Analysis of 42 Cases of Septicemia Caused by an Epidemic Strain of Methicillin-Resistant Staphylococcus aureus: Evidence of Resistance to Vancomycin

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Recent case reports of vancomycin treatment failures in the United States, Japan, and France have prompted a retrospective analysis of 42 cases of septicemia caused by epidemic methicillin-resistant Staphylococcus aureus strain 15 (EMRSA-15), which is the most prevalent epidemic strain of methicillin-resistant S. aureus in the United Kingdom; all cases occurred in a teaching hospital in Manchester, United Kingdom, between 1994 and 1998. Mortality was lowest (4%) in patients with rifampin-susceptible isolates treated with vancomycin and rifampin. It rose to 38% in patients who were treated with both antibiotics but in whom the organism became resistant to rifampin during therapy, and it reached 78% in patients who had rifampin-resistant isolates or in whom rifampin was contraindicated (P < .0001; Fisher exact test, 2-tailed). All isolates were susceptible to vancomycin by conventional laboratory testing, but susceptibility was lost by growth in vancomycin in vitro, becoming resistant at a minimum inhibitory concentration of 8 mg/L. This was associated with accumulation of cell-wall material. The deoxyribonucleic acid fingerprint remained unchanged. This study suggests that rifampin played a key role in the prevention of deaths caused by an epidemic strain of methicillin-resistant S. aureus that readily gave rise to a subpopulation with reduced susceptibility to vancomycin.

Antimicrobial resistance is a major threat to public health [1]. Methicillin-resistant strains of Staphylococcus aureus (MRSA) are of particular concern because of their virulence and resistance to multiple antibiotics [2]. Since emerging in the late 1970s, these bacteria have become increasingly prevalent as nosocomial pathogens. For >30 years, they remained fully susceptible to the glycopeptide class of antibiotics—vancomycin and teicoplanin—which became the mainstay of treatment [3, 4]. Recently, there have been several case reports of failure of vancomycin treatment in Japan, the United States, and France [4–8]. The MRSA that were isolated were of intermediate resistance to vancomycin, with an MIC of 8 mg/L required to inhibit growth on laboratory medium. The similarity between early, susceptible isolates and later, more resistant isolates from the same patient, as shown by antibiotic susceptibility patterns and DNA fingerprinting, suggested that the more resistant isolates were derived from the earlier isolates and that they had been selected out by vancomycin during its evolution.

Despite widespread attempts to control the spread of infection [3, 9], by 1992, >40% of S. aureus infections in large hospitals in the United States were resistant to methicillin [1]. In a recent survey, S. aureus accounted for 16% of nosocomial bloodstream infections, second only to coagulase-negative staphylococci; 29% of these isolates were MRSA [10]. In England and Wales, the reported incidence of S. aureus bacteremia increased from 6010 in 1994 to 10,237 in 1998, with the proportion caused by MRSA increasing 4-fold [11]. Among blood culture and CSF isolates, resistance to methicillin was stable at ~1.5% during 1989–1991, but it increased thereafter to 13.3%, and resistance was associated with a significant increase in the percentage of isolates resistant to other commonly used antibiotics [12].

Because of their propensity to spread, certain strains have come to be called “epidemic MRSA,” or “EMRSA,” in the United Kingdom. When epidemic strains spread in >2 hospitals, they were numbered sequentially. EMRSA-1 was the most prevalent strain found in 1990, but, by 1997, occurrences of the strains EMRSA-15 and EMRSA-16 had increased dramatically. They are now the 2 most prevalent strains in the country; EMRSA-15 affects patients in 167 hospitals, and EMRSA-16 affects patients in 142 hospitals [9]. In a large teaching hospital (the Central Manchester Healthcare Trust) in cen-
rifampin-susceptible isolates that were treated with vancomycin and rifampin (table 1), 8 had rifampin-susceptible isolates that became resistant during therapy with vancomycin and rifampin (table 2), and 9 either had rifampin-resistant isolates (2 patients) or did not receive rifampin because of impaired liver function (7 patients) (table 3).

