Antimicrobial Susceptibilities of a *Corynebacterium* CDC Group I1 Strain Isolated from a Patient with Endocarditis

GREGORY J. MALANOSKI,1,2 RICHARD PARKER,2,3 AND GEORGE M. ELIOPoulos1,2*

Division of Infectious Diseases1 and Division of General Internal Medicine,3 Department of Medicine, New England Deaconess Hospital, and Harvard Medical School2 Boston, Massachusetts 02215

Received 17 December 1991/Accepted 17 March 1992

We encountered a case of native valve endocarditis due to *Corynebacterium* CDC group I1 which was successfully controlled with antimicrobial agents alone. This organism and three other isolates of this group were susceptible to penicillin, vancomycin, and gentamicin. The combination of penicillin with subinhibitory concentrations of gentamicin resulted in a 1,000-fold decrease in CFU per milliliter at 24 h compared with penicillin alone. Augmentation of killing was noted also with vancomycin plus gentamicin but to a degree that did not meet strict criteria for bactericidal synergism.

Diphtheroids are ubiquitous in nature and, in addition to being found on human skin, can be found in soil or fresh or salt water, as well as on plants. They occasionally are encountered as human pathogens (8). In particular, several groups of corynebacteria, some of which have been identified to species level, have been found to cause endocarditis, mostly involving prosthetic or damaged native heart valves. There have been a number of reports identifying *Corynebacterium jeikeium* as a cause of prosthetic valve endocarditis (5, 10). There also have been reports of endocarditis due to other CDC coryneform groups of bacteria which have not been identified to species level, including group G2 (involving a prosthetic valve [1]) and group I1 (involving all four native valves [3]). The group I1 patient, the first described with bacteremia due to this organism, was unusual in that he had no previously apparent valvular damage. He expired despite intensive therapy with vancomycin, gentamicin, and penicillin to which the organism was susceptible as determined by Kirby-Bauer disc diffusion methods.

We also have encountered a case of *Corynebacterium* CDC group I1 native valve endocarditis involving an apparently abnormal aortic valve which was successfully controlled with antibiotics alone. This report describes inhibitory and bactericidal activities of several antimicrobial agents against this isolate as well as against three additional isolates obtained from the Centers for Disease Control. Bactericidal activity was tested by time-kill methods, and potential synergism between penicillin and vancomycin and gentamicin was evaluated.

A 53-year-old male, who was a long-standing resident of a mental health facility and had a history of an aortic insufficiency murmur, was admitted for evaluation of a febrile illness. He was well until 1 month prior to admission, when he was given a 10-day course of cephalaxin (500 mg taken four times daily) for a left-foot cellulitis accompanied by a low-grade fever. He did well until 5 days prior to admission, when fevers recurred, reaching 39°C. A prominent aortic insufficiency murmur was noted. Four sets of blood cultures taken on admission revealed gram-positive rods, later identified as *Corynebacterium* CDC group I1. A transthoracic echocardiogram confirmed the presence of an irregularly shaped, nonpedunculated vegetation of less than 1.0 cm maximum dimension involving the right coronary cusp of the aortic valve and the presence of moderately severe aortic insufficiency.

Because of a penicillin allergy, therapy with vancomycin and gentamicin was initiated. Progressive atrioventricular block developed within 48 h of admission, leading to complete heart block. This necessitated placement of a permanent pacemaker but the patient was not felt to be a candidate for further surgical intervention. However, following 2 weeks of combined antimicrobial therapy, he became afebrile and returned to a normal sinus rhythm with a normal P-R interval. He completed an additional 4 weeks of parenteral therapy with vancomycin alone. Because a cardiac abscess was suspected but evidence for microbiologic cure could not be obtained, he was discharged on oral erythromycin for continued suppression. All follow-up blood cultures remained negative, and the patient remained free of recurrent disease 7 months following discharge.

The patient’s strain was identified as *Corynebacterium* CDC group I1 by the Massachusetts Department of Public Health State Laboratory Institute. Three additional strains, E-8873, F-435, and F-8257, were kindly provided by Robert E. Weaver of the Centers for Disease Control in Atlanta, Ga. These strains had been isolated from orbital fat, uterus, and wound cultures, respectively.

All four strains grew readily in brain heart infusion (Difco Laboratories, Detroit, Mich.) broth to a concentration of greater than 10^8 CFU/ml at 24 h of incubation. Smooth, white alpha-hemolytic colonies approximately 0.5 to 1.0 mm in diameter were clearly visible within 24 h of incubation at 35°C on horse blood agar plates (brucella agar with 5% horse blood). MICs against all four strains were determined by a macrodilution method in brain heart infusion broth with an inoculum of 5 × 10^7 CFU/ml. The MIC was defined as the lowest concentration of antibiotic that inhibited visible growth at 24 h of incubation at 35°C. Samples (0.01 ml) from each test broth were plated onto horse blood agar plates and incubated overnight at 35°C. The MBCs were determined by using the guidelines of Pearson et al. for 99.9% killing (11).

