Efficacy of Short Courses of Oral Novobiocin-Rifampin in Eradicating Carrier State of Methicillin-Resistant Staphylococcus aureus and In Vitro Killing Studies of Clinical Isolates

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Methicillin-resistant Staphylococcus aureus (MRSA) is an important nosocomial infection problem. Colonization appears to be more common than invasive disease. Eradication of colonization or the carrier state could limit the spread of MRSA, thus reducing the potential for mortality and morbidity in other patients. The detection of patients with MRSA infection in a rehabilitation ward led to a study of the combination of novobiocin-rifampin in vivo and in vitro. We found that 300 mg of rifampin plus 500 mg of novobiocin orally twice daily for 5 days, in 18 courses of treatment given to 12 patients, resulted in the clearing of MRSA in 79% of the evaluable courses and 81% of the evaluable sites. A second course cleared MRSA from one of the patients with a treatment failure. Side effects were not noted. All 18 pretherapy isolates were susceptible to either drug in vitro, but 1 of 2 posttherapy isolates was rifampin resistant. Timed-kill studies demonstrated that the rate of killing was the same with either drug alone or both drugs together. Pretherapy isolates from treatment successes or failure were killed at the same rate by the drug combination. However, with the rifampin-resistant isolate killing ceased after 48 h. Results of this study suggest that previously untreated patients are likely to have isolates that are susceptible to the combination of drugs and that the combination is commonly effective in eradicating MRSA carriage. Since the regimen is orally administered, and thus convenient, in conjunction with other measures it has the promise of reducing the spread of MRSA in hospitals.

Staphylococcal resistance to semisynthetic penicillins was first reported in Great Britain (2), but it did not become a significant problem in the United States until the mid-1970s (6, 9, 15). Since then, methicillin-resistant Staphylococcus aureus (MRSA) has been recognized with increasing frequency as a human pathogen. MRSA has been implicated in outbreaks of nosocomial infections in different parts of the world (14, 16).

Although isolation of affected patients is likely the single most important measure to control the spread of MRSA, systemic or local antibiotic therapy is sometimes necessary to treat MRSA carriers, especially if they are to be hospitalized for some period. Successful treatments would also decrease their risk of invasive MRSA disease.

Topical treatment with antiseptics has been proposed as treatment for the carrier state (3), but it commonly does not work (14). There are few drugs with anti-MRSA activity that are available for systemic use, and some drugs, when used in combination, have been found to be antagonistic. Some of these drugs, such as vancomycin, need to be given parenterally, are expensive, do not penetrate well to noninfamed mucosal surfaces, have significant toxicity, or have combinations of these deficiencies, which makes contemplation of their use for only the carrier state problematic. Furthermore, there is a concern in using the few active drugs to eliminate the carrier state because MRSA may develop resistance to them, thus abrogating their use in the treatment of serious MRSA infections.

The persistence of patients with MRSA in a rehabilitation ward enabled us to study the efficacy of short courses of the combination of novobiocin and rifampin in clearing MRSA from colonized sites. The two drugs were chosen because of the ease of oral administration; similar half-lives in serum, which allowed convenience of dosing; good penetration and absorption (19); and their activity in vitro against MRSA (4, 7, 17). They were used together because of the tendency of bacteria to develop resistance to each of these antibiotics when they are used alone (8, 13, 18), in an effort to diminish this problem.