Rifampin, 0.45 g q12h, was given orally or, if this was not possible, intravenously. The conventional adult regimen for vancomycin was used, with 1 g administered by infusion for 60 min every 12 h for patients aged <70 years [15]. The dose was reduced to 750 mg for those aged >70 years, and the dosing interval was increased to every 24–48 h for patients with reduced creatinine clearance. A predose serum level was checked before the fourth or fifth dose and thereafter at intervals of 2–3 days, assayed by the TDx method (Abbott Laboratories, Maidenhead, UK). Dosage was adjusted to keep the predose (trough) level within 5–10 mg/L; if the level was >10 mg/L, the interval between doses was increased. Peak concentrations were expected to be 25–35 mg/L, although analysis of the available data suggests that the drug’s bactericidal activity is independent of its concentration [15, 16].

Characterization and virulence testing. Serial passage of rifampin-susceptible (A) and rifampin-resistant (C) EMRSA-15 isolates in nutrient broth with increasing vancomycin (Sigma Chemical, Poole, UK; dose, 1–30 mg/L, in 1-mg/L increments) [17, 18] produced, respectively, rifampin-susceptible (B) and rifampin-resistant (D) isolates with increased resistance to vancomycin (MIC, 8 mg/L; by E test and broth dilution) [14]. Each was further characterized by phage susceptibility testing [13], genetic fingerprinting (Smal digestion followed by pulsed-field gel electrophoresis), population analysis, and transmission electron microscopy, as previously described [6, 7, 17]. To compare virulence, adult female CD1 mice (Charles River, Maidstone, UK) were given a 100-μL intravenous bolus containing 6 × 10^6 cfu of EMRSA-15 A, B, C, or D (n = 25). Mortality was monitored for up to 28 days; cause of death was confirmed by culture of kidney, liver, and spleen.

Results

Patient mortality. Mortality was lowest among patients with rifampin-susceptible EMRSA septicaemia treated with vancomycin and rifampin (mortality, 4% [1 of 25 patients]). It in-

Table 2. Patients with septicemia caused by epidemic methicillin-resistant *Staphylococcus aureus* strain 15 (EMRSA-15) who were initially susceptible to rifampin but became resistant.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Underlying condition</th>
<th>Antibiotic therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Neuroleptic malignancy</td>
<td>Vm, Rif</td>
<td>Died (24 d)</td>
</tr>
<tr>
<td>27</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>Vm, Rif</td>
<td>Died (6 d)</td>
</tr>
<tr>
<td>28</td>
<td>Diabetes mellitus</td>
<td>Vm, Rif</td>
<td>Died (10 d)</td>
</tr>
<tr>
<td>29</td>
<td>Huntington’s chorea, atypical mycobacterial abscess</td>
<td>Azm, Rif, Vm</td>
<td>Survived</td>
</tr>
<tr>
<td>30</td>
<td>Strangulated hernia</td>
<td>Vm, Rif</td>
<td>Survived</td>
</tr>
<tr>
<td>31</td>
<td>Carcinoid tumor</td>
<td>Vm, Rif</td>
<td>Survived</td>
</tr>
<tr>
<td>32</td>
<td>Renal transplant</td>
<td>Vm, Rif</td>
<td>Survived</td>
</tr>
<tr>
<td>33</td>
<td>Chronic renal failure</td>
<td>Vm, Rif</td>
<td>Survived</td>
</tr>
</tbody>
</table>

NOTE. Azm, azithromycin; Rif, rifampin; Vm, vancomycin.

* The number of days from initial positive blood culture to death is indicated in parentheses.

### Patients and Methods

Patients and isolates. All 42 patients were hospitalized at the Central Manchester Healthcare Trust, Manchester, United Kingdom, with blood-culture-confirmed septicemia caused by the strain EMRSA-15 and identified by use of standard techniques (e.g., pigmentation, biochemistry, antibiogram, phage reactions, toxin production, and pulsed-field gel electrophoresis) [13]. Phage typing was performed by the Public Health Laboratory, Manchester. By means of conventional disk-susceptibility testing [14], all isolates were found to be susceptible to vancomycin but resistant to erythromycin, ciprofloxacin, and clindamycin. Twenty-five patients had
creased to 38% (3 of 8) among patients who were treated with both antibiotics but in whom the organism became rifampin resistant during therapy. It reached 78% (7 of 9) among patients who had rifampin-resistant isolates or in whom rifampin was contraindicated, a highly statistically significant increase ($P < .0001$; Fisher exact test, 2-tailed). For some patients (patients 30, 38, and 40), blood cultures remained positive for 10–28 days, despite therapeutic levels of vancomycin, as determined by serum assay and by changing the peripheral lines. Peripheral lines were present in all patients and were replaced either every 72 h or when they were obviously infected. Among the fatal cases, central lines were present in patients 27 and 39.

