

## The significance of coagulase-negative staphylococci bacteremia in a low resource setting

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### Abstract

**Introduction:** Coagulase-negative staphylococci (CoNS) are a group of micro-organisms that are increasingly implicated as a cause of significant infection and the leading cause of bloodstream infection (BSI). One important predictor of true BSI is the isolation of CoNS from multiple blood cultures, presuming that the isolates represent the same species. Thus the objective of this study was to determine the significance of repeated CoNS isolated from blood cultures.

**Methodology:** This was a prospective laboratory study which was initiated in June 2007 and lasted until July 2008. CoNS isolates were obtained from patients who had two positive blood cultures within a 14-day interval. CoNS were identified to the species level using an API-Staph, and antibiotics susceptibility testing was performed according to Clinical and Laboratory Standards Institute specifications. Strain relatedness was confirmed using pulsed-field gel electrophoresis.

**Results:** During the study period, 202 CoNS-positive samples were isolated from 101 patients. The most common species isolated was *Staphylococcus epidermidis* (59.0%), and 83.2% of the patients isolated the same species of CoNS from repeated blood cultures. Among the isolates of the same species, only 40.7% had the same antibiogram. CoNS with the same species and antibiogram had 93.3% probability of belonging to the same strain. Most (65.5%) of the patients were treated with antibiotics, primarily from the glycopeptides group.

**Conclusion:** Speciation and antibiogram of CoNS from repeated blood cultures are adequate in determining the significance of repeated CoNS isolated from blood cultures.

**Key words:** coagulase-negative staphylococci; blood stream infection; antibiotics susceptibility testing

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### Introduction

Coagulase-negative staphylococci (CoNS) are divided into more than 44 species and more than a dozen subspecies, of which approximately half have been associated with humans [1]. CoNS comprise an ever-expanding group of bacteria whose medical importance has emerged in the past decades. They have become one of the most frequent nosocomial pathogens isolated from blood cultures, often in association with intravascular devices, and as a cause of deep-seated prosthetic implant infections [2,3]. CoNS species have emerged as the most recurrent cause of nosocomial bloodstream infection, accounting for 27% to 32% and 50% of such infections among adult and paediatric patients, respectively [4].

In our institution, hospital Universiti Sains Malaysia, decisions for the commencement of antibiotic treatment for patients suffering from CoNS bacteremia are based on the following criteria: clinical

sepsis, no other obvious documented source of infection, and isolation of CoNS from blood on more than one occasion. Clinical criteria in predicting whether CoNS isolated from blood cultures are associated with bloodstream infection are neither sensitive nor specific [5]. Repeated CoNS should be of the same strain to be clinically significant, and should be confirmed by genotyping, which is not widely available [6]. These uncertainties regarding the significance of CoNS isolated from blood cultures may result in over-diagnosis and, indirectly, overuse of anti-staphylococci drugs, especially vancomycin, which may contribute to the development of resistance that will amplify the likelihood of morbidity, mortality and total hospital costs [7]. Thus the objective of this study was to determine the significance of repeated CoNS isolated from blood cultures.

## Methodology

### Setting

This prospective laboratory study began in June 2007 and lasted until July 2008. The Microbiology and Parasitology laboratory received clinical specimens from Hospital Universiti Sains Malaysia, a tertiary-teaching hospital in the east coast region of Malaysia. It is an 800-bed hospital, with two adult intensive care units (ICUs), both medical and surgical, two neonatal ICUs, 28 medical wards, and 11 surgical wards, including two oncology wards.

### Clinical isolates

The study isolates were collected from in-patients at Universiti Sains Malaysia, who had two or more blood cultures testing positive for CoNS within a 10-day interval. The time interval was based on the published data on various definitions for blood culture contaminant [7]. For blood cultures sent for analysis more than two times, only the first two consecutive isolates were included in the study. Repeated blood cultures were frequently sent based on clinical judgment by the managing team or physician in charge. Record reviews were performed for isolates with the same species of CoNS to correlate with antibiotic management.

