JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2003, p. 3942–3944 Vol. 41, No. 8
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.8.3942–3944.2003

Clinical Impact of a PCR Assay for Identification of *Staphylococcus aureus* and Determination of Methicillin Resistance Directly from Blood Cultures

M. Hallin,[1]* N. Maes,[1] B. Byl,[2] F. Jacobs,[2] Y. De Gheldre,[1]† and M. J. Struelens[1]

Laboratory of Microbiology[1] and Infectious Diseases Clinic,[2] Erasme University Hospital, Université Libre de Bruxelles, Brussels, Belgium

Received 31 January 2003/Returned for modification 19 March 2003/Accepted 22 May 2003

We evaluated the clinical usefulness of a PCR assay that discriminates *Staphylococcus aureus* from coagulase-negative staphylococci and detects methicillin resistance on blood cultures by measuring the adaptation of antimicrobial therapy based on the PCR results. Only 7 of 28 patients (25%) benefited from a modification of antibiotic therapy based on the PCR results, since empirical therapy was appropriate in a majority of cases.

Staphylococci are the most frequent pathogens implicated in bloodstream infections (BSI). In a recent European multicenter study, they represented 36% of all bacteremia events, with 22% due to *Staphylococcus aureus* and 14% to coagulase-negative staphylococci (CoNS) (6). With respect to nosocomial BSI, CoNS are the most common pathogens, with an attributable mortality rate of 13 to 18% (8). In the case of *S. aureus* BSI, the attributed mortality ranges from 22% for methicillin-susceptible *S. aureus* to 42% for methicillin-resistant *S. aureus* (MRSA) (10). Rapid reporting of identification and susceptibility results can significantly improve outcomes for infected patients by enabling rapid adjustment of antibiotherapy, leading to decreased mortality, shortened hospital stay, and lower hospitalization costs (3, 7, 13). Consequently, many methods were developed over recent years to provide more rapid identification and susceptibility results. Only a few studies have determined whether those rapid but expensive and labor-intensive methods could improve patient care. We examined how a PCR assay that discriminates *S. aureus* from CoNS and detects methicillin resistance on positive blood cultures could improve patient care by tracking the following two parameters: (i) turnaround time to results by PCR versus conventional tests and (ii) adaptation of empirical therapy.

During an 8-month period, we prospectively enrolled 35 adult inpatients suspected of having staphylococcal BSI, defined as patients with two or more blood cultures positive for gram-positive cocci in clusters or with a single positive blood culture and another clinical site infected with staphylococci. Only blood cultures becoming positive between 7 p.m. and 9 a.m. on weekdays were included. PCR was performed on DNA directly extracted from the blood culture broth as previously described (9) by using three sets of primers for sequences encoding a staphylococcus-specific region of the 16S rRNA gene, the *S. aureus*-specific thermostable nuclease gene (*nuc*), and the PBP2A gene (*mecA*). Conventional methods consisted of a 24-h subculture on Columbia agar with 5% sheep blood, a coagulase test in human plasma, and an agglutination test with a Pastorex Staph Plus kit (Sanofi Diagnostics Pasteur, Marnes la Coquette, France). For CoNS, susceptibility tests were performed by disk diffusion (Rosco Neosensitabs, Taastrup, Denmark) according to NCCLS criteria. For *S. aureus*, a Rapid ATB-Staph microdilution test (Biomérieux, Marcy l'Etoile, France) was used on weekdays, and a disk diffusion test was used on weekends. CoNS identification to species was performed with the ID 32 Staph system (Biomérieux). PCR showed 100% concordance with conventional methods for all clinically significant isolates causing BSI. PCR was not able to discriminate mixed cultures containing contaminant isolates in three cases (Fig. 1). The turnaround time to result communication was the time elapsed between reporting of the Gram stain result and notification of either PCR or conventional method results. For PCR, the mean turnaround time was 6 h 8 min (range, 4 h 50 min to 7 h 30 min), whereas the conventional methods took a mean time of 36 h 39 min (range, 27 h 35 min to 51 h 1 min) for *S. aureus* and 49 h 16 min (range, 47 h 6 min to 51 h 21 min) for CoNS. Thus, PCR provided results on average 39 h earlier than conventional methods ($P < 0.01$).

Positive blood cultures were considered to be indicative of BSI in 29 patients and CoNS pseudobacteremia in 6 patients (Fig. 1). CoNS pseudobacteremia was considered if only one of the two or three blood culture samples taken within 72 h from a patient was positive for CoNS or if several blood cultures of the same patient were positive for distinct CoNS (based on two or more major or three or more minor discrepancies in the susceptibility profile to 14 antibacterial agents). Patients with CoNS pseudobacteremia and one patient without follow-up because of his transfer to another hospital were excluded from the evaluation of treatment modification.

