Eradication of Colonization by Methicillin-Resistant *Staphylococcus aureus* by Using Oral Minocycline-Rifampin and Topical Mupirocin

RABIH DAROUICHÉ,1,2,3,4* CHARLES WRIGHT,3 RICHARD HAMIL,1,3,5 MAUREEN KOZA,5 DEBORAH LEWIS,3 AND JON MARKOWSKI2,4

Medical Service, Infectious Disease Section,1 Spinal Cord Injury Service,2 and Infection Control Program,5 Veterans Affairs Medical Center, and the Departments of Medicine,1 and Physical Medicine and Rehabilitation,4 Baylor College of Medicine, Houston, Texas 77030

Received 17 December 1990/Accepted 3 June 1991

In an attempt to control the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) within a spinal cord injury unit, we investigated the mode of transmission and implemented a multidisciplinary approach for control that consisted of grouping of patients into cohorts, contact isolation, and antibiotics. Surveillance cultures of patients and nose and hand cultures of medical personnel were performed. Of 11 colonized patients, 6 had MRSA isolates that shared a similar plasmid profile and antibiogram, raising the possibility of interpatient spread of the organism. Medical personnel had no evident role in transmitting MRSA. All patients' pretherapy MRSA isolates were susceptible to minocycline and, except for one, to rifampin. Time-kill studies showed an indifferent interaction of these two antibiotics. Ten colonized patients received a 2-week oral course of 100 mg of minocycline twice daily and 600 mg of rifampin once daily, while the 11th patient was treated for only 1 week. Patients with colonization of the nares also had twice daily nasal application of 2% mupirocin for 5 days. Colonization with MRSA cleared in 10 of 11 patients (91%) and 20 of 21 sites (95%). When the individual circumstances of a medical facility justify eradication of MRSA colonization, a multidisciplinary approach that includes antibiotic therapy with oral minocycline and rifampin, along with topical mupirocin for those with nasal carriage, may be successful.

Since the first large outbreak in the United States in 1968 (1, 13), methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasingly common organism, especially in tertiary-care hospitals (7, 18). Colonization with MRSA, with or without clinical infection, occurs most commonly in acute care units and skilled nursing facilities (24). The latter include spinal cord injury units where the relatively prolonged hospital stay and the frequency with which patients get transferred between such facilities and nursing homes enhance the intrahospital and inter-institutional spread, respectively, of MRSA (13).

Controlling the spread of MRSA in medical facilities has been difficult (20). An abrupt increase in the prevalence of MRSA colonization among patients in a spinal cord injury unit encouraged us to (i) investigate the mode of spread of MRSA and (ii) implement a multidisciplinary regimen in an attempt to interrupt transmission of and eradicate colonization with this organism.

(Continued).

**MATERIALS AND METHODS**

Study population. The Houston Veterans Affairs Medical Center is a 1,040-bed facility affiliated with Baylor College of Medicine. The majority of patients in the 26-bed spinal cord injury unit are transferred from nursing homes or other medical facilities; only a few come from home. By using sterile dacron swabs (Culturette; Marion Scientific, Kansas City, Mo.), surveillance cultures of anterior nares, axillae, groins, anuses, urethrae, urine samples, wounds, and catheter sites were obtained from 17 patients. The hands and anterior nares of 45 medical personnel were also cultured.

**Bacterial cultures.** Clinical specimens were plated onto Trypticase soy agar plates with 5% sheep blood and Mueller-Hinton agar plates containing 5% sheep blood, 4% NaCl, and 6 μg of oxacillin per ml (BBL Microbiology Systems, Cockeysville, Md.). The plates were incubated for 24 h at 35°C in a 5% CO₂ atmosphere. Colonies of *S. aureus* were identified by Gram stain, catalase reaction, and a slide coagulase test (SeroSTAT II; Scott Laboratories, West Warwick, R.I.). Susceptibility testing for each isolate to oxacillin, minocycline, rifampin, ciprofloxacin, tetracycline, cefazolin, clindamycin, erythromycin, gentamicin, novobiocin, and trimethoprim-sulfamethoxazole was done with the Kirby-Bauer disk diffusion method. A zone size of 12 mm around a 1-μg oxacillin disk after 24 h of incubation indicated methicillin resistance (4).

