Antibiotic Disc Susceptibility Tests for Rapid Presumptive Identification of Gram-Negative Anaerobic Bacilli

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A method for identification of gram-negative anaerobic bacilli is described. Based on differences in susceptibility to paper discs containing 10 μg of colistin, 60 μg of erythromycin, 1,000 μg of kanamycin, 1,000 μg of neomycin, 2 units of penicillin, and 15 μg of rifampin, these bacteria may be placed into five groups. Other tests such as colony morphology, production of pigment, growth in bile, esculin hydrolysis, and reaction on egg yolk agar may be used for further identification. The susceptibility tests are rapid and simple to perform and are helpful in characterizing gram-negative anaerobic bacilli. They are not intended for use in predicting clinical effectiveness of the drugs utilized.

The presence of anaerobic bacteria in specimens from infectious processes is being recognized with increasing frequency. In 1958, Stokes (9), found them in 9% of the specimens he examined. More recently, Zabransky (10) found them in 26% of specimens he examined. It is likely that with emphasis on better methods for cultivation and isolation of anaerobes, their incidence in clinical specimens may be found to be still higher. They comprise the major portion of the indigenous flora of man and constitute a likely source of infection when factors such as malignancy, surgery, therapy with immunosuppressants or antimetabolites, and debilitation interfere with natural defense mechanisms.

In the report of Stokes (9) and in that of Zabransky (10), approximately half of the anaerobes isolated were gram-negative bacilli. Others (2) have also found them to occur in infections with much greater frequency than do clostridia. The identification of anaerobic gram-negative bacilli often poses a problem to the diagnostic microbiologist, and many of the tests used for identification require from several days to weeks to obtain results.

Previously reported data (5) have indicated the usefulness of antibacterial susceptibility patterns as an aid in characterizing the anaerobic gram-negative bacilli. These data were based on plate dilution methods which are rather cumbersome. We felt that if these characteristic patterns were to be useful for rapid screening of isolates, a simpler method would be needed. This report presents results of an antibiotic disc susceptibility test for use in rapid identification of groups of anaerobic gram-negative bacilli. The methods and data cannot be used for predicting therapeutic effectiveness of the drugs utilized.

MATERIALS AND METHODS

After preliminary testing of media, antibiotics and concentrations to be used in discs, and incubation time for tests, the method presented below was developed.

One or two colonies of each strain to be tested were inoculated into Fluid Thioglycollate Medium (BBL 11260) enriched with 25% ascitic fluid and incubated for 4 to 6 hr or until barely turbid growth was visible. Occasional strains of Bacteroides melaninogenicus, B. oralis, Fusobacterium fusiforme, and F. nucleatum required overnight incubation to achieve sufficient growth. All strains of B. corrodens required overnight incubation. The inoculum was spread by swab over the surfaces of two 5% sheep blood agar plates (BBL Blood Agar Base, 11037) poured to a depth of 5 mm (28 ml in a plastic petri dish, 15 by 100 mm). Antibiotic discs, three per plate, were then applied.

The following discs were used: colistin, 10 μg; erythromycin, 60 μg; kanamycin, 1,000 μg; neomycin, 1,000 μg; penicillin, 2 units; and rifampin, 15 μg. Only the colistin and penicillin discs are commercially available at present. The others may be prepared as follows: sterile, 6-mm filter paper discs (S & S 740E) are dipped into solutions of erythromycin (Ilotycin, Eli Lilly & Co., Indianapolis, Ind.), 3,000 μg/ml; kanamycin (Bristol Laboratories Inc., Syracuse, N.Y.),
50,000 µg/ml; neomycin (The Upjohn Co., Kalamazoo, Mich.), 50,000 µg/ml; and rifampin (Ciba Pharmaceutical Products, Inc., Summit, N.J.), 750 µg/ml. (These antibiotics may also be obtained from Mann Research Laboratories.) Excess fluid should be drained off, and then the discs should be dried in a vacuum desiccator on sterile, stainless-steel screens. Each disc absorbs 0.02 ml of fluid so that the final concentration is as indicated above. Discs were stored with a desiccant at −20°C and removed from the freezer 1 hr before use as suggested by Griffith and Mullins (6). After discs were applied, the plates were incubated in a Gas-Pak jar (BBL) for 48 to 72 hr. The diameters of zones of inhibition were measured in millimeters. The reproducibility of the method was determined by testing one strain each of B. fragilis, F. fusiforme—nucleatum, Sphaerophorus necrophorus, and S. varius in triplicate on 3 consecutive days.

