Use of quantitative antibiogram analysis to determine the clonality of coagulase-negative *Staphylococcus* species from blood culture

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ABSTRACT

Two phenotypic methods, quantitative antibiogram analysis and colony morphology, were compared to pulsed-field gel electrophoresis (PFGE) for distinguishing the clonality of coagulase-negative *Staphylococcus* (CNS) species. The results of these three methods were correlated with the patients' clinical findings for 23 episodes in which CNS species were isolated from two blood culture bottles within a 24-h period. Quantitative antibiogram and colony morphology at 24 h correlated with PFGE typing in 21 (91%) and 20 (87%) episodes, respectively. All episodes associated with CNS strains with identical PFGE patterns had quantitative antibiogram similarity coefficients <10, whereas most episodes associated with strains with different PFGE patterns had quantitative antibiogram similarity coefficients ≥ 17. The CNS isolate pairs were less likely to be associated with infection if the strains had different PFGE types or a quantitative antibiogram similarity coefficient ≥ 17. Clinical microbiology laboratories should consider use of the quantitative antibiogram similarity coefficient to aid clinicians in distinguishing infection-associated CNS blood isolates from contaminants.

Keywords Bacteraemia, coagulase-negative *Staphylococcus* species, pulsed-field gel electrophoresis, quantitative antibiogram, *Staphylococcus*

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INTRODUCTION

Coagulase-negative *Staphylococcus* (CNS) species have emerged as the most frequent cause of nosocomial bloodstream infection, accounting for 27–32% and 50% of such infections among adult and paediatric patients, respectively [1–4]. However, CNS species are normal skin flora, and, as a result, it can be difficult to determine if CNS species isolated from blood cultures reflect infection or microbial contamination, especially among neonates and neutropenic patients, and when cultures are drawn from a central venous catheter. The high frequency of contamination of blood cultures with CNS results in overuse of antimicrobial agents, especially vancomycin, which may promote antimicrobial resistance [5–7].

Clinical criteria to predict whether CNS strains isolated from blood cultures are associated with bloodstream infections are neither sensitive nor specific [8]. In addition, Mirrett *et al.* [9] found that the number of positive culture bottles in a given culture set cannot reliably predict whether or not an isolate represents true infection or contamination. Both phenotypic and genotypic characteristics of CNS have been examined to differentiate infection-associated isolates from contaminants. Strain typing of CNS by pulsed-field gel electrophoresis (PFGE) appears to have a higher positive predictive value than phenotypic characterisation by speciation, biotype, or resistance profile [10,11]. However, because of the time, expense and labour involved, PFGE is impractical to use in clinical decision-making. Quantitative

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antibiogram determination correlates well with CNS genetic relatedness assessed by PFGE, and could be a rapid and cost-effective technique to aid in characterising CNS blood isolates. In the present study, the microbiological characteristics of CNS strains from 23 episodes in which CNS strains were isolated from two different blood cultures were examined, and the ability of colony morphology, quantitative antibiogram and PFGE to identify CNS-associated bloodstream infection was determined.

MATERIALS AND METHODS

Hospital and background

UCLA Medical Center is a 500-bed tertiary healthcare centre with 12 intensive care units and large solid organ and bone marrow transplantation programmes. During 1995–98, CNS strains were isolated from 35–38% of positive blood cultures.

From 1 February to 30 April 1998, all episodes in which CNS strains were isolated from two blood cultures drawn within a 24-h period were selected for analysis. Episodes when CNS was isolated from three or more blood cultures were excluded, to focus on a clinical situation that presents greater diagnostic uncertainty. Patients were identified by twice-daily surveillance of blood culture logs.

Blood culture and routine microbiological methods

Aerobic and anaerobic blood culture bottles were incubated and monitored continuously in a BacT/Alert Blood Culture System (Organon Teknika, Durham, NC, USA). Samples from positive blood culture bottles were Gram-stained, and an aliquot was plated on 5% sheep blood trypticase soy agar plates (Becton Dickinson, Franklin Lakes, NJ, USA) for isolation. Identification of CNS was confirmed by negative reactions in slide coagulase tests and following overnight incubation at room temperature of tube coagulase tests (rabbit coagulase plasma with EDTA) (Becton Dickinson).