**Infection control procedures.** Patients colonized with MRSA were grouped into cohorts in rooms outside of which isolation carts were stationed. Contact precautions consisted of wearing gloves prior to contact and washing hands with an antiseptic agent after contact, however brief, with colonized patients or their articles. Gowns and/or masks were worn when soiling or splashing of material were likely. Colonized patients were allowed to participate in rehabilitation therapy, receive whirlpool treatment, and undergo diagnostic procedures, provided contact precautions were adhered to; these activities were scheduled as the last ones of the day, just prior to terminal disinfection of the area in use. A 2-week course of oral antibiotics consisting of 100 mg of minocycline twice a day and 600 mg of rifampin each morning was completed by all colonized patients, except for one who received oral antibiotics for only 1 week. This regimen was supplemented by intranasal application of 2% mupirocin ointment twice daily for 5 days in patients with
A large table is shown, detailing the effect of antibiotic treatment on MRSA colonization across different sites and patient numbers. The table includes the patient number, site(s) colonized, plasmid patterns, antibiotic treatment, and sites with cultures positive for MRSA at different time points after treatment.

**Table 1. Effect of antibiotic treatment on MRSA colonization**

| Patient no. | Site(s) colonized | Plasmid pattern | Antibiotic treatment | Site with cultures positive for MRSA at: |
|-------------|-------------------|----------------|---------------------|---------------------------------------|
|             |                   |                |                     | 1 week after starting treatment | 4 days after finishing treatment | 3-4 months after treatment |
| 1           | Nose              | I               | Oral* + topical     | None                                | None                                | ND*                        |
| 2           | Axilla            | I               | Oral               | None                                | None                                | ND*                        |
| 3           | Nose              | I               | Oral + topical     | None                                | None                                | ND*                        |
| 4           | Axilla            | I               | Oral               | None                                | None                                | ND*                        |
| 5           | Nose, wound       | I               | Oral + topical     | None                                | None                                | None                      |
| 6           | Axilla            | I               | Oral               | None                                | None                                | ND*                        |
| 7           | Nose, axilla, anus, urine, wound | I | Oral + topical     | None                                | None                                | ND*                        |
| 8           | Perisuprapubic    | I               | Oral               | None                                | None                                | ND*                        |
| 9           | Nose              | I               | Oral + topical     | None                                | None                                | ND*                        |
| 10          | Nose, tracheostomy, urethra, anus, wound | II | Oral + topical     | Wound                              | Wound                              | Wound                     |
| 11          | Urine             | III              | Oral               | None                                | ND*                                | ND*                        |

* Oral, 2-week course of minocycline and rifampin, except for patient 11, who was treated for only 1 week.

* Topical, 5-day course of 2% mupirocin nasal ointment.

* ND, not done.

colonization of the nares. Follow-up cultures were obtained from all patients on days 7 and 18 after enrollment. A treatment success was defined as the absence of MRSA at previously positive sites on these follow-up cultures. All patients were eventually discharged from the hospital. A third set of cultures could be performed in six patients 3 to 4 months after treatment.

Time-kill studies. Stock solutions of antibiotics were diluted in Mueller-Hinton broth (MHB) to achieve final concentrations of 2.0 μg of minocycline (Merrell Dow Inc., Cincinnati, Ohio) per ml, or 2.0 μg of minocycline and 5 μg of rifampin per ml; these concentrations represent the average levels in serum that are achievable with the doses we administered. Four sets of tubes containing minocycline, rifampin, both antibiotics, or neither antibiotic in 0.9 ml of MHB were prepared. MRSA isolates were suspended overnight in MHB at 37°C in a shaking water bath. After a 1:100 dilution, 0.1-ml portions of each bacterial suspension were added to the antibiotic-containing tubes to achieve bacterial concentrations of 5 x 10^2 to 1 x 10^6 organisms per ml of MHB. The tubes were then incubated at 37°C, and 0.1-ml samples were removed after 0, 4, 8, and 24 h of incubation. These samples were diluted in sterile saline, and 10-μl portions of each dilution were inoculated onto Trypti-case soy agar plates with 5% sheep blood, which were then incubated overnight at 37°C. The number of colonies was counted; the lowest level of surviving organisms that could be detected by this method was 100 CFU/ml.

Plasmid analysis. Plasmid analysis was carried out on all MRSA isolates from patients and medical personnel by using modifications of the method originally described by Birn-boim and Doly (6).

**RESULTS**

Clinical results. Eleven of 17 patients were considered colonized with MRSA after it grew in two consecutive cultures from at least one body site (Table 1); none of the patients was clinically infected with MRSA. MRSA colonization was eradicated in 10 treated subjects (a 91% success rate). When the 21 MRSA-colonized sites were considered separately, a similarly high proportion cleared following treatment (95%). The only MRSA-positive site that remained colonized was a surgical wound in patient 10. Of six patients who could be followed up to 3 to 4 months after treatment, five were still MRSA free (83%), while the sixth patient remained colonized throughout the study period. In no treated patients did MRSA appear at a site that was culture negative prior to therapy. Antibiotics were generally well tolerated, and patients denied vestibular symptoms.