A total of 187 strains of well-characterized, anaerobic, nonsporeforming, nonmotile, gram-negative bacilli were tested in the first part of this study. Most were from human sources; a few were from animal or unknown sources. Most were old stock strains; a few were freshly isolated strains. Most were isolated in the laboratory of the authors; some were contributed by others as shown in Table 1. They were characterized on the basis of morphology, production of lecinthinase and lipase and proteolysis on egg yolk-agar, production of indole, effect of bile, nitrate reduction, fermentation of carbohydrates, esculin hydrolysis, and end products of glucose fermentation (1). The effect of deoxycholate on growth of the bacteria was also determined (K. Shimada and S. M. Finegold, Bacteriol. Proc., p. 87, 1969). Characteristics of species or groups are given in Table 2. The species or group designations used in this study are those used in Bergey’s Manual, 7th ed., except as follows: (i) B. fragilis includes B. fragilis, B. convexus, B. distasonis, B. ovatus, B. thetaiotaomicron, and B. vulgatus since we consider the latter five to be identical to or subspecies of B. fragilis; (ii) B. oralis as described by Loesche, Socransky, and Gibbons (8); (iii) F. fusiforme is not as described and is grouped together with F. nucleatum because of the difficulty in separating them with certainty on the basis of the tests used; (iv) S. moritferus and S. rigoridosus are also grouped together for the same reason. The species in these two groups may be separated on the basis of differences in their nucleic acid base ratios. The per cent of guanine plus cytosine (GC%) of F. fusiforme is 32 to 34, whereas F. nucleatum has a GC% of 28; S. moritferus has a GC% of 27 and S. rigoridosus has a GC% of 40 to 41 (L. V. Holdeman and W. E. C. Moore, personal communication).

The second part of the study consisted of testing 11 incompletely identified anaerobic stock strains, 71 isolates from 11 fecal specimens, and 44 isolates from 34 clinical specimens to evaluate the usefulness of the test as a rapid screening procedure; all of these organisms were originally thought to be anaerobic gram-negative bacilli.

**RESULTS**

Results obtained with 129 strains of Bacteroides species are given in Table 3. The four strains of B. corrodens were susceptible to all six antibiotics. (Only obligately anaerobic strains were considered

| Table 1. Sources of strains contributed by others |
|-----------------------------------------------|
| **Species** | **No. of strains** | **Source** | **Contributor** |
|-----------------------------------------------|
| Bacteroides fragilis | 1 | Unknown | H. R. Ingham (NCTC 9343) |
| | 1 | Unknown | J. F. Farber (ATCC 8482) |
| | 1 | Human infection | T. Bergen (ATCC 23745) |
| | 4 | Unknown | Anaerobe Laboratory-CDC |
| | 2 | Human infection | Anaerobe Laboratory-CDC |
| | 3 | Unknown | J. McDade |
| | 2 | Human infection | A. Sonnenwirth |
| | 1 | Chicken caecum | E. Barnes |
| | 3 | Human oral cavity | R. J. Gibbons |
| | 1 | Unknown | Anaerobe Laboratory-VPI |
| | 1 | Human infection | Anaerobe Laboratory-CDC |
| B. hypermegas | 1 | Unknown | H. R. Ingham (NCTC 9343) |
| B. oralis | 3 | Human oral cavity | Anaerobe Laboratory-CDC |
| B. trichoides | 1 | Unknown | Anaerobe Laboratory-CDC |
| Fusobacterium fusiforme or F. nucleatum | 1 | Human infection | Anaerobe Laboratory-CDC |
| Sphaerophorus necrophages | 1 | Duck intestine | E. Barnes |
| S. necrophorus | 1 | Unknown | A. Prévol |
| S. moritferus | 1 | Human feces | T. Pearson |
| | 1 | Human infection | V. R. Dowell |
| | 3 | Unknown | A. Sonnenwirth |
| S. ridiculosus | 1 | Unknown | Anaerobe Laboratory-VPI |
| S. varius | 7 | Human infection | Anaerobe Laboratory-VPI |

