Evaluation of Quantitative Antibiotic Susceptibility Testing by Vitek 2 as a Routine Method To Predict Strain Relatedness of Coagulase-Negative Staphylococci Isolated from Blood Cultures[▽]

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To test the hypothesis that the strain relatedness of coagulase-negative staphylococci (CoNS) recovered from blood cultures can be inferred from automated antibiotic susceptibility testing (AST) results generated by Vitek 2, concordant or discordant AST results were compared with pulsed-field gel electrophoresis (PFGE) typing results for 119 CoNS blood culture isolate pairs. Concordant AST results were highly predictive of the strain relatedness of CoNS isolates.

Clinicians and microbiologists are frequently facing the difficulty of determining the clinical significance of blood cultures positive for coagulase-negative staphylococci (CoNS). Various approaches and algorithms, including clinical and microbiological criteria, biomarkers, and mathematical models, have been developed to assist in the interpretation of CoNS-positive blood cultures (3, 8–10, 12–14, 18). A frequently used approach is to assess the identity or distinctness of CoNS isolates recovered from sequential blood cultures based on microbiologic routine testing results such as species identification, biotyping, and antimicrobial susceptibility testing (AST) (2, 5, 11, 16). However, no precise criteria to predict the strain relatedness of CoNS based on AST results to help distinguish between true bacteremia and contamination have been developed to date.

We propose a simple approach based on quantitative AST results to predict the strain relatedness of CoNS isolates recovered from two or more blood cultures from a patient with bacteremia. The aim of this study was to evaluate the sensitivity, specificity, and predictive values of the proposed approach by comparing the results obtained by application of the AST criteria with the results of pulsed-field gel electrophoresis (PFGE) of CoNS isolate pairs as the gold standard.

(This work was presented in part at the 15th European Congress of Clinical Microbiology and Infectious Diseases, Copenhagen, Denmark, 2 April to 5 April 2005 [3a].)

Blood culture isolates of all patients were prospectively collected at the Institute for Medical Microbiology, Immunology and Hygiene of the University of Cologne from 1 January 1999 to 31 December 2000 and stored at -70°C . Isolates were selected for this study if two or more blood cultures obtained from a patient within a 7-day interval had yielded CoNS. Stored isolates were subcultured and then identified to the species level and tested for antimicrobial susceptibility with Gram-positive identification (ID-GPC) and susceptibility test-

ing (AST-P526) cards of the Vitek 2 system (bioMérieux, Marcy l'Etoile, France).

Pairs of isolates were classified as concordant or discordant based on MIC data and the interpretative category (susceptible versus resistant) for each of the antimicrobial agents penicillin, oxacillin, ciprofloxacin, erythromycin, trimethoprim-sulfamethoxazole, tetracycline, gentamicin, fosfomycin, fusidic acid, and rifampin. Isolates were considered concordant when they had not more than two 2-fold dilution differences in MIC values and no differences in the resistance category for all antimicrobials tested and discordant when they had more than two 2-fold dilution differences in MIC or a difference in the interpretative category for at least one antimicrobial tested. Concordance or discordance based on AST results was compared with the results of molecular analysis of isolate pairs by PFGE as the gold standard as described previously (20). Interpretation of PFGE patterns was performed visually according to the criteria described by Tenover et al. (17) as follows: for classification of two isolates as identical, no band differences regarding the number and position of bands; for classification of isolates as closely related, 2 to 3 band differences; for classification of isolates as possibly related, 4 to 6 band differences; for classification of isolates as distinct, ≥ 7 band differences.

A total of 119 CoNS isolate pairs from 109 patients were included in this study. Ninety-eight (82.4%) of these pairs were identified as belonging to the same staphylococcal species, while 21 (17.6%) pairs represented different species. The most frequently identified species among the included CoNS isolates were *Staphylococcus epidermidis* (194 [83.6%] isolates), *S. haemolyticus* (14 [6.0%] isolates), *S. warneri* (6 [2.6%] isolates), and *S. hominis* (5 [2.2%] isolates). According to AST results, 63 (52.9%) of 119 CoNS pairs were classified as concordant and 56 (47.1%) isolate pairs as discordant.

Among 119 CoNS pairs, isolates from 66 (55.5%) pairs were found to be genetically identical (60 pairs) or closely related (6 pairs) and 53 (44.5%) pairs comprised isolates that showed at least seven band differences and were considered genetically distinct based on PFGE results (Fig. 1).

