment (days)	Infection source
			Tei	Van	4 µg ml ⁻¹	6 µg ml ⁻¹	Tei	Van					
CoNS13	2008	<i>S. epidermidis</i>	64	2	+	—	64	2	M	67	Van, Fus	13	CVC
CoNS25	2008	<i>S. haemolyticus</i>	64	1	+	+	128	4	F	70	Ceftri, Van, Gen	5	SSI
CoNS46	2008	<i>S. hominis</i>	64	2	+	—	64	2	M	25	Pipe, Clox, Van	17	Blood
CoNS88	2008	<i>S. epidermidis</i>	16	0.5	+	—	16	1	M	51	Van	21	CVC
CoNS94	2008	<i>S. epidermidis</i>	16	0.5	+	—	16	2	F	78	None	0	CVC
CoNS121	2008	<i>S. haemolyticus</i>	16	1	+	—	32	2	M	62	Rif, Gen, Czid	0	Blood
CoNS06	2009	<i>S. epidermidis</i>	128	2	+	+	128	4	M	32	Van, Rif	16	Blood
CoNS29	2009	<i>S. epidermidis</i>	128	2	+	+	128	4	M	3.5	Ceftri, Van	24	Blood
CoNS30	2009	<i>S. caprae</i>	128	1	+	—	64	1	F	57	None	0	Pharynx colonization
CoNS74	2009	<i>S. haemolyticus</i>	128	2	+	+	128	2	M	66	Imi, Van	9	CVC
CoNS86	2009	<i>S. haemolyticus</i>	128	2	+	—	128	2	F	82	Imi-Cilas, Amik	0	Urine
CoNS102	2009	<i>S. warneri</i>	128	2	+	+	128	6	M	42	Pip/Taz, Cefti, Van	13	Wound
CoNS104	2009	<i>S. hominis</i>	128	2	+	+	64	8	F	10	Ceftri, Van	6	CVC
CoNS135	2009	<i>S. epidermidis</i>	64	0.5	+	—	64	1	M	64	Van	19	Blood
CoNS144	2009	<i>S. hominis</i>	64	1	+	+	128	4	F	53	Ceftri, Amik, Van	12	SSI
CoNS147	2009	<i>S. capitis</i>	64	1	+	—	64	2	M	12 days	Mero, Amik, Van	15	Blood
CoNS159	2009	<i>S. haemolyticus</i>	32	2	+	—	16	2	F	46	Imi, Amik, Mtz	0	Wound

Fig. 2. Six CoNS strains that could grow on agar containing 6 µg vancomycin ml⁻¹ were selected as representative strains. Population analysis of these six representative strains in the presence of teicoplanin (a) or vancomycin (b), in comparison with Mu50 and Mu3, was performed. CoNS25, CoNS06, CoNS29 and CoNS144 showed a similar type of hetero-resistance to vancomycin as Mu3. CoNS102, with a vancomycin MIC of 6 µg ml⁻¹, and CoNS104, with a vancomycin MIC of 8 µg ml⁻¹, harboured a subpopulation of cells that could grow on agar plates containing 8 µg vancomycin ml⁻¹ at a frequency of >0.1 and 0.01 %, respectively.

empirical β -lactam therapy for sick febrile patients (Sieradzki *et al.*, 1998). A lack of response to β -lactam therapy is often followed by addition of a glycopeptide because of the high incidence of superinfection with methicillin-resistant staphylococci.

In conclusion, the annual antimicrobial-resistance rates in CoNS strains are increasing. This poses a serious problem for medical treatment. Particularly, CoNS strains with diminished teicoplanin resistance have appeared in recent years. Antibiotic-susceptibility studies are very important from an epidemiological point of view because they allow policies to be established for adequate and rational use of antibiotics, thus making treatment more efficient and more effective. Our study summarizes the incidence and evolution of teicoplanin resistance among CoNS over a

period of 6 years. Both the teicoplanin MIC values and the number of non-susceptible strains increased from 2008 to 2009 (six CoNS strains with MIC values of 16 and 64 µg ml⁻¹ in 2008; seven CoNS strains with a MIC value of 128 µg ml⁻¹ in 2009). More CoNS species were also observed in the teicoplanin-non-susceptible CoNS strains in 2009 than in 2008. A one-step vancomycin agar selection experiment and subsequent population analysis revealed potentially vancomycin-resistant subpopulations that have been selected from the teicoplanin-non-susceptible strains. Cell culture of CoNS102 and CoNS104, which are capable of growing at 6 µg vancomycin ml⁻¹, showed stable vancomycin MICs of 6 and 8 µg ml⁻¹, respectively. *vanA*, *vanB* and *vanC* genes were not detected in these strains by PCR amplification, suggesting that glycopeptide resistance in these strains occurred endogenously, rather than via

acquisition of transferable plasmids, transposons or closely related elements. Further studies on the ultrastructural morphology, glycopeptide-binding capacity, membrane proteins, cell-wall composition and susceptibility to cell-active antibiotics and enzymes are needed to elucidate the mechanism of glycopeptide resistance in these strains.

As the use of teicoplanin has been limited in the Second Affiliated Hospital of China Medical University, the emergence of teicoplanin resistance among CoNS might be the result of selective pressure from the frequent use of vancomycin. Our results show that 12 patients received vancomycin as an antimicrobial-chemotherapy drug, and eight of the 12 patients received β -lactam antibiotics before or concomitantly with vancomycin, verifying the possibility that prior use of β -lactams might serve to induce the expression of vancomycin resistance. The sequential use of vancomycin could then exert its effects through selection of vancomycin-resistant subclones *in vivo*. This is a cause for clinical concern, as vancomycin is still the reasonable choice for the treatment of severe infections due to multiple-resistant CoNS. There is a need for surveillance of nosocomial isolates of CoNS for resistance to glycopeptides.

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