(Materials and Methods)

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Patients. The index case of infection in a patient who was transferred to the rehabilitation ward following an injury in competitive motorcycle racing in Europe and hospitalization in Paris, France, was detected by obtaining a sample for culture from a site suspected to be infected. Infections in subsequent patients were detected by obtaining samples for surveillance cultures from patients on rehabilitation wards. For all patients who entered into our treatment protocol, samples for culture were obtained from wounds; nares and nasopharynx; sputum, throat, or tracheostomy; stool; and any other purulent sites just before the initiation of treatment. Patients were deemed carriers when samples from these sites were positive for MRSA on culturing; this required the absence of invasive disease requiring prolonged

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therapy (in contrast to the protocol, which was directed against colonization). Entry into this protocol required consent of the patient and the patient’s primary care physician. All patients who entered the treatment protocol are reported here. Complete blood counts and an automated chemistry panel (SMA-20) were obtained prior to therapy. Treatment consisting of 300 mg of rifampin plus 500 mg of novobiocin was then given orally twice daily for 5 days; these doses were suggested from results of previous studies of each drug alone (1, 10, 13). The protocol required that all sites that were positive for MRSA therapy be recultured on phenylethyl alcohol agar with 5% (vol/vol) sheep erythrocytes (BBL Microbiology Systems, Cockeysville, Md.) at least 48 h and not more than 10 days after the end of the 5-day regimen and while the patient remained off other antibiotics and in modified “contact isolation.” This consisted of a private room, with all treatments and procedures done in the room whenever possible. When this was not possible, the patient was transported in isolation, and often, special arrangements could be made (e.g., an MRSA-infected patient would be the last one served that day, just prior to extensive cleaning of the treatment or procedure area). Hematology and chemistry studies were repeated after treatment. A treatment success was defined as the absence of MRSA at previously positive sites on the follow-up culture.

Organisms. Patient specimens for culture were plated onto sheep blood agar (BBL Microbiology Systems) and phenylethyl alcohol. S. aureus was identified by colony morphology, Gram stain, catalase test, and clumping factor test by using Staphylo Slide (BBL Microbiology Systems). Methicillin resistance was defined for all isolates in part by disk susceptibility testing, viz., a zone size of ≤10 mm with a 1-μg oxacillin disk at 24 h of incubation (11). All isolates produced growth up to the disk.

Antibiotics. Novobiocin (The Upjohn Co., Kalamazoo, Mich.) and rifampin (CIBA-Geigy Corp., Summit, N.J.) were supplied in powder form for in vitro testing. The stock solution of rifampin was prepared by dissolving powder in 1 ml of methanol and diluting it with 9 ml of distilled water to produce a 250-μg/ml solution. Novobiocin was dissolved in distilled water to produce a solution of 500 μg/ml. The stock solutions were divided and frozen at −70°C.

Microdilution studies. The MicroScan system (Baxter Healthcare Corp., North Sacramento, Calif.) and the MIC panels as prepared by the manufacturer were used to determine the MIC of nafcillin or oxacillin. Two different MIC panels were used; one contained nafcillin (not supplemented with 2% NaCl); the other contained oxacillin (supplemented with 2% NaCl). In addition to the disk testing definition of “methicillin resistance” described above, the remainder of the definition included a nafcillin MIC of ≥2.0 μg/ml and an oxacillin MIC of ≥4.0 μg/ml. Our criteria for resistance were intended to be rigorous and were verified by several methods. For microdilution testing, a no. 0.5 McFarland standard suspension was prepared in Mueller-Hinton broth (BBL Microbiology Systems) from an 18- to 24-h growth of organisms on sheep blood agar. The suspension was diluted according to the recommendations of the MicroScan manufacturer (0.5 ml in 25 ml of 0.2% Tween 80 in water) and was inoculated with the inoculator tray. The plates were examined after 24 h of incubation at 35°C.

Macrodilution studies. The stock solutions of antibiotics were diluted in Mueller-Hinton broth (BBL Microbiology Systems) supplemented with Ca²⁺ and Mg²⁺ (MHBS) according to the methods of the National Committee on Clinical Laboratory Standards (12). The dilution ranges assayed were 0.06 to 4 μg/ml for rifampin (with tubes up to 62.5 μg/ml for one resistant isolate) and 0.25 to 4 μg/ml for novobiocin. A no. 0.5 McFarland standard suspension of organisms was prepared with MHBS from growth on sheep blood agar for 18 to 24 h at 35°C and was then diluted 1:200 in the broth. Portions of 1 ml were inoculated into tubes containing 1-ml portions of the antibiotic dilutions. The tubes were incubated at 35°C for 24 h.