Sixty-three (100%) isolate pairs with concordant AST results belonged to the same species and were genetically identical or closely related based on PFGE, while 53 (94.6%) of 56 isolate

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FIG. 1. Representative PFGE patterns of CoNS isolates from six patients. Lanes 1 and 15, molecular-weight markers. Pairs of CoNS isolates are placed side by side. Isolates from patient B were distinct (lanes 4 and 5); isolates from patient C were had three band differences (lanes 6 and 7); the remaining four patients had identical banding patterns (lanes 2 and 3, 8 and 9, 10 and 11, and 12 to 14, respectively).

pairs with discordant AST results were genetically distinct (Table 1). The AST results were found to be a significant predictor of genetic relatedness. Concordant AST results were predictive of genetic relatedness with 95.5% sensitivity, 100% specificity, a 100% positive predictive value (PPV), and a 94.6% negative predictive value (NPV). Among the 66 pairs of CoNS isolates that were considered genetically identical or closely related, 63 pairs had concordant AST results and three pairs showed discrepancies in only one AST result. For those three isolate pairs, discrepancies were seen for erythromycin (one case) and gentamicin (two cases). In contrast, pairs of genetically unrelated isolates showed up to 8 discrepancies (1 discrepancy for three pairs, 2 discrepancies for 17 pairs, 3 and 4 discrepancies for 10 pairs each, and ≥ 5 discrepancies for 13 pairs). In cases in which a discrepancy in one AST result was used as a cutoff to infer the genetic distinctness of CoNS isolates, AST was only 50% predictive. However, when a threshold of two or more antibiotic discrepancies among the 10 tested antimicrobials was used to define distinctness, discordant AST results were predictive of genetic distinctness with 100% sensitivity, 100% specificity, 100% PPV, and 100% NPV.

Our report shows that an easily applicable approach based on routine AST using Vitek 2 can predict strain relatedness and provide a clue to the clinical relevance of CoNS isolates recovered from sequential blood cultures with high sensitivity, specificity, PPV, and NPV.

Different microbiologic criteria, including colony morphology, species identification, and biotype, have been previously investigated to determine the relatedness of CoNS isolates (1, 5, 16). Comparison of AST patterns has been evaluated previously using different susceptibility testing methods such as

the Dade MicroScan WalkAway-40 system, the Vitek system, and broth microdilution as well as different gold standards such as plasmid typing and PFGE for comparisons (5, 11, 21). Quantitative antibiogram analysis involving the calculation of a similarity coefficient or cluster analysis and based on the measurement of the diameters of agar diffusion inhibition zones of CoNS isolates was also investigated (2, 16). However, those studies did not define specific criteria for determination of the concordance and discordance of AST results or showed conflicting results. Our study was unique in that it provided precise criteria to determine the strain relatedness of CoNS isolates, with automated quantitative AST results that were easily applicable and were verified by PFGE as the gold standard. Additional tests or calculations are not necessary for this approach.

There were no false-positive results in the present study when inferring strain relatedness from concordant AST patterns compared with the results obtained with PFGE as the gold standard. False-negative results occurred in three episodes, with CoNS isolates showing identical PFGE banding patterns but one discordant AST result for erythromycin or gentamicin. Therefore, a single discordant test result for erythromycin or gentamicin should not be used for inferring the distinctness of CoNS isolates recovered from the same patient.

A limitation of our approach is the underlying assumption that the recovery of genetically identical or closely related CoNS isolates from blood cultures represents true bacteremia whereas the recovery of genetically distinct strains represents contamination. Although this assumption is supported by various clinical and microbiologic studies (8, 15), there might be rare exceptions, with classification of strains as identical being the result of repeated contamination and of distinct strains being the result of a polyclonal infection (7, 19). In addition, CoNS colonies were selected from primary subcultures of positive blood cultures based on colony morphology and preliminary agar diffusion susceptibility results; agar plates were not searched systematically for the presence of different strain types as was performed in other studies (4, 6).

In conclusion, AST determined with Vitek 2 is a reliable, easy-to-perform, and time- and cost-efficient tool for prediction of the strain relatedness of CoNS isolates recovered from

TABLE 1. Correlation of antimicrobial susceptibility testing results with pulsed-field gel electrophoresis results for 119 pairs of coagulase-negative staphylococcal isolates^a

AST result ^b	No. of staphylococcal pairs with the indicated PFGE result ^c		Total no. of staphylococcal pairs
	Identical or closely related	Distinct	
Concordant	63	0	63
Discordant	3	53	56
Total	66	53	119

^a AST, antimicrobial susceptibility testing; PFGE, pulsed-field gel electrophoresis.

^b A concordant AST result was defined as a value representing ≤ 2 log₂ dilutions of difference in the MIC and no difference in the interpretative category (susceptible versus resistant) for any antimicrobial tested. All other AST results were considered discordant.

^c Identical, no differences in PFGE banding patterns; closely related, 2 to 3 band differences; distinct, ≥ 4 band differences.

blood cultures. Our criteria may help distinguish true bacteremia from contamination for a patient with two or more blood cultures yielding CoNS. In practice, concordant AST results for CoNS isolates should be reported to the clinician to enable optimal patient management.

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