Colony morphology analysis

Primary blood agar plates were examined under a dissecting microscope after 24 h, 48 h, and 5 days. Colony appearance (colour, size, shape, and border), haemolysis and texture of CNS isolates were noted.

Quantitative antibiograms

Disk diffusion susceptibility tests were performed on each CNS isolate according to National Committee for Clinical Laboratory Standards guidelines by manual measurement of zone sizes [12]. Antimicrobial agents tested included: cefazolin, levofloxacin, clindamycin, oxacillin, trimethoprim–sulphamethoxazole, vancomycin, doxycycline, and gentamicin. Although routinely performed on clinical CNS isolates, penicillin susceptibility testing was not performed in this study. Quantitative antibiogram analysis was performed for each episode by measuring the zones of inhibition for eight antibiotics and calculating a similarity coefficient comparing two individual isolates [13]. The greater the similarity coefficient for two isolates, the smaller the resemblance between them.

PFGE

Cell lysis was performed as described by Leonard and Carroll [14], with slight modifications. A 300-μL aliquot of overnight growth in brain heart–infusion broth was washed twice in TEN (0.1 M Tris, 0.1 M EDTA, 0.15 M NaCl, pH 7.5) and TN (10 mM Tris, 10 mM NaCl, pH 8.0). Cells were resuspended in 100 μL of TN. To the cell suspension, 10 μL (200 U) of achromopeptidase (Wako Chemicals, Richmond, VA, USA) in TE (10 mM Tris, 1 mM EDTA, pH 7.5) and 100 μL of pre-melted SeaPlaque GTG agarose (FMC Bioproducts, Rockland, ME, USA) 2% w/v in TN was added. The cell suspension–agarose mixture was used to prepare two plugs. The plugs were expelled into 300 μL of TN and the cells lysed by incubating at 50°C for 30 min. Plugs were washed three times with 1 mL of TE at room temperature with agitation. Restriction digestion was performed with one-third of a plug and 2 μL (20 U) of *Sma*I (BRL, Gaithersburg, MD, USA) at room temperature for 2–3 h. Plugs were loaded into a SeaKem GTG agarose 1% w/v gel made with ×0.5 TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). To reduce the formation of air bubbles during the loading of the gel, the wells were completely filled with ×0.5 TBE buffer before the gel slice was placed in the well. After loading, the wells were overlaid with SeaKem GTG agarose 1% w/v in TE. The gel was run for 21 h on a CHEF Mapper (Bio-Rad, Hercules, CA, USA) at 6 V, 120° angle, with an initial switch time of 5 s and a final switch time of 35 s. The gel was stained with ethidium bromide for 30 min and destained in water for 1 h. The gel image was captured using a Gel Doc 1000 (Bio-Rad). For PFGE pattern interpretation, isolates were considered to be the same if no band differences were seen and different if there was one or more band variations.

Definition of infection

The criteria for CNS bloodstream infection were adapted from those of Herwaldt *et al.* [8]. CNS isolates were classified as being associated with bloodstream infection when each of the following four criteria were present: (1) patient temperature of >38 °C on the day the first positive blood culture was obtained; (2) vancomycin therapy and/or line removal; (3) diagnosis by a physician or the National Nosocomial Infections Surveillance definition of nosocomial bloodstream infection [15]; and (4) presence of neutropenia or one or more of the following signs or symptoms: chills; systolic blood pressure <80 mmHg; white blood cell count >12 000/mm^3; prothrombin time >13 s; platelet count <100 000/mm^3. All other strains of CNS isolated from two blood cultures were classified as contaminants.

The bloodstream infection was considered to be nosocomial if the signs, symptoms and positive blood culture were first detected >48 h following admission, or community-acquired if the findings were noted within the first 48 h of hospitalisation.

