CASE REPORTS

Bacteremia Due to Clonally Derived Methicillin-Resistant, Gentamicin-Susceptible Isolates and Methicillin-Susceptible, Gentamicin-Resistant Isolates of Staphylococcus aureus

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We report recurrent bacteremia due to mixed infection with two clonally derived isolates of Staphylococcus aureus in a patient with Sezary syndrome. The two isolates, one gentamicin resistant and methicillin susceptible and the other gentamicin susceptible and methicillin resistant, developed by the deletion of the mecA, ant(4')Ia, and aacA-aphD genes from a common gentamicin-resistant and methicillin-susceptible ancestor.

CASE REPORT

Here we report a case of bacteremia in a 63-year-old male patient with a medical history of Sezary syndrome, diagnosed 8 years previously, who was transferred from another institution to the Department of Dermatology at Hospital Universitario Doce de Octubre, Madrid, Spain. At the time of his transfer, the patient had already undergone several rounds of chemotherapy because of his underlying lymphoma. At the time of admission, he demonstrated symptoms of confusion and had chills and fever to 38°C. Upon physical examination, the patient exhibited generalized erythroderma with moderate desquamation and chronic radiodermatitis with multiple surface ulcers. On his right hand, there were many sanious patches that were difficult to remove. Computerized tomography scanning and a thoracic X ray revealed no signs of inflammation or sites of infection. Within the 4 months preceding this presentation, the patient had had two clinical episodes of methicillin-resistant Staphylococcus aureus (MRSA) bacteremia, both of which were treated with at least 2 weeks of therapy. The first was treated with (intravenous [i.v.]) vancomycin and (i.v.) levofloxacin for 18 days, and the second was treated with (i.v.) vancomycin and (i.v.) piperacillin-tazobactam for 15 days. Because the likelihood of recurrence of his bacteremia was deemed to be high, upon presentation, the patient was empirically treated with (i.v.) vancomycin and (i.v.) cefepime. Two sets of blood cultures were taken an hour apart before the initiation of this treatment. The blood was drawn from different peripheral sites, and extensive precautions were taken to avoid contamination because of the poor condition of his skin. Both cultures yielded two isolates of S. aureus with slight differences in colony morphology. The two isolates from both blood cultures showed different antibiotic profiles, one being susceptible to oxacillin and resistant to gentamicin (isolate 7878) (Table 1) and the other homogeneously resistant to oxacillin but susceptible to gentamicin (isolate 7879) (Table 1). This clinical episode was considered to represent bacteremia that probably originated from the patient’s cutaneous lesions. The patient completed a course of antimicrobial treatment with (i.v.) vancomycin and (i.v.) cefepime over a period of 14 days and was discharged in good health without any signs of infection.

In order to understand the origins of the mixed bacteremia involving both gentamicin-resistant and methicillin-susceptible S. aureus (GR-MRSA) and gentamicin-susceptible and methicillin-resistant S. aureus (GS-MRSA), we decided to study the microbiological and molecular characteristics of these isolates as well as others obtained during previous episodes of bacteremia in the same patient (Table 1). All the clinical isolates were subcultured, and their antimicrobial susceptibilities were retested by the disk-agar diffusion method to confirm the susceptibility patterns. In order to determine whether the GR-MRSA and GS-MRSA isolates and those obtained from the previous episodes of bacteremia belonged to the same clonal group, all isolates were characterized by standard pulsed-field gel electrophoresis (PFGE) with the restriction endonuclease Smal. PFGE analysis of the DNA revealed that the isolates exhibited Smal macrorestriction patterns that were indistinguishable except for the loss of a ~225-kb Smal DNA fragment and the concurrent gain of a smaller fragment of ~194 kb in the GR-MSSA variant (Fig. 1A). These data suggested that the most recent GS-MRSA and GR-MSSA isolates were derived from the same parental GR-MRSA strain of the organism involved in the earlier episode of bacteremia. Furthermore, two MRSA isolates (1336 and 7879) were characterized by multilocus sequence typing and staphylo-
coccal cassette chromosome mec typing (6, 11). The results for both isolates were sequence type 125, type IV.

To investigate further, we then used PCR to test all the available isolates for the mecA gene (11) as well as the aacA-aphD and ant(4’)/Ia genes that encode aminoglycoside resistance (12, 13). The PCR results confirmed the phenotypic susceptibility patterns (Table 1). The three isolates obtained before the last episode of bacteremia exhibited identical antimicrobial susceptibility patterns, and all three resistance genes were detected. With regard to the two isolates obtained from the most recent episode of bacteremia, the GS-MRSA isolate (7879) possessed both the mecA and the ant(4’)/Ia genes but the GR-MSSA isolate (7878) carried only the aacA-aphD gene (Table 1).