Time-kill studies. The stock solutions were diluted in MHBS to yield a final concentration of 1.0 μg of novobiocin per ml, 0.25 μg of rifampin per ml, or a combination of these. These concentrations were arbitrarily selected as four times the conventional MIC of each drug; in addition, they represented achievable concentrations in serum with the doses that we studied (5). A no. 0.5 McFarland standard suspension of organisms in MHBS was prepared as described above and diluted in MHBS to provide a final inoculum of 1 × 10⁸ to 3 × 10⁹/ml in antibiotics. Tubes containing 5 ml of the inoculated antibiotic solutions were incubated at 35°C; and 0.1-ml portions were removed after 0, 6, 24, and 48 h or 0, 6, 24, 48, 72, 96, and 120 h of incubation. At each sampling time, the broth culture was mixed with a pipette by aspirating and expelling the broth. A 0.1-ml portion was diluted in sterile saline, and 0.1-ml portions of each dilution were spread over the surface of a sheep blood agar plate and incubated for 48 h at 35°C. The number of surviving organisms was determined for each sampling time. The lowest level of organisms that could be detected by this method was 10 CFU/ml.

**TABLE 1. Clearing of MRSA carriage by novobiocin-rifampin**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Course no.</th>
<th>Site</th>
<th>Day posttreatment sample for culture was obtained</th>
<th>Success or failure*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Tracheostomy</td>
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<td>S</td>
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<td>S</td>
</tr>
<tr>
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<td>Tracheostomy</td>
<td>Unvaluable; not confirmed pretreatment</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>Tracheostomy</td>
<td>5</td>
<td>S</td>
</tr>
<tr>
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<td>Tracheostomy</td>
<td>4</td>
<td>F</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Tracheostomy</td>
<td>Unvaluable; pneumonia, incomplete course, other drugs</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>Tracheostomy</td>
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<tr>
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<td>2</td>
<td>Tracheostomy</td>
<td>Unvaluable; concurrent therapy</td>
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<td>Wound</td>
<td>2</td>
<td>S</td>
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<tr>
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</tr>
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<td>12</td>
<td>1</td>
<td>Amputation wound</td>
<td>3</td>
<td>S</td>
</tr>
</tbody>
</table>

* S, Success; F, failure.

a Then one to two times per week for 30 days.

b Then one to two times per week for 219 days.
c Then two times per week for 29 days.
d Then one to two times per week for 310 days.
e Then one to two times per week for 88 days.
RESULTS

Clinical results. Eighteen courses of treatment were given to 12 patients (Table 1). No patients described any side effects associated with therapy, and no side effects were evident on examination or in laboratory studies. Initial posttherapy cultures of samples from sites infected with MRSA pretherapy were obtained 2 to 9 days (mean, 3.7 days) after therapy.

Of 14 courses, 11 were followed by clearing of all MRSA-colonized sites (79% success rate). Four courses were considered unevaulable for one or more reasons. Two of these were deemed to have MRSA disease after the 5-day regimen for eradication of a presumed carrier state was initiated. Two courses received additional oral or parenteral antibiotics for reasons other than the carrier state, which could have affected the outcome of the colonization study. In one patient a preliminary report of staphylococcal methicillin resistance pretherapy could not be confirmed in our laboratory. One unevaulable patient did not complete the 5-day course.

If all MRSA-colonized sites were considered separately, 13 of 16 (81%) sites were cleared with a 5-day course of therapy. These colonized sites were eight tracheostomy, two throat, two wound, and one external otitis (not previously involved in MRSA). Although it was not required by the protocol, in most patients samples for culture from sites that were routinely cultured pretherapy and which were MRSA-free pretherapy were obtained posttherapy. In no instance did MRSA appear at a site that was negative pretherapy. The three MRSA-positive sites not cleared were a tracheostomy, nasopharynx, and a wound.