Data collection

An investigator (DAP) who was blinded to the phenotyping and genotyping results reviewed the medical records and recorded the following data: patient demographic characteristics, underlying diseases, admission and discharge date, hospital unit, dates and numbers of positive and negative blood cultures, clinical features, presence of intravascular devices, and treatment. Data were entered and analysed by EpiInfo software v. 6.04b (CDC, Atlanta, GA, USA).

Statistical methods

For determination of the quantitative antibiogram, the similarity coefficient (Euclidean distance) was calculated with the formula $E_{jk} = \sqrt{\Sigma(x_{ij} - x_{ik})^2}$, where x_{ij} and x_{ik} are the inhibition diameter values of a given antibiotic i for two distinct isolates j and k [13]. The similarity coefficient was used to determine the resemblance between two isolates. The smaller the similarity coefficient, the greater the resemblance between the isolates.

Proportions were compared using the chi-square or Fisher's exact test, as appropriate. Continuous variables were compared using the Wilcoxon two-sample test. The sensitivity, specificity and positive and negative predictive values of isolates with identical colony morphology, a quantitative antibiogram similarity coefficient <10 and identical PFGE profiles were calculated to determine the correlation of each test with infection. All p values were two-tailed; a p value ≤ 0.05 was considered to be statistically significant.

RESULTS

Patient characteristics

Twenty patients had 23 episodes where CNS was isolated from two blood cultures. For 22 of the episodes, CNS strains were isolated from both bottles of a single blood culture set (vented and non-vented) drawn at the same time. For the remaining episode, CNS strains were isolated from one bottle each from two blood culture sets drawn <24 h apart. The positive blood cultures were drawn from a central venous catheter in 11 (48%) episodes, from a peripheral vein in nine (39%) episodes, from an arterial line in two (9%) episodes, and from an unknown site in one (4%) episode. Blood cultures became positive on day 1 in eight (35%) episodes, on day 2 in seven (30%) episodes, and on day 3 in eight (35%) episodes. In ten (43%) episodes, the positive blood culture was drawn within 48 h of admission.

Patients ranged in age from 3 months to 84 years (median, 42 years); ten (50%) were female. Primary diagnoses included solid organ transplant ($n = 6$; 30%), bone marrow transplant ($n = 3$; 15%), acute myelogenous leukaemia ($n = 2$; 10%), altered mental status ($n = 3$; 15%), congenital heart disease ($n = 2$; 10%), and other diagnoses ($n = 4$; 20%).

Comparison of colony morphology with PFGE pattern

Based on colony morphology from the primary isolation plates, two CNS isolates were selected for further analysis in 18 episodes, and three or more isolates were selected in five episodes. The correlation between CNS colony morphology and PFGE decreased with increasing duration of incubation of the primary isolation plate, from 87% (20 of 23 episodes) at 24 h, to 70% (16 of 23 episodes) at 48 h and after incubation for 5 days. Assessed after 24 h, in 16 (70%) episodes, the pairs of CNS strains had the same colony morphology and identical PFGE patterns, in four (17%) episodes they had both different colony morphology and different PFGE patterns, in two (8%) episodes they had the same colony morphology but different PFGE patterns, and in one (4%) episode they had different colony morphology and the same PFGE pattern (Table 1). The sensitivity (94%) and positive predictive value (80%) were high, but the specificity was moderate (67%) for the colony morphology when differentiating CNS strains with identical or different PFGE patterns.

Comparison of quantitative antibiograms with PFGE pattern for isolates within single episodes

Quantitative antibiogram similarity coefficients calculated for isolates within single episodes were distributed into two groups with values of <10 or ≥ 17 (Fig. 1). All 17 episodes associated with pairs of CNS strains with identical PFGE patterns had quantitative antibiogram similarity coefficients <10 (mean = 3.41; range = 1.00–9.38), while four (67%) of six episodes associated with strains with different PFGE patterns had quantitative antibiogram similarity coefficients ≥ 17 (mean = 19.74; range = 1.73–37.7) (Table 1). With use of these cut-off values, the correlation between quantitative antibiogram and PFGE pattern was 91% (21 of 23 episodes). The sensitivity (100%) and positive predictive value (100%) of the quantitative antibiogram were high, but the specificity (67%) was moderate in discriminating the number of PFGE strain types.