**Characterization and virulence testing.** Passage of EMRSA-15 A and C through increasing concentrations of vancomycin produced subclones B and D, which had intermediate resistance to both vancomycin and teicoplanin; MIC increased from 1 mg/L to 8 mg/L (vancomycin) and from 1 mg/L to 16 mg/L (teicoplanin), respectively. The MIC was stable on repeated subculture. Population analysis profiles showed no evidence of a vancomycin-resistant subpopulation in EMRSA-15 subclones A or C, with all bacteria ceasing to grow at vancomycin concentrations >2 mg/L. Population analysis of subclones B and D showed heterogeneous vancomycin-resistant phenotypes, with bacteria growing at concentrations up to 8 mg/L. Subclones B and D were indistinguishable from subclones A and C on pulsed-field gel electrophoresis (figure 1). Transmission electron microscopy showed accumulation of cell-wall components in subclones B and D, compared with subclones A and C (figure 2). Although A and C were typeable by phage 75, a characteristic feature of EMRSA-15, B and D were non-typeable.

Among mice, the mortality induced by the more resistant subclones (B and D) was significantly lower than that caused by the parent vancomycin-susceptible strains (A and C): 24-h mortality rates were 90% (A), 94% (C), 0% (B), and 20% (D). Subclones A and C, compared with subclones B and D, produced statistically significant differences ($P < .05$; Fisher exact test, 2-tailed).

**Discussion**

EMRSA-15, a virulent strain of MRSA that is the most prevalent of such strains in the United Kingdom, appears to be fully susceptible to vancomycin by conventional laboratory

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**Table 3.** Patients with septicemia caused by epidemic methicillin-resistant *Staphylococcus aureus* strain 15 (EMRSA-15) for which the organism was resistant to rifampin or for which rifampin was not given because of clinical contraindications.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Underlying condition</th>
<th>Rifampin susceptibility</th>
<th>Antibiotic therapy</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>Fractured tibia, cerebrovascular accident</td>
<td>R</td>
<td>Vm, Cfur</td>
<td>Died (1 d)</td>
</tr>
<tr>
<td>35</td>
<td>Diabetes mellitus</td>
<td>R</td>
<td>Vm, Rif</td>
<td>Died (1 d)</td>
</tr>
<tr>
<td>36</td>
<td>Cerebrovascular accident</td>
<td>S</td>
<td>Cpfx, Vm</td>
<td>Died (3 d)</td>
</tr>
<tr>
<td>37</td>
<td>Chronic liver and renal failure</td>
<td>S</td>
<td>Vm</td>
<td>Died (9 d)</td>
</tr>
<tr>
<td>38</td>
<td>Posterior communicating aneurysm</td>
<td>S</td>
<td>Vm</td>
<td>Died (14 d)</td>
</tr>
<tr>
<td>39</td>
<td>Aplastic anemia, liver failure</td>
<td>S</td>
<td>Vm</td>
<td>Died (12 d)</td>
</tr>
<tr>
<td>40</td>
<td>Alcoholic cirrhosis</td>
<td>S</td>
<td>Vm</td>
<td>Died (28 d)</td>
</tr>
<tr>
<td>41</td>
<td>Budd-Chiari syndrome</td>
<td>S</td>
<td>Vm</td>
<td>Survived</td>
</tr>
<tr>
<td>42</td>
<td>Alcoholic cirrhosis</td>
<td>S</td>
<td>Vm</td>
<td>Survived</td>
</tr>
</tbody>
</table>

*NOTE.* Cfur, cefuroxime; Cpfx, ciprofloxacin; R, resistant to rifampin; Rif, rifampin; S, susceptible to rifampin; Vm, vancomycin.