### Bacterial identification

Blood cultures were incubated in an automated blood culture system (BACTEC or BacT-ALERT, Becton, Dickinson and Company, USA) for a total of 5 days or until the system indicated the culture was positive. Both were then sub-cultured on blood agar plates. CoNS were identified on the basis of colony morphology, Gram stain characteristics, a positive catalase test, and a negative tube-coagulase test. In this study, the identification of CoNS was confirmed to the species level using an Analytical Profile Index Staph (API Staph) (BioMerieux, Marcy l'Etoile, France). Subsequently, the blood isolates were stored at -80°C in glycerol-containing tryptone soy broth (Oxoid, Basingstoke Hampshire, United Kingdom) for further analysis.

### Antibiotic susceptibility testing (AST)

Susceptibility testing was performed by a disc diffusion method according to the Clinical Laboratory and Standard Institute (CLSI) recommendations and was interpreted accordingly [20]. The following antibiotics were tested: erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg),

rifampicin (5 µg), ciprofloxacin (5 µg), teicoplanin (30 µg), linezolid (30 µg), oxacillin (1 µg) and vancomycin (30 µg).

### Strain typing by pulsed-field gel electrophoresis (PFGE)

Repeated CoNS isolates with species on an analogous level were subjected to molecular typing by PFGE. PFGE was performed according to published protocols with some modifications [8]. Briefly, 200 µl of the bacterial suspension were added to an equal volume of low-melting point agarose 2%; 10 µl of lysostaphin (1 mg/ml) were added immediately and the suspension was mixed well before being allowed to solidify in a plug mould. The gel plugs were then incubated at 54°C for 2 hours in 2 ml of ES buffer (N-laurylsarcosine 1% in 0.5 ml of EDTA, pH 8.0) containing proteinase K (10 mg/ml) with gentle shaking. The plugs were washed and digested with 30 units of *Sma*I. The DNA samples were then electrophoresed in agarose 1% with a contour-clamped homogeneous electric field (CHEF-DRIII, Bio-Rad, Hercules, CA, USA). Interpretation and analysis of the PFGE patterns were according to previously published guidelines [9]. Bacterial isolates yielding the same PFGE pattern are interpreted as sharing a common PFGE type.

### Ethical approval and patient consent

The study was approved by the ethical committee of the School of Medical Sciences, Universiti Sains Malaysia (USM/PPSP®/Ethics Com./2006(182.3(7)).

## Results

A total of 663 CoNS were isolated from blood cultures during the study period, accounting for 30.8% of total blood isolates. Two hundred and two CoNS isolated from 101 patients were included in the study. Out of these, 83.2% (84 pairs) were of the same species, whereas another 16.8% isolated were disparate, unrelated species. The most common species isolated were *Staphylococcus epidermidis* (52.5%), followed by *S. capitis* (10.4%), *S. chromogens* (7.9%), *S. hominis* (7.4%), *S. haemolyticus* (5.9%) and *S. warneri* (5.0%). Details of *Staphylococcus* species isolated were as shown in Table 1.

Out of 202 isolates, only 194 were tested for antibiotics susceptibility. Based on oxacillin testing, the majority of the isolates were methicillin-resistant CoNS (68.6%). The subsequent analysis focused on the data of 168 isolates of CoNS with the same species

**Table 1.** Prevalence of *Staphylococcus* species isolated from repeated blood cultures

Species	Frequency	Percentage (%)
<i>S. epidermidis</i>	106	52.5
<i>S. capitis</i>	21	10.4
<i>S. chromogens</i>	16	7.9
<i>S. hominis</i>	15	7.4
<i>S. haemolyticus</i>	12	5.9
<i>S. warneri</i>	10	5
<i>S. auricularis</i>	5	2.5
<i>S. lugdunensis</i>	4	2
<i>S. caprae</i>	4	2
<i>S. saprophyticus</i>	3	1.5
<i>S. schleiferi</i>	3	1.5
<i>S. xylosus</i>	3	1.5
<b>Total</b>	<b>202</b>	<b>100</b>