Table 1. Laboratory data and clinical correlation for 23 episodes in which CNS was isolated from blood culture

Episode	Isolates	Colony morphology		Quantitative antibiogram similarity coefficients[a]	PFGE	Bacteraemia vs. contamination[b]
		24 h	48 h			
1	12, 13	Same	Same	1.73	Different	Contaminant
2	14, 15	Same	Same	1.73	Same	Contaminant
3	24, 25, 26	Different	Different	16.94, 30, 37.7	Different	Bacteraemia
4	27, 28	Same	Different	2.24	Same	Contaminant
5	29, 31, 32, 33	Different	Different	1.73, 7.07, 7.55, 6.71, 7.75, 2.65	Different	Bacteraemia
6	40, 41	Same	Same	1.41	Same	Bacteraemia
7	49, 50	Same	Same	5.29	Same	Bacteraemia
8	51, 52	Same	Different	8.54	Same	Bacteraemia
9	53, 54	Same	Same	3.87	Same	Contaminant
10	55, 56	Same	Same	1.73	Same	Contaminant
11	57, 58	Same	Different	2.45	Same	Contaminant
12	66, 67	Same	Different	1.73	Same	Contaminant
13	68, 69a, 69b	Same	Same	3, 4, 1	Same	Contaminant
14	72, 73	Same	Same	32.59	Different	Contaminant
15	74, 75, 76, 77	Different	Different	25.61, 3, 25.94, 26.55, 1.73, 26.94	Different	Contaminant
16	80, 81	Same	Same	3.74	Same	Bacteraemia
17	88, 89	Different	Different	23.28	Different	Contaminant
18	94, 96, 97	Different	Different	6.24, 9.38, 3.87	Same	Contaminant
19	98, 99	Same	Same	3	Same	Bacteraemia
20	109, 110	Same	Same	4.12	Same	Contaminant
21	111, 112	Same	Same	2.45	Same	Contaminant
22	113, 114	Same	Same	2	Same	Contaminant
23	115, 116	Same	Same	4.36	Same	Contaminant

[a]Similarity coefficients were calculated by comparing each isolate from an episode with all other isolates from the same episode.
[b]Bacteraemia vs. contamination was determined based on clinical findings (see Methods for definitions).

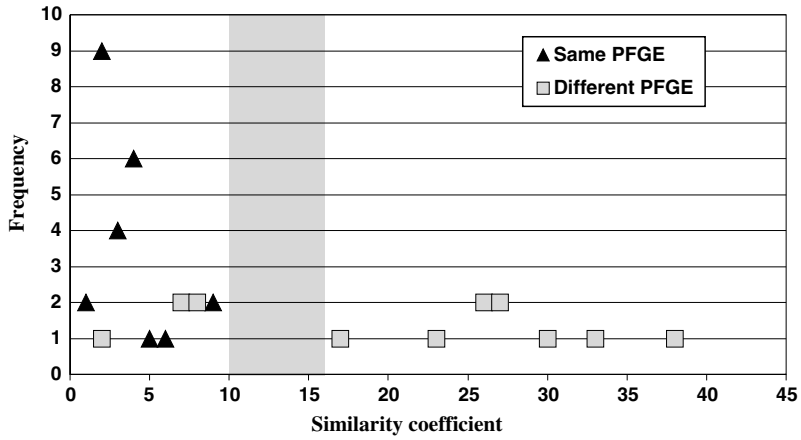

Fig. 1. Comparison of quantitative antibiogram analysis with PFGE patterns for CNS blood culture isolates from each episode. Similarity coefficients from Table 1 were rounded to the nearest whole number and plotted. The frequency is the number of isolate pairs that have a particular similarity coefficient. A window between the smaller and larger similarity coefficients is indicated by the grey shaded area. Based on this window, a cut-off value of ≥17 was assigned to indicate that these isolates are more likely to represent blood culture contaminants.