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as B. corrodens.) The 100 strains of B. fragilis were uniformly resistant to colistin and kanamycin, were usually resistant to neomycin and penicillin, were variable with erythromycin (although all showed some zone of inhibition), and were susceptible to rifampin. The 12 strains of B. melaninogenicus and 11 strains of B. oralis gave essentially the same patterns. All but one strain of B. melaninogenicus showed no zone of inhibition with kanamycin. This one had a zone of 15 mm. All were susceptible to erythromycin, penicillin, and rifampin and were variable with colistin and neomycin. None of the B. melaninogenicus strains required menadione. Only one strain each of B. trichoides and B. hypermegas was available for study. B. trichoides was resistant to neomycin and rifampin and sensitive to the other antibiotics. B. hypermegas was resistant to penicillin but sensitive to the other antibiotics.

Results obtained with 58 strains of Fusobacterium and Sphaerophorus species are given in Table 4. One strain of F. fusiforme-nucleatum was resistant to erythromycin, and one other strain was resistant to neomycin. The remainder were susceptible to some degree to the six antibiotics. The one strain of S. necrophorus and four strains of S. necrophorus were susceptible to all antibiotics tested. S. moriferus (8 strains), S. ridiculosus (1 strain), and S. varius (15 strains) were grouped together because of their similarity in susceptibility patterns. An additional eight strains which are either S. moriferus or S. ridiculosus are also included. They were all susceptible to colistin, kanamycin, and penicillin, most were susceptible to neomycin, and most were resistant to erythromycin and rifampin. The S. moriferus-ridiculosus-varius group always exhibited a thin film of inhibited growth or what appeared to be inhibited colonies within the zone of inhibition around the penicillin disc. Microscopic examination of wet mounts of this material revealed numerous round bodies which were not visible by Gram-stained smear. Subcultures to blood-agar and to osmotically stabilized agar (Brain Heart Infusion agar with 0.5 M sucrose and 0.1 mg of MgSO₄ per liter) did not grow. It seems probable that this phenomenon is similar to that observed with sulfonamide susceptibility testing of aerobes and facultative bacteria, that of limited growth occurring prior to inhibition (7). Occasional strains of the Fusobacterium species show resistant colonies within the zone of inhibition around the penicillin disc with a second zone of inhibited growth at the periphery of the zone.

Table 5 summarizes the data given in Tables 3 and 4, grouping the various species encountered in human infections and as normal flora by their usual susceptibility patterns.

FIG. 1. Schema for rapid presumptive identification of gram-negative anaerobic bacilli. Symbols in parentheses indicate occasional variations.
Tests to determine reproducibility of the method indicated that, in triplicate determinations done on the same day, zone diameters varied from zero to 4 mm. The day to day variation was from 0 to 10 except in the case of the *Fusobacterium* strain with penicillin. On the first day the zones of inhibition were 40 to 44 mm, on the second day they were 28 to 30 mm, and on the third day they were 27 to 28 mm. However, variations that occurred with each strain were not different from the range of zone diameters observed with other bacteria of the same group or species.

Among the 11 unidentified stock strains tested, 6 had patterns of susceptibility of *Bacteroides* species, whereas 5 gave patterns inconsistent with any of the patterns in Table 3. One of these has subsequently been identified as *Clostridium ramosum*; the other four are anaerobic gram-positive nonsporeforming bacilli needing further tests for exact identification. Of the 71 isolates from fecal specimens, 40 were easily identified as *B. fragilis* and 7 as belonging to the *S. mortiferus-ridiculosus* group on the basis of their susceptibility patterns. One strain was identified as a *B. melaninogenicus* on the basis of susceptibility and pigment production. The remaining 23 isolates gave aberrant patterns, and most will require extensive testing to determine their identity. Four have been found to be *Eubacterium*, one was a *Clostridium*, and one was a *Peptostreptococcus*;

