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Molecular Analysis of *Staphylococcus aureus* Blood Isolates Shows Lack of Polyclonal Bacteremia

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Molecular analysis of the first blood culture from 41 patients with *Staphylococcus aureus* bacteremia and 20 bacteremia-associated catheter tip isolates revealed indistinguishable blood colonies in 39 patients (95.1%) and two related variants in two patients (4.9%). Polyclonal bacteremia was not observed in any patient, including four with genetic diversity in the catheter tip isolate.

Staphylococcus aureus is a common cause of community-acquired and nosocomial bacteremia (4, 6). The pathogenesis usually involves the colonization of a site by *S. aureus* followed by its invasion of the bloodstream (15). Whether single or multiple strains of *S. aureus* are involved is unknown. Generally, most infections are thought to be monoclonal (8). However, reports of polyclonal bacteremia are increasing (1, 2, 5, 13, 14, 16). A previous study found that most blood cultures (BC) positive for two or more strains of coagulase-negative *Staphylococcus* resulted from contamination (9). Whether scarcity of polyclonality is also true of other organisms is unknown. Defining the clonal nature of *S. aureus* bacteremia is important for clarifying host-pathogen interactions and strain-related factors involved in invasiveness. It may also have important implications for antibiotic susceptibility testing. We elected to study the clonal makeup of *S. aureus* bacteremia.

(A preliminary report of this work has been presented previously [R. Khatib, M. Sharma, S. A. H. Naqvi, K. Riederer, M. O. Almoujahed, and M. G. Fakih, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. K-1430, 2001.])

The study was conducted at a single 607-bed teaching hospital. BC and intravascular catheter (IVC) tip cultures were prospectively monitored (1 January 2000 to 1 May 2001). All patients with one or more positive BC during the first 2 months and all patients with >15 CFU of *S. aureus* in IVC tip cultures and positive BC during all 5 months of the study were screened for inclusion. The medical records of selected patients were reviewed. All patients with bacteremia (determined by clinical signs of infection and a positive culture obtained from a peripheral blood draw) were included in the study. Patients with contaminated BC (determined by a single positive BC without a clear source of bacteremia, which was judged by the attending physician to be from a contaminant, and where there was no utilization of antibiotics with antistaphylococcal activity) and polymicrobial bacteremia were excluded. Determination of the source of the bacteremia was based on clinical signs of infection and supported by isolation of *S. aureus* with an antibiotic susceptibility pattern identical to that of the implicated source.

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The first blood isolate and the IVC tip isolate were chosen for analysis. Positive BC were plated on a Trypticase soy agar plate (TSA) with 5% sheep blood and incubated for 24 h at 35°C. IVC tips were cultured by a semiquantitative method (3). They were not sonicated. *S. aureus* identification was based on catalase production and Staphaureux latex agglutination (Murex Biotech Ltd., Dartford, England). Susceptibility tests were performed with Vitek gram-positive susceptibility cards (bioMérieux, Hazelwood, Mo.).

The primary blood agar plate of BC and IVC tip cultures was used to minimize in vitro variation in colony populations due to differential growth characteristics. Eight colonies were randomly selected from each BC and IVC isolate and saved individually at −80°C until tested.

Genomic DNA was extracted and subjected to endonuclease digestion and pulsed-field gel electrophoresis (PFGE) as previously described (9). Restriction patterns were compared visually by three independent observers. The isolates were considered indistinguishable if all DNA bands matched, related if there were differences in one to three bands, and distinct if there were differences in more than three bands (11, 12).

Twenty-nine patients with positive BC were encountered; eight patients with bacteremia were excluded—three because of contaminated BC, three because of mixed infections, and two because the BC were not saved. Thirty-three patients had *S. aureus* in an IVC tip culture; 13 of these patients were excluded—six with cultures with <15 CFU and seven without bacteremia. Thus, we studied 21 patients with bacteremia from a variety of sources and 20 patients with IVC-associated bacteremia by using available blood and IVC isolates. The IVC tip and BC isolates from all patients had identical susceptibility patterns.

The mean age (± standard deviation) of the study group was 63.1 ± 19.2 years (range, 5 months to 88 years), and 23 of the patients were males (56.1%). Bacteremia was device associated in 33 patients (74.2%) (IVC, 27 patients; osteomyelitis with hardware, 4 patients; pacemaker, 1 patient; atrial-venous graft, 1 patient) and non-device associated in 8 patients (skin and soft tissue infections, 7 patients; infective endocarditis, 1 patient). The infections were nosocomial in 21 patients (51.2%). The isolates were oxacillin resistant in 61% of the patients.