For one of the sites (tracheostomy, patient 3) that did not respond, we were able to promptly try a second 5-day course of therapy which cleared the infection from that site. Thus, considering the use of this second course, we were able to clear MRSA from all colonized sites with one or two 5-day regimens in 11 of 13 (85%) evaluable attempts.

If first and second courses of treatment are considered separately, success was achieved in 78% (7 of 9) of the first and 80% (4 of 5) of the second courses of therapy. This corresponded to clearing of 80% (8 of 10) of sites in the first course and 83% (5 of 6) in the second course of therapy.

Seven courses with negative posttherapy cultures of samples obtained from sites that were positive pretherapy were followed by culturing of one or more additional samples during the 2- to 9-day period; these additional immediate posttherapy courses were also negative (Table 1). Eight courses with negative cultures of samples obtained 2 to 9 days posttherapy from sites that were positive pretherapy were followed by at least one additional set of cultures obtained in the third week or later posttherapy; these additional late posttherapy samples obtained for culture were also negative in all instances (Table 1). The sole exception to the latter statement was patient 1, who had no MRSA in cultures of samples routinely obtained from sites one to two times per week for a year, at which time his tracheostomy site was again colonized with MRSA. These data for patient 1 are clear evidence of recoinolization rather than recurrence at a site that was positive but from which no MRSA was detected posttherapy (i.e., not a false-negative 2- to 9-day posttherapy culture).

These two additional sets of data, i.e., patients with multiple 2- to 9-day posttherapy cultures and patients with additional samples obtained for culture 3 weeks and more posttherapy (all of which were negative in patients who we deemed to be treatment successes), support our conclusions of treatment success in the remaining patients, from whom we did not obtain more than the one required set of samples for culture posttherapy. One other patient (number 9) from whom samples for regular routine surveillance cultures were not obtained was found to be colonized with MRSA a second time after he had been initially cleared by treatment. This episode, which occurred 3 months after the initial course of therapy, is believed to represent recoinolization.

Susceptibility to novobiocin and rifampin in vitro. Twenty isolates which met our criteria for MRSA were tested by the broth macrodilution method. Eighteen were pretreatment isolates and two were obtained posttherapy from sites that failed to be cleared of MRSA with treatment. All 18 pretherapy isolates and 1 posttherapy isolate were susceptible to novobiocin (MIC, ≤0.25 μg/ml) and rifampin (MIC, ≤0.06 μg/ml). One posttherapy isolate was susceptible to novobiocin but was resistant to rifampin (MIC, >62.5 μg/ml; patient 9; course 2, nasopharynx; Table 1).

Time-kill studies of novobiocin and rifampin together and separately. Time-kill studies were performed with three pretherapy isolates. Samples were removed at 6, 24, and 48 h and quantitated. With each of the three isolates, time-kill curves for the three drug regimens were not significantly different. The CFU per milliliter declined at each sampling time, and by 48 h a reduction that averaged approximately 100-fold from the starting inoculum had occurred. Because these curves were not different from those shown in Fig. 1A and B, they are not shown.

Time-kill studies of isolates from treatment successes and failures. In time-kill of isolates from treatment successes and failures, eight additional isolates were studied with the combined in vitro regimen of 1.0 μg of novobiocin per ml and 0.25 μg of rifampin per ml, and the timed observations were extended to 120 h (the same duration of therapy as that given to the patients). With isolates from two treatment successes (Fig. 1A), dramatic killing, to less than 10 CFU/ml, was achieved within 72 h and was sustained for 120 h.