### Table 2. Characteristics of gram-negative

| Criterion                                      | *Bacteroides corrodens* | *B. fragilis* | *B. hypermegas* | *B. melaninogenicus* | *B. oralis* |
|-----------------------------------------------|-------------------------|---------------|-----------------|----------------------|-------------|
| Colonial morphologya                         | Smooth, translucent, depressed in agar or depression in agar around colonies | Smooth, entire, gray-white | Smooth, entire, translucent | Brown-black | Similar to *B. fragilis* |
| Microscopic morphologyb                      | Rounded ends, rare filaments, occasional bipolar staining | Rounded ends, mod. pleomorphic filaments, bipolar staining | Very large, rounded ends, granular | Cocobacillary to bacillary, rounded ends | Usually — |
| Reaction on egg yolk-agar                    | —                       | —             | —               | —                    | —           |
| Production of indole                         | —                       | —/—/—        | —               | +/—                  | —           |
| Reduction of nitrate                         | +                       | —/—/—        | —               | —/—                  | —           |
| Effect of bile plus deoxycholate on growth   | Inhibited               | Not inhibited, may be stimulated | Inhibited      | Inhibited            | Inhibited |
| Effect of deoxycholate on growth             | Not inhibited, may be stimulated | Inhibited      | Inhibited      | Inhibited            | Inhibited |
| Esculin hydrolysis                           | —                       | +            | +               | +/—                  | +           |
| Butyric acid from glucose fermentation       | —                       | —            | —               | —/—                  | —           |
| Acid reaction in glucose                     | —                       | +            | +               | +/—                  | +           |
| Acid reaction in other carbohydrates         | —                       | +            | +               | +/—                  | +           |

a Blood-agar plate.

b Fluid Thioglycollate Medium.
nonsporeforming nonmotile anaerobic bacilli

| B. trichoides | Fusobacterium fusiforme-nucleatum | Sphaerophorus necrophorus | S. necrophorus | S. mortiferus-ridiculosus | S. varius |
|---------------|---------------------------------|--------------------------|----------------|-------------------------|----------|
| Smooth, entire, translucent | Smooth or rough, entire to irregular, often with internal iridescent flecking | Smooth, entire, white | Matt, umbonate, opaque center, translucent, irregular edge | Smooth to matt, flat to umbonate "fried egg" appearance | Similar to S. mortiferus and S. ridiculosus |
| Similar to B. fragilis | Slender, with tapered ends | Cocacobacillar | Pleomorphic, filaments and swellings | Very pleomorphic with swellings and large round bodies | Similar to S. mortiferus and S. ridiculosus |
| — | — | — | — | — | — |
| — | + | — | + | — | +/— |
| — | — | — | — | — | — |
| Inhibited | Inhibited | Inhibited | Usually inhibited | Not inhibited | Not inhibited |
| Inhibited | Inhibited | Not inhibited, may be stimulated | Inhibited | Not inhibited, may be stimulated | Not inhibited, may be stimulated |
| + | — | + | — | — | — |
| — | + | + | + | + | + |
| + | Weak | Weak | Weak | +/Weak | —/Weak |
| + | —/Weak | —/Weak | —/Weak | +/Weak | —/Weak |

the remaining isolates are probably gram-positive bacteria, but identification is not complete. Table 6 illustrates the aberrant patterns exhibited by the stock strain identified as C. ramosum and the isolates from feces that have been identified.

Of the 44 isolates from clinical specimens, 20 were identified as B. fragilis, 6 as B. oralis-melaninogenicus group, and 3 as F. fusiforme-nucleatum-necrophorus group or B. corrodens on the basis of susceptibility tests. The B. oralis-melaninogenicus strains were distinguished as three B. oralis and three B. melaninogenicus on the basis of pigment production on laked blood-agar. Two strains in the last group were further identified as F. fusiforme-nucleatum on the basis of cellular morphology and failure to produce lipase on egg yolk-agar; one was identified as B. corrodens by the characteristic pitting of agar around the colonies. The remaining 15 strains require further identification.