Comparison of quantitative antibiograms with PFGE pattern for all episodes

Quantitative antibiogram analysis was performed to compare all CNS isolates ($n = 53$) within the study (Fig. 2). There were 1349 comparisons where the PFGE patterns were different for the two individual isolates being analysed. The quantitative antibiogram similarity coefficients for the 1349 evaluations ranged from 1.41 to 61.81, with a mean similarity coefficient of 28.03.

Clinical correlation

Of the 23 episodes involving CNS, 15 (65%) were associated with fever, with a median maximal temperature of 38.8 °C on the date of culture. Seven (30%) episodes were associated with leukocytosis (white blood cell count >12 000), three (13%) with neutropenia (neutrophil count < 500), and four (17%) with hypotension and other signs of sepsis. Seven (30%) episodes were classified as infection-associated and 16 (70%) as contaminants, based on the clinical criteria. Of the seven infection-associated episodes, four (57%) were classified as community-acquired and three (43%) as nosocomial. Vancomycin was administered for 12 (52%) episodes (median = 11 days). The duration of vancomycin therapy was similar whether the episode was classified as infection-associated or as a contaminant (median days, 12 vs. 8; p0.40).

The probability that the episode of CNS was infection-associated was significantly higher among patients with a haematological malignancy than among those with another primary diagnosis (4/5 vs. 3/18; p0.17). The probability that the episode was infection-associated did not vary significantly by the number of days taken for the blood cultures to become positive, the vascular site or device the culture was drawn from, the number and type of intravenous access devices present, hospital location, or whether the episode was classified as nosocomial or community-acquired.

The genotype, colony morphology and quantitative antibiogram discriminated poorly between infection-associated CNS isolates and contaminants (Table 2). Similar results were observed if

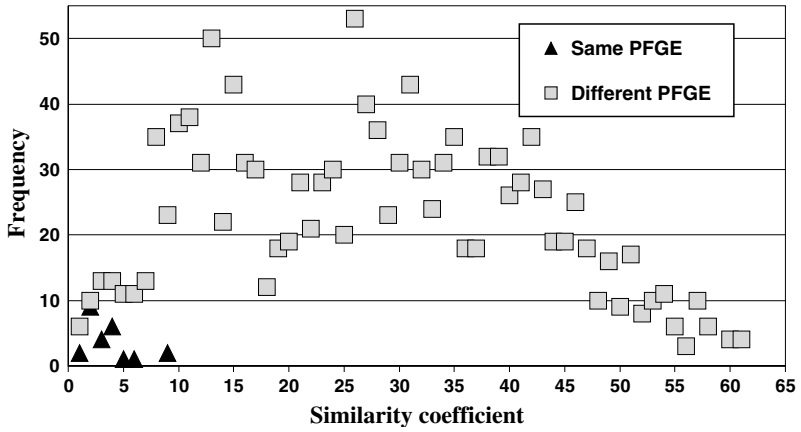

Fig. 2. Comparison of quantitative antibiogram analysis with PFGE patterns for all CNS blood culture isolates. Similarity coefficients for each CNS isolate compared with all other isolates in the study were plotted. Similarity coefficients were rounded to the nearest whole number. The frequency is the number of isolate pairs that have a particular similarity coefficient.

Table 2. Results of laboratory tests of infection-associated CNS isolates and contaminants

Characteristics	Infection-associated isolates ($n = 7$)	Contaminants ($n = 16$)	p value	Sensitivity (%)	Specificity (%)	Predictive value (%)	
						Positive	Negative
Identical PFGE	5	12	1	71	25	29	67
Identical colony morphology	5	13	0.62	71	23	28	60
QA distance <10	6	13	1	86	19	32	75

QA, Quantitative antibiogram.

the analysis was performed using a physician diagnosis or the National Nosocomial Infections Surveillance definition of nosocomial bloodstream infection (data not shown). Although each of the three tests was reasonably sensitive (> 70%) in identifying infection-associated isolates, the specificity and positive predictive value of each test were low (< 30%). In contrast, the negative predictive value of PFGE was 67%, and that for the quantitative antibiogram was 75%, suggesting that CNS isolate pairs were more likely to be contaminants if the strains had different PFGE types or a quantitative antibiogram similarity coefficient ≥ 17.

