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Infection due to methicillin-resistant *Staphylococcus aureus*

Report of an Unusual Case

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METHICILLIN-resistant strains of coagulase-positive *Staphylococcus aureus* were first isolated in Great Britain in 1960.[1] The incidence of such strains has been low, but appears to be increasing in England, in Europe, and in the Scandinavian countries.[2-5] In the United States, there have been few well-documented cases of serious infection due to methicillin-resistant, coagulase-positive staphylococci.[6-9] We recently treated a patient with an infection due to an unusual strain of *Staphylococcus aureus* that was highly resistant to methicillin; findings in the case are the basis of this report.

Report of a case

A 21-year-old Caucasian housewife was admitted to the Cleveland Clinic Hospital on February 22, 1968, for treatment of a large gluteal abscess. The patient had been residing in India and was in apparent good health until the last week of November 1967. At that time, low-grade fever, malaise, and icterus developed. On November 29, 1967, she was admitted to a hospital in New Delhi, India, for treatment of infectious hepatitis. Between December 3 and December 8, 1967, the patient was comatose and required a cutdown in the right saphenous vein for intravenous fluid therapy. On December 12, fever and evidence of septic thrombophlebitis of the right saphenous vein developed. Between December 12 and December 27, the patient received a number of antibiotics including penicillin G, a tetracycline, erythromycin, oxacillin, and chloramphenicol, without noticeable improvement. During that interval, metastatic abscesses developed in the right sternoclavicular joint, in the upper lobe of the right lung, and in subcutaneous tissues of the abdomen, chest, and right thigh. Cultures of blood and fluid from abscesses revealed coagulase-positive staphylococci that were reported as being sensitive to "all antibiotics" tested. On December 27, treatment with kanamycin, 0.5 g intramuscularly every 12 hr, was started. On December 30, treatment with methicillin, 1 g parenterally every 4 hr was initiated. On December 31, an abscess in the region of the cutdown site in the right ankle was incised and drained; a large amount of purulent exudate was recovered and yielded coagulase-positive staphylococci on culture. These organisms were reported as being sensitive to

methicillin. Methicillin was administered for a total of 29 days and kanamycin for 20 days. The patient remained afebrile after January 8, 1968, and inflammatory lesions gradually resolved. There was no evidence of infection when the patient was discharged from the hospital on January 27, 1968. Subsequently she remained afebrile, but consulted her physician on February 8, because of a delay in the onset of her menstrual period. An intramuscular injection was administered in the left gluteal region, and vaginal bleeding began about 24 hr later. On February 10, the patient began to notice tenderness and pain in the left gluteal region. On February 14, a course of methicillin, 1 g every 6 hr, was initiated. On February 17, an abscess in the left gluteal region was incised and partially drained. Subsequently the patient elected to return to the United States for further care.

Physical examination on admission to the Cleveland Clinic Hospital disclosed a thin, Caucasian woman who did not appear to be acutely ill. The blood pressure was 130/80 mm Hg, the pulse was 80 beats per minute and regular, and the temperature was 98.4 F. A dry eschar was in the region of the previous cutdown in the right ankle. A large, tender, indurated area was in the left buttock. In the left buttock were several sinuses with a profuse amount of purulent exudate. The results of the remainder of the physical examination were within normal limits. Urinalysis, blood hemoglobin content, leukocyte count, determination of serum bilirubin, of glutamic oxaloacetic transaminase, and of alkaline phosphatase, and the serum protein electrophoretic pattern were reported as being within normal limits. Roentgenograms of the chest, abdomen, kidneys, and pelvis revealed no abnormalities. A hepatic scintigram showed no evidence of abnormality. Cultures of the blood and urine were sterile. Pretreatment cultures of the exudate from the left gluteal region disclosed numerous colonies of bacteria identified as coagulase-positive *Staphylococcus aureus*. The in vitro determination of susceptibility of the organism was performed initially by means of the routine antibiotic-disk technic. The organism was reported as being resistant to methicillin, penicillin G, chloramphenicol, tetracycline, and dihydrostreptomycin, and susceptible to cephalothin, lincomycin, and erythromycin.