Seven of 45 medical personnel (16%) transiently carried MRSA in the nose (four) or hands (three) and were counselled by the Infection Control Team; adherence to contact precautions was emphasized, and the option of bacterial decolonization with antibiotics was considered. Only one individual consented to treatment, after which he became MRSA free. MRSA colonization spontaneously cleared in the other six medical personnel within a month of the initial positive culture.

Antimicrobial susceptibility. By the disk diffusion method, all patients’ pretherapy MRSA isolates were susceptible to minocycline and, except for one, to rifampin. Pretherapy MRSA organisms isolated from the single persistently colonized site were susceptible to rifampin but became resistant after treatment; posttherapy MRSA isolates remained susceptible to minocycline. Of interest was the uniform resistance of patients’ MRSA isolates to ciprofloxacin, whereas six of seven colonized personnel carried strains that were susceptible to this drug. All patients colonized with MRSA had received extensive antimicrobial therapy in the past, including quinolones in four cases (37%).

Antibiograms of MRSA strains were constructed on the basis of susceptibility to the nine antibiotics discussed above (other than oxacillin). Except for patient 10, MRSA isolates from individual patients who were colonized at more than one site had similar antibiograms. Bacterial strains in 6 of 11 colonized patients shared a similar antibiogram.

Plasmid profiles. Pretherapy MRSA isolates had one of three plasmid profiles (Table 1). MRSA isolates from the group of six patients with an identical antibiogram had the same plasmid profile (I). This plasmid profile was also shared by MRSA isolates from three other patients, each with a different antibiogram. Each of the remaining two patients was colonized by MRSA strains with different antibiograms and plasmid profiles (II and III). None of the medical personnel shared with each other or with the patients iso-
lates of MRSA that had the same antibiogram and plasmid profile.

Concurrent with the development of resistance to rifampin, pre- and posttherapy MRSA isolates from the surgical wound in patient 10 had different plasmid profiles; this raises the possibility that they may have been different strains. None of the MRSA isolates shared a plasmid profile with methicillin-susceptible S. aureus strains.

**Time-kill studies.** Time-kill assays were performed with six pretherapy MRSA isolates, one of which was from the single site that failed to clear with therapy. These six isolates were sensitive to both minocycline and rifampin. With each isolate, the rapid decline in the number of surviving organisms in the presence of rifampin, whether alone or in combination with minocycline, produced an indifferent (or additive) interaction between these two antibiotics.

**DISCUSSION**

This study shows that a multidisciplinary approach which includes geographic segregation, contact isolation, and antibiotics is successful in eradicating colonization with MRSA. In particular, the results obtained by using an antimicrobial regimen that consisted of oral minocycline and rifampin combined with mupirocin nasal ointment compare favorably with the results achieved by other single- or multiple-agent regimens, such as those including skin disinfectants (hexachlorophene and chlorhexidine gluconate) and topical (bacitracin and mupirocin), oral (trimethoprim-sulfamethoxazole, rifampin, ciprofloxacin, and novobiocin), and parenteral (vancomycin) antibiotics (2, 5, 7, 8, 10, 14, 18, 22).

The rapid development of resistance to rifampin when used alone has precluded its use as a single oral agent (11). Combining rifampin with minocycline (30) or other antibiotics (2, 15, 16) helps prevent the development of resistance to rifampin. A combination of minocycline and rifampin is probably more effective in vivo when MRSA isolates are susceptible to both drugs, as opposed to being susceptible to minocycline only; this may be substantiated by finding that our sole clinical failure was encountered with a posttherapy MRSA isolate that was susceptible to minocycline but resistant to rifampin.

Time-kill assays performed with pretherapy MRSA strains, including the single isolate that persisted despite treatment, demonstrated an indifferent interaction between minocycline and rifampin. Results of other in vitro studies with combinations of antibiotics that included rifampin revealed synergy (26), indifference (2, 10, 26), or even antagonism (29) against MRSA. In accordance with our observation, however, the nature of this in vitro interaction frequently fails to predict treatment success or failure (27).