To understand the evolution of these isolates and to determine the significance of the change in PFGE patterns, we proceeded to conduct Southern blot hybridizations of the PFGE-separated SmaI DNA fragments. The pulsed-field gel was blotted onto a Hybond N+/H11001 membrane which was probed with PCR-amplified mecA-, aacA-aphD-, and ant(4’)/Ia-specific probes (11–13). Hybridization was visualized by exposing the blots to photographic films for various time periods. For all MRSA isolates, the mecA and ant(4’)/Ia sequences were present on the same ~225-kb SmaI macrorestriction fragment that was absent from the GR-MSSA variant (Fig. 1B and D). The aacA-aphD gene probe hybridized to the ~339.5-kb fragment from all gentamicin-resistant isolates but not the GS-MRSA strain (Fig. 1C), thus confirming the absence of the bifunctional aacA-aphD modifying enzyme in this organism. As has been reported in other studies, the loss of the aacA-aphD gene did not yield any apparent change in the PFGE patterns (Fig. 1A) because PFGE analysis is not sensitive enough to detect the deletion of small fragments (9). The aacA-aphD gene in gram-positive cocci is known to be flanked by inverted copies of the insertion sequence IS256, forming the 4,566-bp transposon Tn4001 (3). The well-known instability of this element probably provoked a deletion in the genome of the GR-MRSA isolate, giving rise to a new GS-MRSA population (2, 9, 10, 14). Resistance to tobramycin in this new GS-MRSA strain was preserved through the conservation of the ant(4’)/Ia gene (2, 9, 10, 14). Our results confirmed the simultaneous deletion of a fragment containing the mecA and the ant(4’)/Ia genes together with the deletion of the aacA-aphD gene, producing two new

### Table 1. Characteristics of clinical isolates obtained from a patient with recurrent bacteremia

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sample</th>
<th>Date of isolation (mo/day/yr)</th>
<th>Resistance pattern</th>
<th>Result of PCR test for: mecA</th>
<th>aacA-aphD</th>
<th>ant(4’)/Ia</th>
</tr>
</thead>
<tbody>
<tr>
<td>2878</td>
<td>Blood</td>
<td>12/17/2002</td>
<td>Met, Gen, Tob, Lev, Eri, SXT, Mup</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>3618</td>
<td>Naris specimen</td>
<td>12/23/2002</td>
<td>Met, Gen, Tob, Lev, Eri, SXT, Mup</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>1336</td>
<td>Blood</td>
<td>01/10/2003</td>
<td>Met, Gen, Tob, Lev, Eri, SXT, Mup</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>0595</td>
<td>Wound specimen</td>
<td>01/11/2003</td>
<td>Met, Gen, Tob, Lev, Eri, SXT, Mup</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6206</td>
<td>Naris specimen</td>
<td>02/10/2003</td>
<td>Met, Gen, Tob, Lev, Eri, SXT, Mup</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>7879</td>
<td>Blood</td>
<td>04/23/2003</td>
<td>Met, Tob, Lev, Eri, SXT, Mup</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>7878</td>
<td>Blood</td>
<td>04/23/2003</td>
<td>Gen, Tob, Lev, Eri, SXT, Mup</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
</tr>
</tbody>
</table>

*Met, methicillin; Gen, gentamicin; Tob, tobramycin; Lev, levofloxacin; Eri, erythromycin; SXT, cotrimoxazole; Mup, mupirocin; Pos, positive; Neg, negative; NA, not available.*

![FIG. 1. Images from PFGE (A) and from Southern blotting and hybridization with mecA (B), aacA-aphD (C), and ant(4’)/Ia (D) probes. Lane 1 in panel A contains the molecular mass marker (lambda ladder). Lanes: 2, isolate 2878 (GR-MRSA); 3, isolate 1336 (GR-MRSA); 4, isolate 6206 (GR-MRSA); 5, isolate 7879 (GS-MRSA); and 6, isolate 7878 (GR-MSSA).](http://jcm.asm.org/)

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populations which had originated directly from the same ancestor.

In *S. aureus*, resistance to methicillin is encoded by the mecA gene, which is carried on a mobile genetic element termed the staphylococcal cassette chromosome mec. Different studies have demonstrated that the genetic instability of this element can result not only from the absence of antibiotic pressure (7) but also from vancomycin-induced stress that has been shown in vitro to lead to the deletion of mecA (1).

Although reports of similar cases involving the in vivo deletion of the mecA region have already been published (4, 5, 7, 8), to the best of our knowledge this is the first report of a case of recurrent *S. aureus* bacteraemia in which such a deletion of the mecA, ant(4′)A, and aacA-aphD genes has taken place to produce two distinct populations of GR-MSSA and GS-MRSA with the same GR-MRSA ancestor. Our patient had received several courses of antimicrobial therapy, including vancomycin, for three clinical episodes of recurrent bacteremia in a 4-month period. In vitro studies have shown that vancomycin-induced stress can lead to the deletion of mecA in some strains of MRSA (1). The protracted duration of antimicrobial therapy in this immunosuppressed patient could therefore have provoked these deletions. The frequency with which simultaneous infections with mixed variants of MRSA and MSSA occur is unknown. The clinical implications of infection with a mixture of susceptible and resistant variants of *S. aureus* are serious if the condition is not recognized in a timely manner and the appropriate therapy is not provided. The administration of inappropriate treatment may lead to selective growth of the resistant population and severe complications for the patient. Our observations highlight the importance of considering the evolution of *S. aureus* strains in immunosuppressed patients with recurrent MRSA infections in which the bacteria are under prolonged selective pressure from antimicrobial agents.

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