The patient was placed in strict isolation, and a large abscess in the left buttock was incised and drained. No evidence of osteomyelitis was found. Suction irrigation was initiated with streptokinase and streptodornase in solution. Cephalothin, 1 g intramuscularly, was administered every 6 hr from February 23 to February 28, but was discontinued because of persistent fever and inadequate clinical response. Subsequently, lincomycin was administered orally or intravenously in doses ranging from 1.8 to 4.0 g every 24 hr. Fever and signs of inflammation subsided and the wound showed evidence of healing. Mild leukopenia developed on March 11, and administration of lincomycin was discontinued. The patient was discharged from the hospital on March 12, and erythromycin stearate was administered for 24 days. By March 21, the wound on the left buttock had healed completely. Subsequent physical examinations on April 5, 18, and 21, disclosed no evidence of infection. The patient returned to India, and indicated by postcard on May 8, 1968, that she continued to feel well.

Additional bacteriologic and clinical studies

The organism isolated from this patient was identified on the basis of colonial morphology, gram-stain, coagulase-test, and biochemical tests as being *Staphylococcus aureus*. It was coagulase-positive, catalase-positive, nitrate-positive, and it fermented mannitol anaerobically. The phage type was 15 W.* The results of in vitro susceptibility tests by means of the cup-plate dilution method are shown in *Table 1*. The organism was resistant to penicillin G, methicillin, cloxacillin, cephalothin, chloramphenicol, tetracycline, and streptomycin; it was susceptible to kanamycin, vancomycin, gentamicin, and lincomycin.

Population analysis of the methicillin-resistant *Staphylococcus* was performed

* *Phage-typing of this isolate was performed through the courtesy of Emanuel Wolinsky, M.D., from the Case Western Reserve University School of Medicine, Department of Microbiology, at Cleveland Metropolitan Hospital, 3395 Scranton Road, Cleveland, Ohio.*

according to a method similar to that described by Sutherland and Rolinson.[10] This was accomplished by spreading 0.1-ml amounts of diluted or undiluted overnight broth cultures on infusion agar containing graded concentrations of methicillin, as well as on control agar plates with no antibiotic. Two experiments were performed. In the first experiment (*Table 2*) a heavy inoculum was used; three strains of staphylococci with various degrees of suceptibility to methicillin were compared to the methicillin-resistant strain of *Staphylococcus aureus* isolated from the patient. The methicillin-resistant strain exhibited visible growth of innumerable colonies after 24 hr and 48 hr of incubation at concentrations of methicillin as high as 1000 μg per milliliter. In contrast, the other strains of staphylococci were eradicated by methicillin in concentrations of 16 μg per milliliter or less after incubation periods of from 24 to 48 hr.

In the second experiment (*Table 3*), analysis of the population of the methicillin-resistant strain of *Staphylococcus aureus* from the patient was conducted with a large and a small inoculum of organisms. Results of this study revealed an alteration in the minimal inhibitory concentration of methicillin with a change in the size of the inoculum. With the large inoculum (0.1 ml of an undiluted overnight broth culture), innumerable colonies of *Staphylococcus aureus* grew on agar containing methicillin in concentrations as high as 1000 μg per milliliter. With the small inoculum (0.1 ml of a 10^{-6} dilution of an overnight broth culture) no visible growth occurred on plates containing methicillin in concentrations of 500 or 1000 μg per milliliter. Relatively uniform growth of normal-appearing colonies was observed after 48 hr of incubation on plates containing methicillin in concentrations up to and including 32 μg per milliliter. At a concentration of 62.5 μg per milliliter of methicillin, the number of colonies was slightly diminished, and the majority of these were considerably smaller (0.5 mm