The identity of all gram-negative anaerobic bacilli defined above from both fecal and clinical specimens was confirmed by colonial and microscopic morphology, pigment production, their ability to grow in bile broth and deoxycholate broth, production of indole, reduction of nitrate, hydrolysis of esculin, by the end pH in glucose broth, and by reaction on egg yolk-agar. Analysis of end products of glucose fermentation was done on selected strains.

**DISCUSSION**

The presently recommended schema for identification of gram-negative anaerobic bacilli is
based on numerous biochemical characteristics (1, 3, 4); this may represent a burden for many diagnostic laboratories. Clinical specimens from which anaerobic bacteria are isolated often con-

**Table 3. Susceptibility patterns of Bacteroides species**

| Antibiotic disc | Zone diameter | B. corrodens | B. fragilis | B. melaninogenicus | B. oralis |
|-----------------|---------------|--------------|-------------|--------------------|----------|
| Colistin (10 µg) | No zone <10   | 100          | 3           | 4                  |          |
|                 | 10-19         | 100          | 3           | 4                  |          |
|                 | 20-29         | 3            | 2           | 5                  |          |
|                 | 30-39         | 1            | 2           | 5                  |          |
| Erythromycin (60 µg) | 10-19   | 100          | 3           | 4                  |          |
|                 | 20-29         | 2            | 2           | 4                  |          |
|                 | 30-39         | 2            | 1           | 1                  |          |
|                 | >39           | 2            | 1           | 1                  |          |
| Kanamycin (1,000 µg) | No zone <10   | 100          | 1           | 1                  |          |
|                 | 10-19         | 100          | 1           | 1                  |          |
|                 | 20-29         | 2            | 1           | 1                  |          |
|                 | 30-39         | 2            | 1           | 1                  |          |
| Neomycin (1,000 µg) | No zone <10   | 78           | 2           | 1                  |          |
|                 | 10-19         | 78           | 2           | 1                  |          |
|                 | 20-29         | 78           | 2           | 1                  |          |
|                 | 30-39         | 78           | 2           | 1                  |          |
| Penicillin (2 units) | No zone <10   | 93           | 3           | 4                  |          |
|                 | 10-19         | 93           | 3           | 4                  |          |
|                 | 20-29         | 93           | 3           | 4                  |          |
|                 | 30-39         | 93           | 3           | 4                  |          |
| Rifampin (15 µg) | No zone <10   | 93           | 3           | 4                  |          |
|                 | 10-19         | 93           | 3           | 4                  |          |
|                 | 20-29         | 93           | 3           | 4                  |          |
|                 | 30-39         | 93           | 3           | 4                  |          |

* Number of strains tested were as follows: B. corrodens, 4; B. fragilis, 100; B. melaninogenicus, 12; B. oralis, 11.

* The largest zone diameter observed was 20 mm.

* Usually resistant colonies or inhibited growth within zone of inhibition.

tain mixtures of both aerobes, facultatives, and anaerobes (9, 10). Even after the individual strains are isolated and studied by these schema, there remain a number which cannot be identified adequately. In addition, on initial isolation, colony and cellular morphology are sufficiently variable that one may isolate two or more colonies which later are found to be the same strain. Screening of isolates by rapid tests would allow presumptive identification with an early preliminary report, and further tests would then be required on only a proportion of the cultures.

The differences in susceptibility to discs containing selected antibiotics as described have proved to be valuable in our laboratory in rapid screening of isolates from clinical specimens as well as fecal specimens. Within 48 hr after isolation of bacteria thought to be gram-negative anaerobic bacilli, two-thirds of them were grouped by the patterns noted in Table 5. Of the remaining strains, reexamination of Gram reaction, morphology, a more diligent search for spores, and other tests revealed most were either clostridia or nonsporulating anaerobic gram-positive bacteria. Thus far, none of the strains exhibiting aberrant susceptibility patterns has proved to be a species of Bacteroides, Fusobacterium, or Sphaero-

Phorus of human origin.