Time-kill curves and synergism studies were performed by inoculating 20 ml of brain heart infusion broth in Erlenmeyer flasks with approximately 5 × 10^6 CFU of our patient’s isolate per ml. The flasks were incubated at 35°C. Antimicrobial agents utilized for killing curves included vancomy-
TABLE 1. Antimicrobial susceptibilities of four strains of Corynebacterium CDC group I1

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.125-0.25</td>
<td>≥64</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.5-1</td>
<td>4-64*</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.06-0.125</td>
<td>0.25-0.5</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2</td>
<td>≥32</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.06-0.125</td>
<td>4-16</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2</td>
<td>≥128</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>4</td>
<td>≥64</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5-1</td>
<td>1-2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25-0.5</td>
<td>≥32</td>
</tr>
</tbody>
</table>

* MBCs for individual isolates were 4, 8, 32, and 64 µg/ml.

Antimicrobial, penicillin, and gentamicin. Aliquots were removed for colony counts at 0, 4, 7, and 24 h of incubation, serially diluted into sterile saline, plated onto blood agar, and incubated overnight. Synergism studies were performed with vancomycin and with penicillin at clinically achievable levels above their respective MICs, alone or combined with subinhibitory concentrations of gentamicin. Synergism was defined as a 100-fold or greater decrease in CFU per milliliter at 24 h achieved by the combinations compared with that achieved by either vancomycin or penicillin alone.

MIC and MBC data for the four strains are summarized in Table 1. The susceptibilities of the four strains to each of the antimicrobial agents were quite uniform. The MBC of vancomycin, however, varied from 4 µg/ml for our isolate to 64 µg/ml for one of the other three strains. Penicillin, clindamycin, chloramphenicol, ceftriaxone, and tetracycline all were bacteriostatic at clinically achievable concentrations, with ceftriaxone significantly less active than penicillin. Both gentamicin and ciprofloxacin were bactericidal at concentrations near the MIC. Erythromycin also exerted bactericidal activity but to a lesser degree. Rifampin initially appeared to be bactericidal at very low concentrations. However, at an inoculum of 5 × 10^8 CFU/ml, a "skip-tube" phenomenon frequently was noted during repeated MIC testing. This suggested a high rate of mutation to resistance, making interpretation of results unpredictable and unreliable.

Results of synergism studies for our isolate are displayed in Fig. 1 and 2. The bacteriostatic nature of penicillin is well represented in Fig. 1, in which only a minor decrease in CFU per milliliter is observed following 24 h of incubation with 10 µg of this agent per ml. Most striking was the extreme susceptibility of each strain to very low concentrations of gentamicin. At a concentration of 3 µg/ml, no colonies were detected following 4 h of incubation (data not shown). A concentration of 0.03 µg/ml was needed for evaluation of synergy because any concentration above this had a measurable effect on growth. When penicillin was combined with this dose of gentamicin, a 1,000-fold decrease in CFU per milliliter was noted at 24 h relative to the initial inoculum and to final colony counts observed with vancomycin alone. Time (hours)

FIG. 1. Effects of penicillin utilized alone (10 µg/ml) or in combination with subinhibitory concentrations of gentamicin (0.03 µg/ml) for our Corynebacterium CDC group I1 isolate. For this strain, the MIC of penicillin was 0.125 µg/ml and that of gentamicin was 0.06 µg/ml. Symbols: , control; , gentamicin; , penicillin; , penicillin plus gentamicin.

Antimicrobial, penicillin, and gentamicin. Aliquots were removed for colony counts at 0, 4, 7, and 24 h of incubation, serially diluted into sterile saline, plated onto blood agar, and incubated overnight. Synergism studies were performed with vancomycin and with penicillin at clinically achievable levels above their respective MICs, alone or combined with subinhibitory concentrations of gentamicin. Synergism was defined as a 100-fold or greater decrease in CFU per milliliter at 24 h achieved by the combinations compared with that achieved by either vancomycin or penicillin alone.