DISCUSSION

With increased central venous access and the increasing proportion of blood culture isolates that are CNS, it is increasingly difficult for physicians to determine whether the isolation of CNS from blood cultures represents bloodstream infection or contamination. Although multiple strains of CNS may colonise a patient's skin or intravascular device, most CNS bacteraemias are caused by a single strain [16]. Additionally, a recent study indicates that the number of culture bottles positive for CNS in a given culture set should not be used as a criterion to predict the clinical significance of CNS [9]. Thus, rapidly determining whether single or multiple strains of CNS are present in two or more blood cultures could assist clinicians in determining whether antimicrobial therapy for CNS should be initiated, continued, or stopped. Similar to the findings of Herwaldt *et al.* [8], it was found that only 30% of episodes where two blood cultures grew CNS met the criteria for bloodstream infection, and that vancomycin therapy was as likely to be administered for episodes classified as contaminants as for those classified as bacteraemia. This finding clearly supports the need for additional diagnostic tools to limit the inappropriate use of vancomycin.

Although PFGE typing is considered to be the standard for determining strain relatedness of CNS, it is expensive, and requires proficient personnel and at least 3 days' turnaround time, thus limiting its usefulness in clinical decision-making. In the present study, CNS colony morphology assessed after incubation for 24 h of the primary isolation plate, and the quantitative antibiogram, correlated closely with the PFGE pattern of CNS strains obtained from two blood culture bottles. This finding suggested that these rapid and cost-effective phenotypic methods could potentially substitute for PFGE in determining the relatedness of CNS blood isolates. Although a limited number of colonies was examined for each episode, most episodes of CNS infection-associated bacteraemia involved a single clone of CNS with identical colony morphology, similar quantitative antibiogram, and identical PFGE profile. In contrast, episodes associated with contamination involved single or multiple CNS clones. As a result, the positive predictive values of each of the three typing methods were poor.

It is of note that PFGE and the quantitative antibiogram were associated with moderately high negative predictive values (67% and 75%, respectively), suggesting that CNS isolates were more likely to be contaminants if the strains had different PFGE types or a quantitative antibiogram similarity coefficient ≥ 17. Determining the quantitative antibiogram similarity coefficient of CNS isolates from multiple blood cultures may increase the likelihood of detecting multiple strains. One potential approach suggested by this analysis is to notify the physician of the possibility that the isolates represent contaminants when the quantitative antibiogram similarity coefficient is greater than the cut-off value assigned by the laboratory. Whether such an approach could reduce the use of vancomycin and other antimicrobials would require evaluation in a larger, prospective study.

Although our study examined only 23 episodes, the six episodes that involved strains with different PFGE patterns had a mean similarity coefficient of 19.74. In addition, the comparison of each of the 53 isolates to the other isolates in the study with different PFGE patterns resulted in a mean similarity coefficient of 28.03 for the 1349 comparisons. Taken together, these findings indicated that, in most quantitative antibiogram comparisons, isolates with different PFGE patterns will have a high similarity coefficient, indicating that the two strains are unrelated. This supports the hypothesis that CNS isolates with similarity coefficients above a certain cut-off value are more likely to be blood culture contaminants.

Strict criteria for PFGE interpretation were imposed. In this interpretation, one or more band

differences in PFGE pattern between two isolates was regarded as indicating different strain types. Although this classification is different from the criteria established by Tenover *et al.* [17] for epidemiological investigations, the present analysis is not a typical epidemiological investigation. The guidelines were established for epidemiological investigations of outbreaks that occur days or even weeks apart. Under these circumstances, genetic mutations may occur which alter the banding pattern of the genomic DNA by PFGE. With use of the criteria of Tenover *et al.*, isolates can be epidemiologically linked on the basis of a few band differences. However, the present analysis focused on CNS isolates obtained from a single patient within a 24-h period. Under these conditions, genetic mutations may be occurring, but a mutation that would alter the PFGE banding pattern would be a rare event. The temporal occurrence of the genetic event would also be crucial for the genetic difference to be seen; that is, the mutation would need to occur early during incubation for most of the bacterial population to manifest the new genotype. In addition, Sharma *et al.* [16] indicated that almost all CNS bacteraemias were monoclonal in their report of the prevalence of genotypic variation and polyclonal bacteraemia of CNS isolates from blood culture.