The typing of eight colonies or isolates was completed for 39 blood and 18 IVC isolates. Of the remainder, only seven col-

TABLE 1. Molecular analysis of *S. aureus* isolates from IVCs and BC from individual patients with IVC-associated bacteremia

Patient	Isolate	No. of clones in isolates from[a]:		Similarity of IVC to BC clone[a]
		IVC	BC	
1	MRSA	1 (8)	1 (8)	Identical
2	MRSA	1 (8)	1 (8)	Related
3	MSSA	1 (8)	1 (8)	Identical
4	MSSA	1 (8)	1 (8)	Identical
5	MRSA	1 (8)	1 (8)	Identical
6[b]	MRSA	1 (8)	1 (8)	Identical
7	MRSA	1 (8)	1 (8)	Identical
8[b]	MRSA	1 (8)	1 (8)	Identical
9	MSSA	1 (8)	1 (8)	Identical
10	MRSA	1 (7)[c]	2 (7; 1) [1]	Identical[d]
11	MSSA	1 (8)	1 (8)	Identical
12[b]	MRSA	1 (8)	1 (8)	Identical
13	MRSA	2 (5; 1) [6][c]	1 (8)	Identical[e]
14	MRSA	1 (8)	2 (7; 1) [2]	Identical[d]
15	MRSA	1 (8)	1 (8)	Identical
16	MRSA	1 (8)	1 (8)	Identical
17	MRSA	2 (7; 1) [2]	1 (8)	Identical[d]
18	MRSA	1 (8)	1 (8)	Identical
19	MRSA	2 (5; 3) [10]	1 (8)	Identical[f]
20	MSSA	2 (7; 1) [1]	1 (8)	Identical[d]

[a] Numbers in parentheses indicate the number of colonies per clone; numbers in brackets indicate the number of the DNA band difference.
[b] Patients 6, 8, and 12 had indistinguishable PFGE patterns.
[c] The remaining colonies were coagulase negative staphylococci and were considered contaminants.
[d] Matched the predominant clone.
[e] Matched the predominant IVC strain.
[f] Matched the less-frequent IVC strain.

onies were typed in two blood isolates and one IVC isolate and six colonies were typed in one IVC isolate. The remaining colonies proved to be *Staphylococcus epidermidis* in three instances and a *Micrococcus* species in one instance; these colonies were considered contaminants.

Analysis of the entire group (41 patients) revealed 22 PFGE types. Thirteen types were detected among 25 methicillin-resistant *S. aureus* (MRSA) isolates (nine types of each were unique to one patient, two types of each were noted in three patients, and two more types of each were encountered among five patients), and nine types were detected among 16 methicillin-susceptible (MSSA) *S. aureus* isolates (five types of each were unique to a single patient, three types of each were detected in two patients, and one type was detected in five patients).

Individual case analysis of blood isolates revealed indistinguishable banding patterns in all of the colonies (100% monoclonal population; 95% confidence interval, 63.1 to 100%) from 39 patients (95.1%) and in 7 of 8 colonies (87.5% monoclonal population; 95% confidence interval, 47.4 to 99.7%) from 2 patients (4.9%) with IVC-associated bacteremia. Colony morphologies were identical in all patients.

Analysis of the IVC isolates revealed variation in colony morphology and/or hemolytic characteristics in four patients—two patients with MRSA infections and two patients with MSSA infections. All morphological variants had identical banding patterns. All patients with diversified IVC populations had monoclonal blood isolates (Table 1).

Genetic variation was noted in 5 of 25 (20%) oxacillin-resistant isolates and 1 of 16 (6.3%) oxacillin-sensitive isolates. The difference was not significant ($P = 0.4$). Delineating the clonal nature of infections may help to clarify the host-pathogen interaction, set the ground for additional studies to define the virulence factors associated with strain selection, and have important implications for current methods of antibiotic susceptibility testing. Inherent to all DNA-based methods is the use of a single colony as the DNA source (8, 12). Therefore, the possibility of strain variation within individual isolates can easily be missed. Our method allows a more accurate assessment of the clonal makeup. PFGE was chosen because its results compare favorably with those of other tests (10, 11). Additional tests did not yield any meaningful extra distinction (12).

Our findings demonstrate that all of the *S. aureus* blood isolates were monoclonal, with occasional minor clone variation. The monoclonal nature of the isolates was also demonstrable in patients whose IVC isolates showed genotypic diversity. Thus, the current practice of selecting 3 to 5 representative colonies of *S. aureus* from BC for antimicrobial susceptibility testing remains reliable.

