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Frequency of Susceptibility Testing for Patients with Persistent Methicillin-Resistant *Staphylococcus aureus* Bacteremia

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Currently, no standards exist for determining the optimal frequency of repeat antimicrobial susceptibility testing (AST) when an organism is recurrently isolated from the same specimen source. Although testing every 2 to 5 days is thought sufficient, we present three cases of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia where current laboratory protocol for repeating AST every 5 days was inadequate to identify resistant organisms.

CASE REPORTS

We present three cases of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia, where the laboratory policy of repeat testing of blood isolates for antimicrobial susceptibility only once every 5 days resulted in significant delays in the recognition of antimicrobial resistance. All MRSA isolates were recovered from blood incubated in a BacT/Alert 3D system (bioMérieux, Durham, NC) using standard aerobic and anaerobic bottles. Isolates were identified as *S. aureus* by a positive tube coagulase reaction and a characteristic Gram stain and saved on tryptic soy agar slants at room temperature for up to 1 year. For each culture, the length of incubation in the BacT/Alert 3D system prior to signaling a positive result was recorded from the BacT/Alert 3D software. Retrospective antimicrobial susceptibility testing was performed on *S. aureus* isolated from every blood culture in our three patients for the purpose of this study. However, at the time of isolation, susceptibility testing was performed only at 5-day intervals, from the time of blood collection. All retrospective testing was performed subsequent to the hospital stay, and therefore these data were not used in clinical treatment decisions (see Table 1). Susceptibility testing, for both routine and retrospective testing, was performed using the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) MIC method (1) with panels prepared in-house using cation-adjusted Mueller-Hinton Broth (CA-MHB) (Difco, Detroit MI) supplemented with 50 mg/liter calcium for daptomycin testing. BMD panels were prepared as described previously (1) and incubated at 35°C. Following 16 to 20 h of incubation, plates were examined visually for MIC determination and then reincubated to 24 h and examined visually for final MIC determination of vancomycin (2). Correlations between MICs and time to positivity were calculated using the software program GraphPad Prism 5.0 and Pearson's coefficient. Clonality of sensitive and resistant isolates was tested by repetitive-element PCR (repPCR) using the DiversiLab *Staphylococcus* kit (bioMérieux Inc., Durham, NC) as described elsewhere (3). All protocols were approved by the UCLA institutional review board.

Case 1. A 53-year-old female with a history of end-stage renal disease, transhepatic catheter, and 49 days of daptomycin (10 mg/kg of body weight every 48 h [q48h]) suppressive therapy for persistent bacteremia from the indwelling catheter was admitted to our facility with new-onset fever. On admission, blood was collected (culture 1-1; Table 1), and MRSA that was daptomycin nonsusceptible (NS) (MIC, 4.0 μg/ml) was ultimately identified on retrospective testing. Blood collected the following day (culture 1-2; Table 1) grew MRSA that was daptomycin susceptible (MIC, 0.5 μg/ml). Because the daptomycin-NS isolate from culture 1-1 took 31 h to signal positive in the BacT/Alert system whereas the daptomycin-susceptible isolate from culture 1-2 took 16 h to signal positive, the susceptible isolate was the first to be tested for susceptibilities and reported by the laboratory (Table 1), and isolate 1-1 was not tested, per laboratory policy.

Over the course of 20 days, 12 of 18 blood culture sets yielded MRSA with daptomycin MICs that ranged from 0.5 μg/ml to 8 μg/ml (Table 1). Daptomycin-NS isolates (MIC $>$ 1 μg/ml) were recovered in cultures 1-4, 1-5, 1-8, and 1-9, whereas daptomycin-susceptible isolates (MIC $\leq$ 1 μg/ml) were recovered from cultures 1-2, 1-3, 1-6, 1-7, 1-10, 1-11, and 1-12 (Table 1). Three of three blood culture sets from blood drawn on hospital day 15 were positive, two with a daptomycin-susceptible and one with a daptomycin-NS MRSA (isolates 1-9, 1-10, and 1-11; Table 1). Daptomycin-NS MRSA was not recognized in this case until hospital day 10 because of a laboratory policy of testing repeat isolates only once every 5 days, even though blood from the day of admission harbored a daptomycin-NS MRSA. For this patient, alternative therapy (tigecycline, 50 mg intravenously (i.v.) twice a day [b.i.d.]) was not started until day 10, following the first report of daptomycin nonsusceptibility for the patient's MRSA isolate.