The resistance of S. aureus to penicillinase-resistant penicillins is primarily due to alterations in bacterial penicillin-binding proteins that are chromosomally determined (12) and not plasmid determined (23). However, plasmid analysis, together with antibiograms, may help recognize the acquisition of additional resistance markers and define patterns of nosocomial transmission of MRSA (3). MRSA isolates in about half of our patients shared a similar antibiogram and plasmid profile; this may have been caused by interpatient transmission of bacteria (19) or acquisition of the organisms from a common source that was not identified. Contrary to some reports (9, 21), we and others (20) could not demonstrate evidence for spread of MRSA via medical personnel.

If the needs of a medical facility justify an attempt at eliminating MRSA colonization, a coordinated medical and administrative approach should be used. An approach that is successful in eradicating MRSA colonization in one facility may not necessarily work in other facilities (20, 28). This is probably due to institutional variations in the vigor of implementation of infection control programs and to the susceptibility of the resident organisms to antibiotics. Obviously, each medical facility has to define its own needs, goals, and plans on the basis of individual circumstances.

Even though this study was not conducted in a comparative fashion, the similarity of eradication rates midway through the treatment and after completion of oral antibiotic treatment implies that giving minocycline and rifampin for 1 week might have been sufficient. Our results suggest the need for multicenter controlled studies to compare the efficacy of this antimicrobial combination with those of other regimens.

As MRSA spreads in medical facilities and increasingly causes nosocomial infections, methicillin-susceptible S. aureus infections may decline (17, 25) or remain as frequent (17). The impact of using this antimicrobial regimen to contain the spread of MRSA in health care facilities on the frequency of methicillin-susceptible S. aureus infections and hence, on the overall incidence of staphylococcal infections due to both methicillin-resistant and methicillin-sensitive organisms, remains to be evaluated.

**ACKNOWLEDGMENT**

Funds for this study were provided by the Department of Veterans Affairs.

**REFERENCES**

1. Aslits, G. D., F. L. Sapico, H. N. Canawati, G. M. Malik, and J. Z. Montonger. 1982. Methicillin-resistant Staphylococcus aureus colonization and infection in a rehabilitation facility. J. Clin. Microbiol. 16:218–223.

2. Arathoon, E. G., J. R. Hamilton, C. E. Hench, and D. A. Stevens. 1990. 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. Antimicrob. Agents Chemother. 34:1655–1659.

3. Archer, G. L., and C. G. Mayhall. 1983. Comparison of epidemiological markers used in the investigation of an outbreak of methicillin-resistant Staphylococcus aureus infections. J. Clin. Microbiol. 18:395–399.

4. Barry, A. L., and C. Thornberry. 1985. Susceptibility tests: diffusion test procedures, p. 978–987. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

5. Bartozkas, C. A., J. H. Patton, M. F. Gibson, R. Graham, G. A. McLoughlin, and R. S. Croton. 1984. Control and eradication of methicillin-resistant Staphylococcus aureus on a surgical unit. N. Engl. J. Med. 311:1422–1425.

6. Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7:1513–1523.

7. Boyce, J. M. 1989. Methicillin-resistant Staphylococcus aureus. Detection, epidemiology and control measures. Infect. Dis. Clin. N. Am. 3:901–913.

8. Cadera, J. E., M. S. Terpenning, M. Ensberg, S. F. Bradley, and C. A. Kauffman. 1990. Staphylococcus aureus nasal colonization in a nursing home: eradication with mupirocin. Infect. Control Hosp. Epidemiol. 11:13–16.

9. Cockson, B., B. Peters, M. Webster, J. Phillips, M. Rahman, and W. Noble. 1989. Staff carriage of epidemic methicillin-resistant Staphylococcus aureus. J. Clin. Microbiol. 27:1471–1476.