MIC and MBC data for the four strains are summarized in Table 1. The susceptibilities of the four strains to each of the antimicrobial agents were quite uniform. The MBC of vancomycin, however, varied from 4 µg/ml for our isolate to 64 µg/ml for one of the other three strains. Penicillin, clindamycin, chloramphenicol, ceftriaxone, and tetracycline all were bacteriostatic at clinically achievable concentrations, with ceftriaxone significantly less active than penicillin. Both gentamicin and ciprofloxacin were bactericidal at concentrations near the MIC. Erythromycin also exerted bactericidal activity but to a lesser degree. Rifampin initially appeared to be bactericidal at very low concentrations. However, at an inoculum of 5 × 10^8 CFU/ml, a "skip-tube" phenomenon frequently was noted during repeated MIC testing. This suggested a high rate of mutation to resistance, making interpretation of results unpredictable and unreliable.

Results of synergism studies for our isolate are displayed in Fig. 1 and 2. The bacteriostatic nature of penicillin is well represented in Fig. 1, in which only a minor decrease in CFU per milliliter is observed following 24 h of incubation with 10 µg of this agent per ml. Most striking was the extreme susceptibility of each strain to very low concentrations of gentamicin. At a concentration of 3 µg/ml, no colonies were detected following 4 h of incubation (data not shown). A concentration of 0.03 µg/ml was needed for evaluation of synergy because any concentration above this had a measurable effect on growth. When penicillin was combined with this dose of gentamicin, a 1,000-fold decrease in CFU per milliliter was noted at 24 h relative to the initial inoculum and to final colony counts observed with penicillin alone. Vancomycin appeared significantly bactericidal at a dose of 10 µg/ml (Fig. 2), and combination with gentamicin resulted in a 30-fold augmentation of killing, but this did not meet strict criteria for synergism. Similar results of synergism were obtained with CDC isolate F-8257, for which the MBCs of penicillin and vancomycin were greater than 128 and equal to 64 µg/ml, respectively. Combination of vancomycin with subinhibitory concentrations of gentamicin resulted in a 50-fold decrease in CFU per milliliter at 24 h relative to the initial inoculum and to final colony counts observed with vancomycin alone. Though again this did not meet strict criteria for bacterial synergism, an overall 1,000-fold decrease in CFU per milliliter was noted at 24 h relative to the initial inoculum.

Diphtheroids now are a well-known cause of endocarditis, primarily involving prosthetic or damaged heart valves (2, 4, 6, 7, 9, 12). C. jeikeium appears to be the most common of these organisms implicated in endocarditis. In a review of 184 patients, diphtheroids accounted for 9% of early-onset and 4% of late-onset prosthetic valve endocarditis cases (13). Murray et al. (10) found that most isolates recovered from patients with prosthetic valve endocarditis belonged to the group JK and had an associated mortality of 42%, often...
requiring valve replacement for cure. Many isolates were
found to have intermediate- to high-level resistance to pen-
icillin, but combinations of penicillin with gentamicin were
uniformly synergistic, regardless of the penicillin MIC. The
organisms also were uniformly susceptible to vancomycin,
which was bactericidal, with MBCs ≤8 μg/ml.

There have been a number of reports of endocarditis with
organisms other than C. jeikeium, including one previous
report of native valve endocarditis with Corynebacterium
CDC group I (3). The present study provides detailed
susceptibility data for this group. Though the CDC group I
isolates appear quite susceptible to penicillin by MIC testing,
they are resistant to killing by this agent. These data, along
with the knowledge that C. jeikeium can be resistant to
penicillin, support initial combination therapy with gentami-
cin pending further identification and susceptibility testing.
Also, vancomycin appears to be uniformly active against all
groups studied thus far, including group II, and might be
considered for initial therapy, but it may not be bactericidal
against some isolates, as we have shown. Vancomycin and
gentamicin might be used in combination for a brief period,
not so much for potential synergism as for the benefits of
the highly bactericidal activity of gentamicin at low concentra-

tions.

REFERENCES
2. Eliakim, R., P. Silkoff, G. Lugassy, and J. Michel. 1983. Cory-
3. Farrer, W. 1987. Four valve endocarditis caused by Corynebact-
prosthetic valve endocarditis due to Corynebacterium xerosis.
5. Gerry, J. L., and W. B. Greenough. 1976. Diphtheroid endo-
carditis. Report of nine cases and a review of the literature.
Diphtheroid endocarditis after insertion of a prosthetic heart
rium pyogenes endocarditis. Report of a case with necropsy and
Infections caused by nondiphtheria corynebacteria. Rev. Infect.
Dis. 4:1220–1235.
Infective endocarditis due to Corynebacterium diphtheriae.
Report of a case and review of the literature. Johns Hopkins
Diphtheroid prosthetic valve endocarditis. A study of clinical
lethal antibiotic concentrations. Antimicrob. Agents Chemo-
ther. 18:699–708.
161.