No differences in colony morphology or growth rates on agar plates with 5% sheep's blood (BD, Sparks, MD) were noted be-

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TABLE 1 Results of extended testing on MRSA isolated from three patients with persistent bacteremia

Patient	Isolate no.	Day of hospitalization		Routine AST performed?[b]	MIC, μg/ml (interpretation)[d]			Notes
		Blood collected[a]	Blood culture positive		DAP	LNZ	VAN	
1	1-1	0	2	No	**4 (NS)**	1 (S)	2 (S)	Recovered after MRSA from blood collected on day 1 was isolated
	1-2	1	2	Yes	0.5 (S)	1 (S)	1 (S)	
		3			—	—	—	
	1-3	4	5	No	1 (S)	1 (S)	1 (S)	
	1-4	8	9	Yes	**2 (NS)**	1 (S)	2 (S)	Reported on hospital day 10
	1-5	10	11	No	**2 (NS)**	1 (S)	1 (S)	
	1-6	11	13	No	0.5 (S)	1 (S)	1 (S)	
	1-7	12	14	No	1 (S)	1 (S)	1 (S)	
	1-8	13	15	No	**2 (NS)**	1 (S)	1 (S)	
		14			—	—	—	
	1-9	15	18	Yes	**8 (NS)**	1 (S)	2 (S)	
	1-10	15	17	No	1 (S)	1 (S)	1 (S)	
	1-11	15	17	No	1 (S)	1 (S)	1 (S)	
		16			—	—	—	
		17			—	—	—	
		18			—	—	—	
	1-12	19	21	Yes	1 (S)	1 (S)	1 (S)	All blood cultures drawn after day 19 were negative
2	2–1	15	20	No	0.5 (S)	**8 (R)**	1 (S)	Recovered after MRSA from blood collected on day 17 was isolated
	2-2	17	18	Yes	0.5 (S)	1 (S)	1 (S)	2 colony morphologies
	2-3	17	18	No	1 (S)	1 (S)	1 (S)	
	2-4	17	20	No	0.5 (S)	4 (S)	2 (S)	
	2-5	17	20	No	0.5 (S)	2 (S)	1 (S)	
	2-6	18	22	No	0.25 (S)	**8 (R)**	1 (S)	
	2-7	18	22	No	0.5 (S)	4 (S)	1 (S)	All blood cultures drawn after day 18 were negative
3	3-1	6	7	Yes	0.5 (S)	1 (S)	1 (S)	
	3-2	6	8	No	0.5 (S)	1 (S)	1 (S)	
		7			—	—	—	
	3-3	7	8	No	0.5 (S)	1 (S)	1 (S)	
	3-4	8	9	No	1 (S)	1 (S)	2 (S)	
		8			—	—	—	
		8			—	—	—	
		9			—	—	—	
		9			—	—	—	
	3-5	9	10	No	1 (S)	1 (S)	**4 (I)**	
	3-6	9	10	No	1 (S)	1 (S)	**4 (I)**	
		10			—	—	—	
		10			—	—	—	
		11			—	—	—	
		11			—	—	—	
	3-7	40[c]	41	Yes	1 (S)	1 (S)	**4 (I)**	Reported on hospital day 43
	3-8	40	41	Yes	1 (S)	1 (S)	**4 (I)**	All blood cultures drawn after day 40 were negative

[a] Values represent blood drawn for one blood culture set consisting of one aerobic and one anaerobic bottle.
[b] Per laboratory policy, repeat testing of blood isolates for antimicrobial susceptibility (AST) was performed only once every 5 days. Results highlighted in gray were reported to the physician; all other results were completed retrospectively following case resolution and were not available for clinical use. Boldface indicates nonsusceptibility.
[c] The patient was discharged from our facility to a skilled nursing facility on day 12 and readmitted to our facility on day 40.
[d] DAP, daptomycin; LNZ, linezolid; VAN, vancomycin; R, resistant; S, susceptible; NS, nonsusceptible; —, no bacteria isolated.

tween daptomycin-NS and daptomycin-susceptible isolates (not shown). However, repPCR analysis of isolates 1-1 (daptomycin NS; MIC, 4 μg/ml) and 1-2 (daptomycin sensitive; MIC, 0.5 μg/ml) shows a <50% similarity between strains (not shown). Daptomycin and vancomycin MICs were directly correlated (Pearson $r = 0.758$; $P = 0.0043$), whereas no association was found between oxacillin and daptomycin MICs ($P > 0.05$). Time to blood culture positivity was directly correlated with the daptomycin MICs (Pearson $r = 0.727$; $P = 0.0074$).

Ultimately, the patient's transhepatic catheter was replaced, and she was treated with nafcillin-daptomycin combination therapy; bacteremia resolved on hospital day 31.