We then tested four pretherapy isolates obtained from patients in whom therapy failed to eradicate MRSA with the novobiocin-rifampin treatment (Fig. 1B). One of these isolates was from a patient who was not considered to be a treatment failure to eliminate carriage, as this patient was discovered to have an MRSA infection after entry into the study. These four isolates were killed at the same rate as isolates from treatment successes were.

Two isolates were obtained posttherapy from patients who failed to respond to novobiocin-rifampin therapy and were studied (Fig. 1C). One of these two isolates had a time-kill curve that was not different from those described above (Fig. 1A and B). The other isolate, which was the rifampin-resistant isolate from patient 9 (see above), had the same kinetics as the other isolates for 48 h, but it subsequently began logarithmic growth. Subcultures were obtained from the time-kill curve experiment tubes at the end of the experiment (120 h). This isolate also became resistant to novobiocin (MIC, >4 μg/ml) as a result of exposure to and incubation with the two drugs in the in vitro experiment.

DISCUSSION

Results of this study show the efficacy and lack of toxicity of a short course of oral therapy in eradicating colonization of MRSA in patients. Success, tabulating cases or sites, occurred in about 80% of instances. These results compare favorably with those obtained by other regimens (14, 16, 18).
The protocol required only a single set of posttherapy samples for culture to assess the efficacy of treatment. Even though a minimum of 2 days after therapy was required before reculturing, to allow samples for culture to be taken that were free of residual antibiotics, based on the half-lives of the antibiotics (5), and even though a sensitive and selective culturing technique was used, it is theoretically possible that a single set of cultures could miss a therapeutic failure. This would particularly be the case if the therapy merely suppressed colonization temporarily. However, multiple samples from culturing were obtained from many of our patients posttherapy; these confirmed the earlier posttherapy results, and in fact, all of the treatment failures that we detected were uncovered in the first posttherapy culture. If the inability to detect a treatment failure on a single set of cultures is shown to be small, it would be a benefit to be able to rely on a few cultures when, as in our institution, there is a pressure for available beds that is thwarted by the need for patient isolation.

Our susceptibility testing results make the epidemiologic suggestion, at least in our institution, that MRSA-colonized patients who have not received the antibiotic combination previously have isolates that are susceptible to the antibiotics (18 of 18). We also demonstrated that resistance may develop in isolates in patients who were initial treatment failures.

Time-kill studies demonstrated an indifference in the interaction between the antibiotics. This result was also shown by Walsh et al. (17). In our sample, the time-kill studies did not predict treatment success or failure (Fig. 1A and B). However, these time-kill studies did show that, in a patient who was a clinical failure, development of resistance to rifampin correlated with failure of the two drugs to sustain continued killing in vitro. It required >48 h of incubation to show the lack of killing with the drug combination. As a result of this further in vitro exposure to the antibiotics, resistance to novobiocin also developed. This provides substantiation for data which suggest that the antibiotic combination, when isolates are susceptible to both drugs, prevents the development of resistance to either drug (4). Results of other in vitro studies, however, have shown that the presence of rifampin, even when the isolate is resistant to rifampin, prevents the development of resistance to novobiocin (7).

Effective chemotherapy of MRSA carriers may only be part of the approach needed to terminate MRSA transmission in hospitals; other measures which have been suggested to play a role are surveillance cultures of patients and staff on affected wards, patient isolation procedures, cohorting of patients or staff or both, and attention to handwashing. Although results of pharmacokinetic studies (5) of the two drugs that we studied have suggested that when novobiocin and rifampin are coadministered, novobiocin clearance is accelerated, our clinical results suggest that this may not be an important concern. The need for a second course of therapy to eradicate the carrier state in one of our patients may suggest that longer courses of treatment than those that we used might be more efficacious. Other dose combinations could also be studied. Our results suggest the need for further studies of this combination, preferably in comparative trials with other treatment modalities; multicenter studies may be particularly useful, especially so that a spectrum of MRSA isolates could be studied.

LITERATURE CITED
ERADICATING CARRIER STATE OF MRSA