Table 1.—*Susceptibility of coagulase-positive Staphylococcus aureus to 11 antibiotics by the cup-plate dilution method*

Antibiotic	M.I.C.,* units or μg/ml	M.B.C.,† units or μg/ml
Penicillin G	25‡	50‡
Methicillin	>1000	>1000
Cloxacillin	62.5	250
Kanamycin	3.125	3.125
Vancomycin	0.39	0.39
Gentamicin	0.39	0.78
Cephalothin	25	25
Lincomycin	0.195	0.195
Chloramphenicol	>1000	>1000
Tetracycline	250	250
Streptomycin	>1000	>1000

* M.I.C. = minimal inhibitory concentration.
† M.B.C. = minimal bactericidal concentration.
‡ Units per milliliter.

Table 2.—*Population analysis of four strains of staphylococci with various degrees of susceptibility to methicillin*

*Staphylococcus**	M.I.C.,† μg/ml	Incubation period, hr	Concentration of methicillin, μg/ml‡										
			1000	500	250	125	62.5	32	16	8	4	2	0
Coagulase-positive,	>1000	24	±	±	+	+	+	+	+	+	+	+	+
aureus, phage type		48	±	±	+	+	+	+	+	+	+	+	+
W15													
Coagulase-negative	12.5	24	−	−	−	−	−	−	−	−	±	+	+
epidermidis, phage type		48	−	−	−	−	−	−	−	84	±	+	+
untypable													
Coagulase-positive,	6.25	24	−	−	−	−	−	−	−	−	3	+	+
aureus, phage type 3A/		48	−	−	−	−	−	−	−	−	20	+	+
3B/3C/55/71/(44A)/													
15W/5W													
Coagulase-positive	0.78	24	−	−	−	−	−	−	−	−	−	±	+
aureus, phage type 81		48	−	−	−	−	−	−	−	−	1	22	+

* Inoculum: 0.1 ml of an undiluted overnight broth culture spread over surface of nutrient agar plates containing graded concentrations of methicillin.
† M.I.C.: Minimal inhibitory concentration as determined by the cup-plate dilution method.
‡ Symbols: + = confluent growth; ± = discrete colonies (too numerous to count); numerals = number of colonies visible; − = no growth.

Table 3.—*Population analysis of the methicillin-resistant strain of coagulase-positive Staphylococcus aureus*

Dilution of* inoculum	Bacterial cells, number	Incubation period, hr	Concentration of methicillin, μg/ml†										
			1000	500	250	125	62.5	32	16	8	4	2	0
None	8.3×10^7	24	±	±	+	+	+	+	+	+	+	+	+
Heavy inoculum		48	±	±	+	+	+	+	+	+	+	+	+
10^{-6}	1.75×10^2	24	−	−	−	−	−	−	−	−	−	−	−
Light inoculum		48	−	−	1	3	106	157	169	187	228	181	133

* Inoculum, 0.1 ml of an overnight broth culture, diluted as specified and spread over surface of nutrient agar plates containing graded concentrations of methicillin as indicated.
† Symbols: + = confluent growth; ± = discrete colonies (too numerous to count); numerals = number of colonies visible; − = no growth.

or less in diameter) than the typical staphylococcal colonies (1.5 to 2.0 mm in diameter) on the control plates and on plates containing lower concentrations of methicillin. At concentrations of 125 and 250 μg per milliliter of methicillin, only a small proportion of the original inoculum (less than from 1 to 2 percent) was able to survive after 48 hr of incubation; this finding suggests that the bacterial population was composed of individual cells that varied in the degree of resistance to methicillin. Nevertheless, the majority of cells in the population were highly resistant to the action of methicillin.

