Elution of rabies virus RNA from the filter paper was performed by rocking each dried brain spot in 9 mL of lysis buffer (NucliSens; BioMerieux) at room temperature with a rotator-mixer (rotor size, 60; BioSan) at 60 rpm for a period of 2 h. After the filter paper was removed from the buffer solution, RNA extraction was performed in accordance with a silica-guanidine thiocyanate protocol described elsewhere [4]. Dried brain spots were examined for rabies virus RNA on days 7, 14, 21, 28 and 222 of storage by NASBA and RT-PCR analysis with or without nested primers (LISEBL1F and LISEBL2R), as described elsewhere [4, 5].

All of the rabies-infected brain samples tested positive for rabies virus by NASBA and RT-PCR at all time points. However, nested primers were required to detect rabies virus RNA in samples on day 222. NASBA and RT-PCR analyses of control samples were negative for rabies virus.

Since clinical diagnosis can be unreliable, brain tissue should be used in the postmortem diagnosis of rabies [6, 7]. Although RT-PCR can identify rabies virus RNA in naturally decomposed brains, the time limit for detection may be variable [2, 8]. The use of the filter paper technique we have described may provide further advantage, since specific results can be obtained for samples stored for 222 days. Samples of brain stem should also be included [6]. The length of the amplification product may also be crucial. Product lengths in our study were 180 and 260 base pairs by NASBA and RT-PCR, respectively. Selection of appropriate primer sets is valuable, not only for diagnostic purposes, but also for identification of the viral genotype or variant [5, 9]. The dried brain spot can be shipped in an envelope to a diagnostic laboratory for NASBA or RT-PCR analysis. Although this technique may not aid treatment, it should maximize the reliability of epidemiological data.

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**References**

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**Persistent MRSA Bacteremia in a Patient with Low Linezolid Levels**

Sr—We read with interest the report by Ruiz et al. [1] of 2 cases of endocarditis caused by methicillin-resistant *Staphylococcus aureus* (MRSA) that failed to respond to therapy with intravenous linezolid. Failure of therapy due to the development of linezolid resistance has been reported by Tsiodras et al. [2], but the 2 patients described by Ruiz et al. [1] had isolates that remained susceptible to linezolid. At the 2001 Infectious Diseases Society of America annual meeting, we described a patient who had persistent MRSA bacteremia and low linezolid blood levels during both oral and intravenous linezolid therapy [3]. A 49-year-old man, who weighed 85 kg and had a history of insulin-dependent diabetes mellitus, coronary artery disease, hepatitis C, and intravenous drug abuse, and who was receiving oral methadone at the time of admission, initially had cellulitis of the toe, with drainage that grew MRSA on culture. He developed a femoral pseudoaneurysm infected with MRSA, which required an axillopopliteal bypass graft and subsequent revision with a ring Gore-Tex graft (W. L. Gore & Associates). Because of a rash due to vancomycin, treatment with linezolid, 600 mg po q12h, was initiated. After 2 weeks, the patient developed renal insufficiency that required dialysis. (Subsequent antibiotic doses were not administered immediately prior to the start of dialysis.) Therapy was changed to quinupristin/dalfopristin, 700 mg iv q12h, to complete a 4-week total treatment course.

Two weeks after completion of the first quinupristin/dalfopristin course, the pa-
tient developed tenderness at the dialysis catheter site and purulence at the pseudoaneurysm site. Cultures of blood samples, fluid from the pseudoaneurysm site, and the dialysis catheter were positive for MRSA. Treatment with quinupristin/dalfopristin, 700 mg iv q8h, was restarted. Blood cultures were positive for MRSA at day 4 but negative at day 7 of the quinupristin/dalfopristin course. Eight days after quinupristin/dalfopristin therapy was restarted, a new rash appeared, and quinupristin/dalfopristin was changed to linezolid, 600 mg po q12h, to complete a 3-week total treatment course. A transeosophageal echocardiogram (TEE) revealed no vegetations. A renal biopsy revealed crescentic glomerulonephritis. Methylprednisolone succinate therapy was started, and, 2 weeks later, blood cultures were again positive for MRSA, and the dialysis catheter site was tender. Linezolid therapy, 600 mg iv q12h, was initiated. Both the catheter tip and specimens from the catheter site grew MRSA on culture. Because of poor venous access, the linezolid dosage was changed to 600 mg po q12h.

Blood cultures were persistently positive, despite catheter removal and 3 weeks of oral linezolid therapy. A second TEE revealed no vegetations. Radiographic evaluation for a graft infection by CT scan and WBC-labeled nuclear medicine scan yielded inconclusive results. The patient and surgeon declined surgical intervention. Susceptibility testing confirmed that the isolate was sensitive to linezolid (MIC, <4 μg/mL; diffusion disk inhibition zone diameter, >21 mm). A Schlichter test performed during oral linezolid therapy at the time of peak drug levels revealed the lack of bacteriostatic or bactericidal activity at a serum dilution of 1:2. Because of persistent bacteremia, linezolid levels were measured by high-pressure liquid chromatography assay (see table 1). Linezolid blood levels were also measured subsequently, while the patient was receiving linezolid at a dosage of 600 mg iv q12h and when the dosage was increased to 900 mg iv q12h. The patient was eventually treated with linezolid, 900 mg iv q12h, for 5 weeks, with resolution of bacteremia.

In this patient, a vascular graft infection may have led to persistent MRSA bacteremia. Since the MIC₉₀ of linezolid for S. aureus is usually in the range of 2–4 μg/mL (the MIC of linezolid for the isolate obtained from the patient was <4 μg/mL by disk diffusion), the low levels achieved in the patient during both oral and intravenous linezolid therapy at a dosage of 600 mg q12h may have contributed to his persistent bacteremia. It is unclear why the linezolid levels were low. Dialysis may remove ~30% of a dose, but for this patient the drug was not administered immediately prior to the start of dialysis. Low linezolid levels such as those we observed, although uncommon, may have contributed to the results described by Ruiz et al. [1]. In patients who do not respond to linezolid therapy, it may be prudent to check linezolid levels. At this time, we would not recommend use of higher dosages or decreased dosage intervals without further study.

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References

Table 1. Linezolid blood levels achieved at various dosages in a patient treated with linezolid every 12 hours.

<table>
<thead>
<tr>
<th>Level achieved (expected, mean ± SD), μg/mL, by dosage</th>
<th>600 mg po</th>
<th>600 mg iv</th>
<th>900 mg iv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peakᵃ</td>
<td>1.73 (21.20 ± 5.78)</td>
<td>3.15 (15.10 ± 2.52)</td>
<td>9.14</td>
</tr>
<tr>
<td>Troughᵇ</td>
<td>0.10 (6.15 ± 2.94)</td>
<td>Trace (3.68 ± 2.36)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**NOTE.** High-pressure liquid chromatography assay was performed at the National Jewish Medical and Research Center, Denver, Colorado.

ᵃ Sample was obtained immediately after completion of administration of intravenous dose and 2 h after administration of oral dose.
ᵇ Sample was obtained just prior to administration of dose.