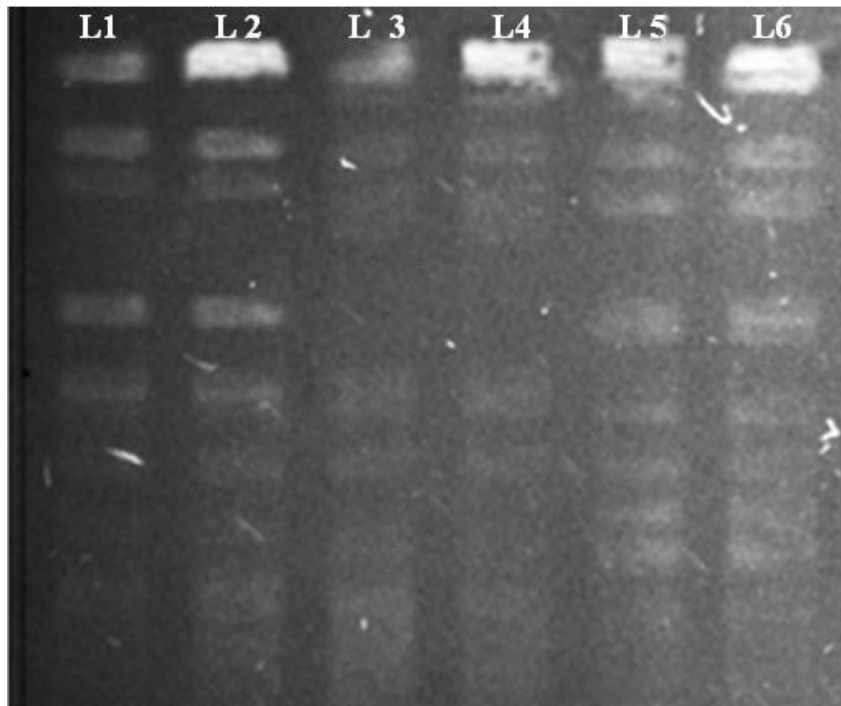
from 84 patients. Among these, 40.7% of the isolates had similar antibiotic susceptibility patterns. 65.5% of patients were treated with antibiotics. Anti-staphylococci drugs used were cloxacillin, vancomycin, teicoplanin or a combination of antibiotics. Among these antibiotics, glycopeptides (vancomycin and teicoplanin) were the most commonly used accounting for 60% of cases. Sixty-six isolates of repeated CoNS with the same species were subjected to PFGE analysis. Among these isolates, 87.9% (58 isolates) demonstrated indistinguishable patterns which denote similar genotype and strain (Figure 1). On further analysis, CoNS with the same species and antibiogram had 93.3% probability of the same strain, proven by PFGE.

## Discussion

Blood for culture is a routine procedure for investigating the cause of fever or suspected infection in the majority of hospitalized patients and certain patients attending an emergency department. Isolation of a true pathogen from blood culture ultimately warrants treatment with an appropriate antibiotic. Problems occur when the isolated organism is of doubtful significance, such as CoNS, which require further clinical assessment and extra laboratory tests to help the physician in appropriate patient management. For a clinical microbiologist, interpretation of the clinical significance of isolated CoNS from blood culture continues to be complex. The isolation of CoNS from blood on more than one occasion and clinical parameters are used habitually in determining the clinical significance of the isolate. However, repeated isolates of CoNS could be of species varying in diversity and strain since speciation is not a routine laboratory procedure.