Other investigators have also examined CNS strain relatedness to predict bacteraemia and contamination. Zaidi *et al.* [10] used PFGE, speciation and antibiograms to characterise sequential blood isolates of CNS collected within a 7-day period from neonates and children with bacteraemia. In their analysis, speciation and antibiograms did not reliably distinguish CNS isolates, whereas PFGE was an excellent technique to study differences among CNS strains. In another study, identical antibiograms to ten antibiotics were highly predictive of strain relatedness, and a four-fold difference in a single MIC was always predictive of strain variation [18]. Sloos *et al.* [11] examined six methods (biotyping, quantitative antibiogram typing, plasmid typing, randomly amplified polymorphic DNA analysis, PFGE, and amplified fragment length polymorphism analysis) for typing multiple blood isolates of *S. epidermidis*. Randomly amplified polymorphic DNA analysis was unsuitable as a single typing method, and the discriminatory power of both biotyping and plasmid typing was low. In contrast, the results of quantitative antibiogram typing, PFGE and amplified fragment length polymorphism analysis were highly discriminatory and correlated with the epidemiological features for each case. Similar to the present study, Pierry *et al.* [19] compared quantitative antibiogram analysis with PFGE to determine the likelihood that CNS blood culture isolates are genetically related. Their correlation between quantitative antibiogram analysis and PFGE pattern was 94%. The present analysis focused on the utilisation of phenotypic methods that could be easily incorporated into the workflow of a clinical microbiology laboratory and the predictive value of these tests in clinical practice. Similarly, strains of CNS were not speciated in the present study, because the goal was to assess the phenotyping methods as they would be applied in clinical laboratory practice.

Although the correlation of colony morphology with PFGE at 24 h (87%) was similar to that of the quantitative antibiogram (91%), the use of the quantitative antibiogram was preferred for CNS strain analysis, for several reasons. CNS colony morphology analysis is limited by the lack of laboratory standardisation, and is a subjective method where subtle differences in colony morphology may not be appreciated by independent observers, especially when they are inexperienced. In contrast, quantitative antibiogram analysis utilises disk diffusion, a common, inexpensive and standardised method for antibiotic susceptibility testing. The use of computer software, such as Microsoft Excel, simplifies the calculation of the similarity coefficient while reducing the likelihood of a mathematical error. To perform quantitative antibiogram typing, each laboratory should first select the antimicrobials with variable activity against CNS (that is, strains should not be predominantly resistant or susceptible to the agent). Then, a pilot study should be performed to determine the quantitative antibiogram cut-off value for interpretation, based on the correlation with PFGE strain typing results. Because of emerging antimicrobial resistance, the antimicrobials chosen for disk diffusion susceptibility testing should be reviewed annually, and the quantitative antibiogram cut-off value should be re-calculated. The results of the quantitative antibiogram, like the results of all laboratory tests, must be interpreted carefully and cannot substitute for clinical decision-making.

The present analysis of CNS blood culture isolates was limited to episodes with the greatest clinical uncertainty (i.e., where two blood culture bottles were positive). In this situation, the microbiology laboratory can have the greatest clinical impact by providing the physician with information about the clonality of CNS isolates from blood cultures. Although this study set was small, the data suggest that laboratory information concerning quantitative antibiogram analysis could aid the physician in making clinical decisions. This approach is supported by a recent study from Kim *et al*. [20], where the use of both speciation and PFGE would have reduced by 26% the number of patients with retrospectively diagnosed CNS bloodstream infection based on the presence of symptoms and two or more positive blood cultures. Thus, larger, prospective studies need to be performed, utilising quantitative antibiogram analysis or other rapid phenotyping methods, to assess the potential impact of these techniques in reducing the inappropriate use of vancomycin for treating CNS blood culture contaminants.

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