Case 2. A 48-year-old man with type 2 diabetes, hypertension, and end-stage renal disease presented to an outside hos-

pital with MRSA bacteremia, endocarditis, and multiple pulmonary nodules. The patient was treated with levofloxacin (500 mg i.v. q24h) and vancomycin (1 g i.v. q24h) for 11 days and transferred to our facility for mitral valve replacement. The patient was continued on vancomycin (1 g q24h) for 17 additional days. Following 16 days of vancomycin therapy, 2 of 4 blood culture sets drawn on hospital day 17 yielded MRSA displaying multiple colony morphologies (e.g., pinpoint beta-hemolytic and large beta-hemolytic, both resembling staphylococci) after <24 h of incubation in the BacT/Alert system. Prompted by this finding and our laboratory's previous experiences with the detection of vancomycin-intermediate *S. aureus* (VISA) (4), susceptibility testing was performed on both colony morphologies, which were susceptible to vancomycin, daptomycin, and linezolid (Table 1). Because all other MRSA subsequently isolated from blood was collected within 5 days of the initial blood culture (isolate number 2-2; Table 1), no other routine susceptibilities were performed for this patient. The patient was transitioned to linezolid (600 mg i.v. q12h) on hospital day 18, since it was felt that the recurrent MRSA bacteremia may have been related to seeding from a pulmonary source and would preclude daptomycin treatment. Retrospective testing of isolates 2-1, 2-3, 2-4, 2-5, 2-6, and 2-7 revealed that both isolates 2-1 and 2-6 were resistant to linezolid (MIC = 8 μg/ml), while isolates 2-4 and 2-7 demonstrated an elevated (but susceptible) linezolid MIC of 4 μg/ml (Table 1). Had susceptibility testing been performed on each isolate at the time of recovery from blood, linezolid resistance would have been detected on hospital day 20, as opposed to day 23. Analysis of isolates 2-1 (linezolid resistant) and 2-2 (linezolid sensitive) by repPCR showed a >95% similarity between the strains (not shown), suggesting a clonal origin of resistance. In spite of the presence of linezolid-resistant isolates, the patient's bacteremia resolved on hospital day 19, and he was ultimately transitioned to daptomycin (6 mg/kg q48h) and discharged on a 6-week course of daptomycin. Time to positivity in the BacT/Alert system was directly correlated to the linezolid MIC (Pearson $r = 0.861$; $P = 0.0029$).

Case 3. A 58-year-old man with a history of colorectal cancer, status posthemicolectomy, with a recurrence of liver metastatic disease complicated by biliary stenosis and obstruction following hepatic metastasectomy, end-stage kidney disease, and a recent history of MRSA bacteremia treated with 29 days of vancomycin (1 g q24h) presented to the emergency room with fever and hypotension. MRSA was isolated from blood collected on hospital day 6 (isolate 3-1), and found to be vancomycin susceptible (MIC = 1 μg/ml); however, a vancomycin-intermediate (MIC = 4 μg/ml) isolate was identified on retrospective testing from blood drawn on hospital day 9 (isolates 3-5 and 3-6; Table 1); this vancomycin-intermediate *S. aureus* (VISA) isolate went undetected by the laboratory at the time of isolation, because it was not tested for antimicrobial susceptibilities. Subsequent blood cultures were negative on hospital days 10 and 11, and the patient was discharged to a skilled nursing facility on a 3-week course of vancomycin (1 g q24h). The patient presented to the emergency room at week 7 with MRSA bacteremia. In this instance, VISA was recovered on the day of admission (isolates 3-7 and 3-8; Table 1), and the patient was transitioned to daptomycin (8 mg/kg q48h) and cefepime (1g q24h). A delay of 31 days (from isolate 3-5 to isolate 3-7; Table 1) occurred before the VISA isolate was identified, due to a repeat isolate testing policy. More than 95% similarity between isolate 3-1 (vancomycin sensitive) and 3-5 (vancomycin intermediate) was observed with repPCR analysis (not shown), suggesting that the elevated MIC can be attributed to clonal strains. There was no correlation between vancomycin MICs and time to positivity in the BacT/Alert system for this patient's isolates (Pearson's $r = -0.436$; $P = 0.2802$).

In the balance between quality patient care and resource allocation, antimicrobial susceptibility testing is frequently performed only on a periodic basis when an organism is repeatedly isolated from the same specimen source collected from a patient over multiple days. No formal standards exist to indicate the frequency with which bacterial isolates should be reidentified and retested for antimicrobial susceptibility. Initially susceptible isolates may develop resistance over the course of therapy, since quinolone resistance has been detected within 3 days in staphylococcus species (1). It has been shown that some bacteria, including *S. aureus*, have a propensity to develop antimicrobial resistance following long-term therapy (5, 6) or prolonged persistence in the host, possibly due to selective pressure from the innate immune system (7). In addition, deep-seated infections, such as endocarditis or bacteremia originating from a colonized intravascular line or hardware, are associated with bacterial biofilms from which different bacterial subpopulations or strains (as seen in case 1) with multiple susceptibility profiles may be shed intermittently into the bloodstream (8). As such, susceptibility testing may be warranted on a more frequent basis for these patients.