In this study, *S. epidermidis* appeared to be the predominant species isolated which accounted for 53% of the total isolates. This finding was in keeping with previously published data. Bates *et al.* reported *S. epidermidis* as the most frequent species associated with bacteraemia [10]. A study was conducted to determine the clinical significance of CoNS isolated from neonates and found *S. epidermidis* was the major species isolated from 46 infants (85.2%). *S. haemolyticus* was responsible for infection in two infants (3.7%), *S. lugdunensis* in three infants (5.6%), and *S. simulans* (1.8%), *S. warneri* (1.8%) and *S. xylosus* (1.8%) in one infant each [11]. In 2007, Senger *et al.* also demonstrated a high percentage of *S. epidermidis* isolated in their study, which surprisingly contributed to 68% of the total CoNS isolates [12]. The occurrence of more than one positive blood culture has been used as a first-rate predictor of true bacteraemia and isolating the same species further increases the probability of true bacteraemia. However, two blood cultures proving positive for CoNS for up to 20% of the patients were due to contamination; therefore, the number of positive blood cultures is insufficient as a sole parameter when considering and predicting CoNS bacteraemia [13]. In this study, we found that about 83% of repeated isolates of CoNS belonged to the same species, and the remaining residual of 17% most probably were caused by contaminants [5]. Herwaldt *et al.* in 1996 found a strong association between identical species and a noteworthy significance in CoNS bacteraemia in repeated blood cultures with a sensitivity of 85% and a specificity of 45%. Hence diagnoses of true bacteraemia due to CoNS in patients with two positive blood cultures require species identification and critical evaluation of the antimicrobial susceptibility testing results [14].

**Figure 1.** Representative PFGE patterns of coagulase-negative *Staphylococcus* (CoNS) strains from three patients



Lane 1-2: CoNS from patient A; Lane 3-4: CoNS from patient B; Lane 5-6: CoNS from patient C

In this study, the percentage of CoNS with the same species and the same antibiogram was a great deal lower (40.7%) compared to those with different antibiogram patterns (59.3%). There were two pairs of CoNS (12.5%) with different species that showed similar antibiograms. These findings indicate that the antibiogram alone is not a reliable enough tool to distinguish CoNS isolates. PFGE is a well-established method used to determine strain relatedness [15]. Here we demonstrated that isolates with the same species and antibiogram actually obtained a 93.3% probability of belonging to the same strain, proven by the PFGE. Thus, in a laboratory with limited resources, the speciation and antibiotics susceptibility pattern were adequate enough to alert the managing team to the probability of significant isolates for optimal antibiotic therapy. However, the correlation of CoNS with similar species, antibiogram and genotype with clinical sepsis was beyond the scope of the study. Nonetheless, in the year 2000 Kim *et al.* demonstrated that patients with symptoms of bloodstream infections (BSI) who isolated similar CoNS species and antibiogram, had true BSI in 74% of cases [16].

Misinterpretation of contaminated blood cultures as true bacteraemia has two major consequences; it initiates an increase in unnecessary health-care

expenditures and contributes to the emergence of vancomycin resistance staphylococci [10,17,18]. We found that 65% of our patients with repeated isolates were treated with antibiotics, and glycopeptides (vancomycin and teicoplanin) were the most commonly used and accounted for 60% of cases. This result was in keeping with a study by Senger *et al.* in 2007, which reported widespread misuse of glycopeptides in the treatment of CoNS bacteraemia [11]. Vancomycin therapy was as likely to be administered for episodes classified as contaminants as for those classified as bacteraemia [4]. According to the CDC recommendations for preventing the spread of vancomycin resistance, vancomycin is recommended when CoNS is isolated from multiple blood cultures; vancomycin is not the most suitable option when only one in a series of blood cultures is CoNS positive [19].

### Conclusion

This study shows that only 40% of repeated isolation of CoNS represents significant isolates; therefore, speciation and antibiotics susceptibility testing for repeated isolates from blood cultures are strongly recommended. This practice will lessen superfluous antibiotic treatment and thwart the

emergence of resistant strains that indirectly reduce total hospital costs. Ideally, the molecular approach is for the most part a consistent method in determining the significant isolates of CoNS. However, in countries with inadequate resources, speciation and antibiogram are recommended when determining significant isolates.

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