Cultures of the anterior nares were obtained from physicians, nurses, laboratory technicians, and other persons known to have been in contact with the pa-

tient or the organism. None of those cultures revealed methicillin-resistant staphylococci. However, a second strain of methicillin-resistant *Staphylococcus aureus* (M.I.C. = 100 μg per milliliter) was recently isolated from the disk-sensitivity plate (of a patient treated by us) by the random selection of a single small colony growing within the inhibitory zone of the methicillin disk.[11] Several strains of coagulase-negative staphylococci resistant to methicillin also have been isolated.

Discussion

One of the most troublesome problems in the management of staphylococcal infections has been the emergence and prevalence of organisms resistant to previously effective antibiotics.[12] Before the introduction of methicillin and other penicillinase-resistant semisynthetic penicillins, there was grave concern regarding the future of therapy of staphylococcal infections. Antibiotic-resistant strains of staphylococci flourished in hospitals, produced rising numbers of serious and fatal infections, and caused epidemics in nurseries and hospital wards.[13-15] After the introduction of methicillin in 1959 and the subsequent availability of other penicillinase-resistant penicillins and cephalothin, the mortality rate from serious staphylococcal infections was reduced dramatically.[16] As a result, an attitude of optimism seems to prevail at this time. However, it would appear that optimism should be tempered with caution and continued surveillance of antibiotic susceptibility of staphyloccal strains. Coagulase-negative staphylococci frequently have been found to be resistant to methicillin;[17] although these organisms are relatively avirulent,[11] they may occasionally cause life-threatening infections.[18,19] The incidence of methicillin-resistant strains of coagulase-positive *Staphylococcus aureus* is currently low,[2] but regrettably appears to be increasing.[2-5, 9] These organisms have become endemic in some hospitals,[20] and have produced serious and fatal infections.[7, 20, 21] Accordingly, stringent efforts should be made to detect, isolate, and adequately treat patients with infections due to methicillin-resistant staphylococci.

The ability of staphylococci to resist the action of penicillin G is due to the elaboration of penicillinase, a beta-lactamase enzyme that inactivates the penicillin molecule. Virtually all staphylococci that are not inhibited by 1 μg (1.6 units) of penicillin G produce penicillinase and are resistant to the drug.[14] Methicillin is resistant to degradation by staphylococcal penicillinase; most strains of coagulase-positive staphylococci are inhibited by methicillin in concentrations ranging from 1 to 5 μg per milliliter.[22] Staphylococci that are not inhibited by methicillin in concentrations of 12.5 μg or more per milliliter are regarded as being insensitive to the drug.[4] Resistance of staphylococci to methicillin does not appear to be due to elaboration of a methicillin-destroying enzyme but to intrinsic insensitivity to the drug.[4, 7, 10, 23]

Strains of methicillin-resistant staphylococci isolated from clinical sources demonstrate penicillinase production, cross-resistance with other penicillinase-resistant penicillins, and a notable alteration in susceptibility to methicillin

with a change in inoculum size.[1, 10, 11] These naturally occurring, methicillin-resistant staphylococci typically are composed of a heterogeneous population of cells; the majority of the bacterial cells are susceptible to methicillin in low concentrations, and only a small fraction of the population is resistant to the drug.[10] In contrast, the strain of *Staphylococcus aureus* from the patient we treated had a more uniform type of resistance to methicillin, with the majority of cells being highly resistant to this antibiotic. This pattern of resistance is somewhat similar to that described in regard to penicillinase-producing strains of *Staphylococcus aureus* which are made tolerant to methicillin in the laboratory.[24] Such strains exhibit a uniform type of insusceptibility to the drug, and the degree of resistance is not greatly dependent upon the size of the inoculum.[24] Laboratory-induced, methicillin-resistant strains of penicillinase-producing staphylococci are fully virulent and retain their capacity to circumvent the action of methicillin after repeated transfers in antibiotic-free media.[24] Whether the methicillin-resistant *Staphylococcus* in the patient emerged from an initially susceptible or from a heteroresistant* strain as a result of prolonged therapy with methicillin is a matter of speculation. Unfortunately the organism producing the initial infection in the patient we treated was not available for comparison with the strain isolated from the gluteal abscess. Most available evidence indicates that administration of methicillin or other penicillinase-resistant penicillins does not lead to the emergence of resistant staphylococci under the usual conditions of clinical therapy.[25, 26] However, Chabbert and his associates[27] observed that antibiotic treatment of patients with infections due to "methicillin-heteroresistant" strains of staphylococci led to the selection of highly resistant cells during therapy.