The results of Gram stain, PCR, and conventional tests were communicated to the clinician in charge of the patient by a microbiologist, who recorded the time at which the results were communicated. At each step of the process, the implementation or adaptation of the treatment was evaluated according to local antimicrobial therapy guidelines in collaboration with an infectious disease specialist (Table 1). Before PCR

* Corresponding author. Mailing address: Service de Microbiologie, Hôpital Erasme, 808 route de Lennik, 1070 Brussels, Belgium. Phone: 32 2 555 34 84. Fax: 32 2 555 31 10. E-mail: mahallin@ulb.ac.be.

† Present address: Laboratoire de Microbiologie, Cliniques Universitaires Mont-Godinne, 5530 Yvoir, Belgium.

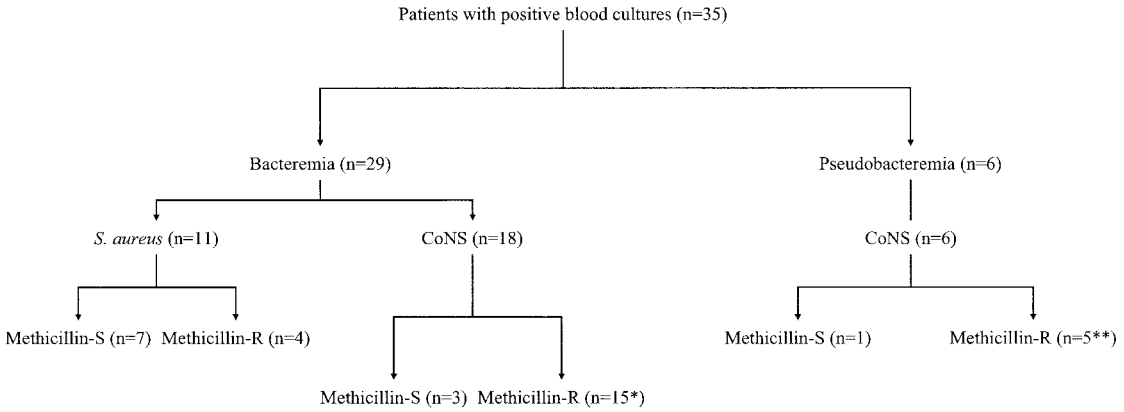

FIG. 1. Repartition of the patients with staphylococcal-positive blood cultures by type of isolate. *, one case with methicillin-resistant CoNS bacteremia plus methicillin-resistant CoNS contamination; **, two cases with mixed contamination by two distinct methicillin-resistant CoNS; Methicillin-R, methicillin-resistant; Methicillin-S, methicillin-susceptible.

results were available, 25 of the 28 evaluated patients (89%) were already receiving an appropriate antibiotic treatment or had a CoNS-infected device removed, while three patients were untreated. Based on PCR results, treatment was altered in seven patients: five patients with methicillin-susceptible staphylococci were shifted from vancomycin to oxacillin therapy, vancomycin treatment was started for one patient with methicillin-resistant CoNS, and treatment with cefepime plus vancomycin was streamlined to vancomycin only for one patient with methicillin-resistant CoNS. Conversely, two patients with methicillin-susceptible staphylococci remained under vancomycin despite notification of PCR results (Table 1).

Tan et al. (12) have retrospectively evaluated the potential clinical impact of a PCR assay for MRSA detection on 52 staphylococcal BSI. They report that 39% of MRSA bacteremia under ineffective empirical therapy could benefit from an adaptation of treatment based on PCR results. Allaouchiche et al. (1) studied 145 patients with an *S. aureus* bacteremia in an intensive care unit. The patients were randomly assigned to two groups, one in which methicillin susceptibility tests were performed by the conventional overnight method and the other in which a same-day PCR assay was used. They found no significant difference between the two groups in terms of outcome of the infection or general clinical outcome. In the present study, our PCR assay was confirmed to be as accurate as conventional methods (9). Results were available about 39 h earlier. However, this method is labor intensive and relatively expensive (approximately $30 per assay in our laboratory, including material and labor). This PCR does not discriminate mixed cultures and neither provides a complete susceptibility profile nor identifies CoNS to the species level. Only 25% of the treatments were modified following notification of the PCR results for our patient population. This is related to the high proportion of methicillin-resistant staphylococci causing bacteremia as well as to the high frequency of appropriate empirical antibiotic therapy in our institution (4).