The detection of related variants in a small number of IVC and blood isolates was an interesting finding. Whether this finding reflects minor genotypic changes at the implicated site, in the bloodstream, or in vitro during culture is uncertain.

There are a few limitations to our study. We attempted to minimize in vitro variation in colony populations due to differential growth characteristics by selecting colonies from the primary blood agar plate of the BC and catheter tip cultures. However, blood was first cultured in a broth medium. Therefore, we cannot be certain that selection did not occur. Additionally, the number of patients with polyclonal source isolates was small. Examining additional patients and including isolates from other sources might have disclosed more frequent polyclonal bacteremia. Finally, utilizing additional typing methods might have provided better discrimination, but performing extra analysis of 488 colonies would be prohibitive.

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REFERENCES

1. **Arbeit, R., D., A. Slutsky, T. W. Barber, et al.** 1993. Genetic diversity among strains of *Mycobacterium avium* causing monoclonal and polyclonal bacteremia in patients with AIDS. J. Infect. Dis. **167:**1384–1390.
2. **Hakim, A., A. Deplano, N. Maes, A. Kentos, C. Rossi, and M. J. Struelens.** 1999. Polyclonal coagulase-negative staphylococcal catheter-related bacteremia documented by molecular identification and typing. Clin. Microbiol. Infect. **5:**224–227.
3. **Maki, D. G., C. E. Weiss, and H. W. Sarafin.** 1977. A semiquantitative culture method for identifying intravenous catheter-related infection. N. Engl. J. Med. **296:**1305–1309.
4. **Morin, G. A., and J. L. Hadler.** 2001. Population-based incidence and characteristics of community-onset *Staphylococcus aureus* infections with bacteremia in 4 metropolitan Connecticut areas, 1998. J. Infect. Dis. **184:**1029–1034.
5. **Murdoch, D. R., S. A. Roberts, V. G. Fowle, M. A. Shah, Jr., S. L. Taylor, A. J. Morris, and G. R. Corey.** 2001. Infection of orthopedic prostheses after *Staphylococcus aureus* bacteremia. Clin. Infect. Dis **32:**647–649.
6. **Mylotte, J. M., and A. Tayara.** 2000. *Staphylococcus aureus* bacteremia: predictors of 30-day mortality in a large cohort. Clin. Infect. Dis. **31:**1170–1174.
7. **Na'was, T., A. Hawwari, E. Hendrix, J. Hebden, R. Edelman, M. Martin, W. Campbell, R. Naso, R. Schwable, and A. I. Fattom.** 1998. Phenotypic and genotypic characterization of nosocomial *Staphylococcus aureus* isolates from trauma patients. J. Clin. Microbiol. **36:**414–420.

8. **Relman, D. A., and S. Falkow.** 1999. A molecular prospective of microbial pathogenicity, p. 2–13. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Philadelphia, Pa.
9. **Sharma, M., K. Riederer, L. B. Johnson, and R. Khatib.** 2001. Molecular analysis of coagulase-negative *Staphylococcus* isolates from blood cultures: prevalence of genotypic variation and polyclonal bacteremia. Clin. Infect. Dis. **33:**1317–1323.
10. **Tenover, F. C., R. D. Arbeit, R. V. Goering, et al.** 1997. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infect. Control Hosp. Epidemiol. **18:**426–429.
11. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
12. **Tenover, F. C., R. D. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hébert, B. Hill, R. Hollis, W. R. Jarvis, B. Kreiswirth, W. Eisner, J. Maslow, L. K. McDougal, J. M. Miller, M. Mulligan, and M. A. Pfaller.** 1994. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **32:**407–415.
13. **Van Eldre, J., W. E. Peetermans, M. J. Struelens, A. Deplano, and H. Bobbaers.** 2000. Polyclonal staphylococcal endocarditis caused by genetic variability. Clin. Infect. Dis. **31:**24–30.
14. **Van Wijngaerden, E., W. E. Peetermans, S. Van Lierde, and J. Van Eldere.** 1997. Polyclonal staphylococcal endocarditis. Clin. Infect. Dis. **25:**69–71.
15. **Von Eiff, C., K. Becker, K. Machka, H. Stammer, and G. Peters.** 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N. Engl. J. Med. **344:**11–16.
16. **Wendt, C., S. A. Messar, R. J. Hollis, M. A. Pfaller, and L. A. Herwaldt.** 1998. Epidemiology of polyclonal gram-negative bacteremia. Diagn. Microbiol. Infect. Dis. **32:**9–13.