Selection of appropriate antibacterial agents for therapy of infection due to methicillin-resistant staphylococci must be determined largely from the results of in vitro susceptibility tests. Those strains of staphylococci exhibit cross-resistance to other penicillinase-resistant semisynthetic penicillins, and frequently are insusceptible to many other antibiotics. Most strains are insensitive to penicillin G, streptomycin, erythromycin, kanamycin, chloramphenicol, and the tetracyclines;[8] some strains are insensitive to cephalothin and lincomycin.[28] Benner and Morthland[8] found that methicillin-resistant staphylococci were uniformly susceptible to vancomycin, and suggested that this might be the best single antimicrobial agent for therapy of infections due to these organisms. Bulger[29] found that kanamycin and cephalothin exhibited a synergistic effect in vitro against strains of methicillin-resistant staphylococci, and suggested that this combination of antibiotics might be useful for treatment while tests of drug susceptibility are being performed. The *Staphylococcus* isolated from the patient we treated was moderately resistant to cephalothin in vitro and in vivo. The organism was susceptible to lincomycin, and the results of therapy with this

* *The term "heteroresistant" refers to naturally occurring strains of methicillin-resistant staphylococci. These strains are composed of a heterogeneous population of cells and may appear to be susceptible to methicillin if sensitivity testing is performed with a small inoculum and read after overnight incubation.*[3, 8]

agent were favorable. Administration of lincomycin was discontinued because of the development of transient leukopenia, and subsequently erythromycin was used.

Summary

There have been few reported cases of infection due to methicillin-resistant staphylococci in the United States, but the incidence of this type of infection appears to be increasing in other parts of the world. One case of infection due to methicillin-resistant, coagulase-positive *Staphylococcus aureus* is reported. The patient acquired the infection in India, and subsequently she returned to the United States for treatment. The organism was resistant to methicillin, cloxacillin, penicillin G, cephalothin, chloramphenicol, streptomycin, and tetracycline, and was susceptible to lincomycin, erythromycin, kanamycin, gentamicin, and vancomycin. Analysis of the bacterial population of this strain disclosed that a majority of the cells were highly resistant to methicillin. Incision and drainage of a gluteal abscess, suction irrigation, and administration of appropriate antibiotics resulted in cure. The patient was placed in strict isolation during the acute illness, and cultures of the anterior nares of attending personnel subsequently did not reveal methicillin-resistant staphylococci. The need for continued surveillance of the in vitro susceptibility of staphylococci is emphasized. In the future, infection due to methicillin-resistant staphylococci may become a problem in hospitalized patients in the United States.

Addendum

After this paper was submitted for publication, a patient with persistent bacteremia due to a methicillin-resistant strain of coagulase-positive *Staphylococcus aureus* was treated by one of us (M. C. M.), Penn G. Skillern, M.D., Department of Endocrinology, and Robert E. Hermann, M.D., Department of General Surgery, at the Cleveland Clinic Hospital. The minimal inhibitory concentration of methicillin for the causative organism was 25.0 μg per milliliter, and the minimal bactericidal concentration was more than 100 μg per milliliter. Blood cultures were repeatedly positive for coagulase-positive staphylococci while the patient was receiving 2 g of methicillin intravenously every 4 hr. Currently the patient appears to be responding satisfactorily to the administration of 0.5 g of vancomycin intravenously every 6 hr.

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