This PCR assay thus showed limited clinical benefits in our setting. The cost effectiveness of this assay may be better in

TABLE 1. Evolution of antistaphylococcal treatment in 28 patients with staphylococcal bacteremia by diagnostic step

Infectious agent (no. of patients)	Drug	No. of patients receiving the indicated drug at the indicated diagnostic stage[a]			
		Empirical[b]	Gram stain	PCR	Conventional methods
Methicillin-susceptible *S. aureus* (7)	Vancomycin	4	5	1	1
	Oxacillin	0	2	6	6
	No antibiotic	3	0	0	0
MRSA (4)	Vancomycin	2	3	3	3
	Oxacillin	0	0	0	0
	No antibiotic	2	1	1	1
Methicillin-susceptible CoNS (3)	Vancomycin	1	2	1	0
	Oxacillin	0	0	1	2
	No antibiotic	2 (1)	1 (1)	1 (1)	1 (1)
Methicillin-resistant CoNS (15)	Vancomycin	4	9	10	10
	Oxacillin	0	0	0	0
	No antibiotic	10	5 (3)	4 (3)	4 (3)

[a] Values in parentheses indicate the number of patients with removal of an intravenous device.
[b] Values represent the number of patients treated before any result from blood cultures was available.

other institutions with lower prevalences of methicillin resistance or less frequently appropriate empirical therapy (12). Improved DNA detection methods, allowing the simultaneous probing of several bacterial targets, such as oligonucleotide arrays (2), and faster methods, like PCR performed directly from the blood of septic patients (5, 11), warrant further investigation of their impact on the management and outcome of infection in hospitalized patients.

We gratefully thank Sylvianne Rottiers and Soultana Pavlov for their excellent technical support.

REFERENCES

1. **Allaouchiche, B., H. Jaumain, G. Zambardi, D. Chassard, and J. Freney.** 1999. Clinical impact of rapid oxacillin susceptibility testing using a PCR assay in *Staphylococcus aureus* bactaeremia. J. Infect. **39:**198–204.
2. **Anthony, R. M., T. J. Brown, and G. L. French.** 2000. Rapid diagnosis of bacteremia by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. J. Clin. Microbiol. **38:**781–788.
3. **Barenfanger, J., C. Drake, and G. Kacich.** 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. J. Clin. Microbiol. **37:**1415–1418.
4. **Byl, B., P. Clevenbergh, F. Jacobs, M. J. Struelens, F. Zech, A. Kentos, and J.-P. Thys.** 1999. Impact of infectious disease specialists and microbiological data on the appropriateness of antimicrobial therapy for bacteremia. Clin. Infect. Dis. **29:**60–66.
5. **Cursons, R. T., E. Jeyerajah, and J. W. Sleigh.** 1999. The use of polymerase chain reaction to detect septicemia in critically ill patients. Crit. Care Med. **27:**937–940.
6. **Diekema, D. J., F. J. Schmitz, M. A. Pfaller, J. Bell, J. Smayevsky, M. Beach, R. N. Jones, and the SENTRY Participants Group.** 2001. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997–1999. Clin. Infect. Dis. **32**(Suppl. 2)**:**S114–S132.
7. **Doern, G., R. Vautour, M. Gaudet, and B. Levy.** 1994. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. J. Clin. Microbiol. **32:**1757–1762.
8. **Karchmer, A. W.** 2000. Nosocomial bloodstream infections: organisms, risk factors, and implications. Clin. Infect. Dis. **31**(Suppl. 4)**:**S139–S143.
9. **Maes, N., J. Magdalena, S. Rottiers, Y. De Gheldre, and M. J. Struelens.** 2002. Evaluation of a triplex PCR assay to discriminate *Staphylococcus aureus* from coagulase-negative staphylococci and determine methicillin resistance from blood cultures. J. Clin. Microbiol. **40:**1514–1517.
10. **Romero-Vivas, J., M. Rubio, C. Fernandez, and J. J. Picazo.** 1995. Mortality associated with nosocomial bacteremia due to methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **21:**1417–1423.
11. **Sleigh, J., R. Cursons, and M. La Pine.** 2001. Detection of bacteraemia in critically ill patients using 16S rDNA polymerase chain reaction and DNA sequencing. Int. Care Med. **27:**1269–1273.
12. **Tan, T. Y., S. Corden, R. Barnes, and B. Cookson.** 2001. Rapid identification of methicillin-resistant *Staphylococcus aureus* from positive blood cultures by real-time fluorescence PCR. J. Clin. Microbiol. **39:**4529–4531.
13. **Trenholme, G. M., R. L. Kaplan, P. H. Karakusis, T. Stine, J. Fuhrer, W. Landau, and S. Levin.** 1989. Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. J. Clin. Microbiol. **27:**1342–1345.