* The number of days from initial positive blood culture to death is indicated in parentheses.
Vancomycin treatment failures are usually attributed to the patient’s underlying disease, rather than to clinical resistance to vancomycin. However, in this study, the success of vancomycin for the treatment of septicemia caused by EMRSA-15 was strongly associated with cotherapy with rifampin. In the absence of rifampin or in instances in which the EMRSA-15 was rifampin resistant, mortality increased from 4% to 78%. This suggests that the success of vancomycin for the treatment of EMRSA-15 septicemia was critically dependent on the presence of rifampin. It must be noted that, for patients 34, 35, and 36, death was rapid and may have occurred even if the strain had been susceptible to rifampin or if the drug had been given. The in vitro data showed that EMRSA-15, although appearing to be fully susceptible to vancomycin by conventional laboratory tests, was capable of producing subclones with reduced susceptibility to vancomycin. If such subclones emerged among seriously infected patients receiving vancomycin, it could account for the need for a second antibiotic, such as rifampin, to achieve elimination of the infection.

During a 9-month period, serial passage of the vancomycin-susceptible EMRSA-15 in medium containing vancomycin led to a marked increase in resistance. Both rifampin-susceptible and rifampin-resistant variants of EMRSA-15 acquired intermediate resistance to vancomycin and teicoplanin. Susceptibility to phage 75 was lost, but DNA fingerprinting showed genetic similarity between all 4 strains. Accumulation of cell-wall components was evident on electron microscopy, a feature typical of MRSA with reduced susceptibility to vancomycin, either produced in vitro by serial passage through vancomycin [17, 18] or isolated from patients [6, 7]. Loss of phage typing has also previously been observed after acquisition of vancomycin resistance [19]. Activation of cell-wall synthesis, re-
organization of the cell wall, and increased production of penicillin-binding proteins have all been implicated as possible mechanisms underlying vancomycin resistance [19–22].

The blood culture isolates of EMRSA-15 were fully susceptible to vancomycin, according to standard laboratory tests, and did not display heterogeneous resistance to vancomycin in a population analysis. In this respect, EMRSA-15 differed from the previously described isolates of intermediate resistance, obtained from patients for whom vancomycin treatment had failed, which had an MIC of 8 mg/L and which showed heterogeneous resistance on population analysis [5–7]. Hiramatsu et al. [23] examined >1000 clinical isolates of MRSA from Japan and found that the prevalence of heterogeneous resistance varied from 1.3% to 20% in different hospitals. There have also been sporadic reports of vancomycin-tolerant strains of *S. aureus*, particularly MRSA, which are thought to be responsible for some treatment failures [24]. These isolates are readily inhibited by vancomycin but show increased resistance to killing; therefore, the MBC is high (32 mg/L) but the MIC remains low (2 mg/L). In the case of the Tupelo strain of *Streptococcus pneumoniae*, lysis-kill curves demonstrated vancomycin tolerance, whereas the MIC was 0.5 mg/L [25].

To our knowledge, the pathogenicity of vancomycin-resistant subclones in animal models of infection has not been previously examined. The more resistant subclones were found to be significantly less virulent after intravenous challenge than were the parent EMRSA-15 strains. Because tissue penetration of vancomycin is often poor [26], resistant subclones are more likely to arise sequestered in the tissues, rather than in the bloodstream. Perhaps once they are established in a patient’s tissues, their contribution to lethality is as a persistent source of infection, rather than intrinsic virulence. A similar concept has been advanced to explain a persistent peritoneal dialysis infection caused by a methicillin-resistant strain of *Staphylococcus epidermidis*, despite virtually continuous vancomycin therapy [27].

This study shows that the epidemic strain EMRSA-15 could give rise to subclones with increased resistance to vancomycin (MIC, 8 mg/L). This appears to be of clinical significance because in the absence of cotherapy with rifampin, mortality increased from 4% to 78%. Conventional laboratory testing could not detect the ability of vancomycin-susceptible EMRSA-15 to give rise to vancomycin-resistant subclones, because this required serial passage in vancomycin. In well-documented cases of vancomycin treatment failures from the United States, Japan, and France, the isolates were of intermediate resistance to vancomycin (MIC, 8 mg/L) [5–8], a level of resistance easily missed by conventional disk diffusion susceptibility testing and microtiter assay [14]. We suggest that a more easily detectable marker of clinical outcome in life-threatening infections is the organism’s susceptibility to other antibiotics, particularly rifampin. This is a cidal antibiotic with good tissue penetration and a significant impact on mortality. At present, the great majority of MRSA isolates are susceptible to rifampin [28]. It is essential that we monitor for loss of susceptibility not only to vancomycin but, also, to rifampin; and it is also essential that rigorous cross-infection control measures be instigated against strains of MRSA that are resistant to rifampin, to prevent their spread.

Acknowledgment

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References