As these three cases demonstrate, laboratory detection of resistance in MRSA can be challenging, even in patients with a high index of suspicion, such as those with persistent bacteremia while on therapy (9). Further complicating this issue is the lack of a consensus on what constitutes persistent bacteremia: patients with complicated *S. aureus* bacteremia have a median time of 8 or 9 days on treatment before bacteremia clears (10). In these cases, persistent bacteremia does not reflect antimicrobial failure but rather a residual source of infection. Regardless, the most common element associated with decreased susceptibility to daptomycin, vancomycin, and linezolid is previous or current therapy with the respective antimicrobial agent (11–13), and this was documented in all three cases herein.

We present two important findings from this study: (i) identification of a susceptible isolate early in a clinical infection does not exclude later development of resistance to vancomycin, daptomycin, or linezolid, nor the presence of an unrecognized resistant subpopulation in the patient, even within a short time frame (e.g., 1 to 2 days); and (ii) resistant isolates may grow more slowly than susceptible isolates in primary culture. These two findings have important implications for laboratory testing, primarily that antimicrobial susceptibility testing may be considered for every isolate of *S. aureus* recovered from blood. This is especially important for patients with residual sources of infection, such as abscesses, catheters, and end-stage renal disease, where altered pharmacokinetics for certain antibiotics may predispose for the development of resistance (14–17). In our laboratory, we now perform susceptibility testing on every *S. aureus* isolated from blood. This change resulted in an additional 180 susceptibility tests performed in

2012, a 6.4% overall increase in susceptibility tests performed on blood isolates, and an overall 0.08% increase in susceptibility tests performed. While additional testing may be warranted in our laboratory due to the complexity of the patient population, this level of testing may not be required in small hospitals or community laboratories where the patient populations do not have multiple comorbidities. We therefore recommend testing every *S. aureus* isolate from sterile sites in patients who are on long-term antibiotic therapy or who have infections with a high bacterial burden.

It is interesting to note that the daptomycin-NS and linezolid-resistant isolates took longer to grow in blood cultures than did the susceptible isolates in cases 1 and 2. This fitness cost associated with antimicrobial resistance in *S. aureus* is well documented. *vanA* expression by vancomycin-resistant *S. aureus* is associated with significant growth reduction (18), and oxacillin resistance frequently appears as heteroresistance (19–21). Similarly, we have previously documented that VISA take longer to grow in primary bacterial culture and in susceptibility tests than do isolates with vancomycin-susceptible MICs (4), which is likely associated with the metabolic cost of generating a thicker cell wall (22). Daptomycin-NS *S. aureus* may also display a thickened cell wall (23), which may be associated with a longer time to recovery in culture from clinical specimens, as seen in case 1. However, it should be noted that not all daptomycin-NS *S. aureus* isolates express a thickened cell wall, and the growth kinetics of daptomycin-NS *S. aureus* have not been well studied. With regard to linezolid resistance, accumulation of the most frequently described 23S rRNA mutation, G2576T, across multiple gene copies has been shown to result in a successive decline in the biological fitness of *S. aureus* (24).

In all cases, resistant isolates were recovered intermittently from blood (Table 1). This may relate to the inability of the resistant isolates to compete with susceptible isolates in the blood culture. Alternatively, the inoculum used for antimicrobial susceptibility testing may have been inadequate to detect resistant subpopulations. Finally, since all patients had infections with a high bacterial burden (e.g., a colonized transhepatic catheter or endocarditis), it is also tempting to speculate that resistant and susceptible subpopulations were alternately shed from these foci of infection. In particular, for patient 1, two different strains of *S. aureus* were isolated from her blood, but no phenotypic differences between the two strains (daptomycin-S and -NS) were noted. This further highlights the need to test every *S. aureus* isolate from blood, since mixed populations that are not detectable by morphological evaluation may be present. Conversely, identical antimicrobial resistance patterns were observed for the two different morphologies identified in case 3. We have found previously that careful evaluation of colony morphology may help identify resistant subpopulations, and laboratories should be aware that resistant isolates may grow more slowly than susceptible organisms (4). Physicians should be aware of these limitations in the detection of resistance in MRSA and consult with the laboratory to determine if more frequent susceptibility testing is warranted for patients with complications presenting with bacteremia.

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