10. Ellison, R. T., III, F. N. Judson, L. C. Peterson, D. L. Cohn, and J. M. Ehret. 1984. Oral rifampin and trimethoprim/sulfamethoxazole therapy in asymptomatic carriers of methicillin-resistant S. au
Staphylococcus aureus infections. West. J. Med. 140:735–740.
11. Eng, R. H., S. M. Smith, M. Tillem, and C. Cherubin. 1985. Rifampin resistance. Development during the therapy of methicillin-resistant Staphylococcus aureus infections. Rev. Infect. Dis. 145:146–148.
12. Hackbart, C. J., and H. F. Chambers. 1989. Methicillin-resistant staphylococci: genetics and mechanisms of resistance. Antimicrob. Agents Chemother. 33:991–994.
13. Haley, R. W., A. W. Rightower, R. F. Khbahaz, C. Thorusesberry, W. J. Martone, J. R. Allen, and J. M. Hughes. 1982. The emergence of methicillin-resistant Staphylococcus aureus in United States hospitals. Ann. Intern. Med. 97:297–308.
14. Locksley, R. M., M. L. Cohen, T. C. Quinn, L. S. Tompkins, M. B. Coyle, J. M. Kirihara, and G. W. Counts. 1982. Multiply antibiotic-resistant Staphylococcus aureus: introduction, transmission and evolution of nosocomial infection. Ann. Intern. Med. 97:317–324.
15. Lorian, V., B. Atkinson, and Y. Kim. 1983. Effect of rifampin and oxacillin on the ultrastructure and growth of Staphylococcus aureus. Rev. Infect. Dis. 5(Suppl. 3):418–427.
16. Mandell, G. L., and D. R. Moorman. 1980. Treatment of experimental staphylococcal infections: effect of rifampin alone and in combination on development of rifampin resistance. Antimicrob. Agents Chemother. 17:658–662.
17. Muder, R. R., C. Brennan, M. M. Wagener, R. M. Vickers, J. D. Rihs, G. A. Hancock, Y. C. Yee, J. M. Miller, and Y. L. Yu. 1991. Methicillin-resistant staphylococcal colonization and infection in a long-term care facility. Ann. Intern. Med. 114:107–112.
18. Murray-Leisure, K. A., S. Geib, D. Graceley, A. B. Rubin-Slutsky, N. Saxena, H. A. Muller, and B. H. Hamory. 1990. Control of epidemic methicillin-resistant Staphylococcus aureus. Infect. Control Hosp. Epidemiol. 11:343–350.
19. Peacock, J. E., Jr., F. J. Marsik, and R. P. Wenzel. 1980. Methicillin-resistant Staphylococcus aureus: introduction and spread within a hospital. Ann. Intern. Med. 93:526–532.
20. Preheim, L. C., D. Kimland, and M. J. Bittner. 1987. Methicillin-resistant Staphylococcus aureus in Veterans Administration Medical Centers. Infect. Control 8:191–194.
21. Rebolli, A. C., J. F. John, Jr., C. G. Platt, and J. R. Cantey. 1990. Methicillin-resistant Staphylococcus aureus outbreak at a Veterans Affairs Medical Center: importance of carriage of the organism by hospital personnel. Infect. Control Hosp. Epidemiol. 11:291–295.
22. Smith, S. M., R. H. Eng, and F. Tecson-Tumang. 1989. Ciprofloxacin therapy for methicillin-resistant Staphylococcus aureus infections or colonizations. Antimicrob. Agents Chemother. 33:181–184.
23. Stiller, P. W., H. M. Sweeney, and S. Cohen. 1973. Absence of circular plasmid deoxyribonucleic acid attributable to a genetic determinant for methicillin resistance in Staphylococcus aureus. J. Bacteriol. 116:771–777.
24. Thomas, J. C., J. Bridge, S. Waterman, J. Vogt, L. Kilman, and G. Hancock. 1989. Transmission and control of methicillin-resistant Staphylococcus aureus in a skilled nursing facility. Infect. Control Hosp. Epidemiol. 10:106–110.
25. Thompson, R. L., I. Cabezudo, and R. F. Wenzel. 1982. Epidemiology of nosocomial infections caused by methicillin-resistant Staphylococcus aureus. Ann. Intern. Med. 97:309–317.
26. Tuazon, C. U., M. Y. Lin, and J. N. Sheagren. 1978. In vitro activity of rifampin alone and in combination with nafcillin and vancomycin against pathogenic strains of Staphylococcus aureus. Antimicrob. Agents Chemother. 13:759–761.
27. Van Der Auwera, P., F. Mesnier-Carpentier, and J. Klasterksy. 1983. Clinical study of combination therapy with oxacillin and rifampin for staphylococcal infections. Rev. Infect. Dis. 5(Suppl. 3):515–522.
28. Wakefield, D. S., M. Pfaller, R. M. Massanari, and G. T. Hammens. 1987. Variation in methicillin-resistant Staphylococcus aureus occurrence by geographic location and hospital characteristics. Infect. Control 8:151–157.
29. Watananakorn, C., and J. C. Guerriero. 1981. Interaction between vancomycin and rifampin against Staphylococcus aureus. Antimicrob. Agents Chemother. 19:1089–1091.
30. Yourassowsky, E., M. P. Van Der Linden, M. J. Lismont, and F. Crokaert. 1981. Combination of minocycline and rifampin against methicillin- and gentamicin-resistant Staphylococcus aureus. J. Clin. Pathol. 34:559–563.