Examination of a few additional characteristics allowed further and more reliable differentiation of the groups. For example, the Clostridium sp. shown in Table 6 had a susceptibility pattern suggestive of the B. melaninogenicus-oralis group, but in confirmatory tests this strain grew without inhibition in 20% bile plus 0.1% deoxycholate and was found to form spores in older cultures. Other characteristics such as colony morphology, production of black pigment on laked blood-agar, inhibition of growth in 0.1% deoxycholate, esculin hydrolysis, and production of lipase on egg yolk-agar are also necessary for more reliable differen-

tiation of anaerobic gram-negative bacilli. A suggested schema is presented in Fig. 1.

As with any schema based on a few characteristics, there are obvious limitations to this schema. Strains aberrant in any of the key features and confirmatory tests could not be identified, and additional tests would be required. In our experience with 126 isolates, only 43 were not identifiable by the schema and required extensive testing to establish their identity.

This schema appears to provide a rapid, rea-

sonably reliable differentiation of gram-negative anaerobic bacilli for routine clinical purposes and for initial screening of the numerous isolates made in studies of intestinal flora. It is not intended to
### Table 4. Susceptibility patterns of Fusobacterium and Sphaerophorus species

| Antibiotic disc | Zone diameter | Fusobacterium nucleatum-fusiforme | Sphaerophorus necrogenes | S. necrophorus | S. mortiferus-ridiculosus-varius |
|----------------|---------------|-----------------------------------|--------------------------|----------------|----------------------------------|
| Colistin (10 μg) | 10–19         | 4                                 | 2                        | 23             | 9                               |
|                | 20–29         | 17                                | 1                        | 2              | 9                               |
| Erythromycin (60 μg) | No zone       | 1                                 | 31                       |                |                                  |
|                | 10–19         | 6                                 | 1                        |                |                                  |
|                | 20–29         | 4                                 | 4                        |                |                                  |
|                | 30–39         | 4                                 | 4                        |                |                                  |
|                | >39           | 6                                 |                          |                |                                  |
| Kanamycin (1,000 μg) | 10–19         | 1                                 | 1                        |                |                                  |
|                | 20–29         | 1                                 | 25                       |                |                                  |
|                | 30–39         | 14                                | 3                        | 3              |                                  |
|                | >39           | 4                                 | 1                        |                |                                  |
| Neomycin (1,000 μg) | No zone       | 1                                 | 1                        |                |                                  |
|                | 10–19         | 15                                | 3                        | 10<sup>a</sup> |                                  |
|                | 20–29         | 4                                 | 1                        | 20<sup>a</sup> |                                  |
|                | 30–39         | 4                                 | 1                        | 1<sup>b</sup>  |                                  |
|                | >39           | 1                                 |                          |                |                                  |
| Penicillin (2 units) | 10–19         | 1                                 | 1                        | 7<sup>c</sup>  |                                  |
|                | 20–29         | 1                                 | 22<sup>c</sup>           |                |                                  |
|                | 30–39         | 4                                 | 1                        | 3<sup>c</sup>  |                                  |
|                | >39           | 16                                | 3                        |                |                                  |
| Rifampin (15 μg) | No zone       | 2                                 | 2                        | 3              |                                  |
|                | 10–19         | 2                                 | 2                        |                |                                  |
|                | 20–29         | 6                                 | 1                        |                |                                  |
|                | 30–39         | 9                                 | 2                        |                |                                  |
|                | >39           | 4                                 |                          |                |                                  |

<sup>a</sup> Number of strains tested were as follows: *Fusobacterium fusiforme* and *F. nucleatum*, 21; *Sphaerophorus necrogenes*, 1; *S. necrophorus*, 4; *S. mortiferus*, *S. ridiculosus*, and *S. varius*, 32.

<sup>b</sup> Frequently with resistant colonies within the zone of inhibition.

<sup>c</sup> Always inhibited growth or resistant colonies within the zone of inhibition.

### Table 5. Most frequently observed patterns of susceptibility of major groups of Bacteroidaceae

| Antibiotic disc | Bacteroides corrodens | B. fragilis | B. melaninogenicus-oralis | P. fusiforme-nucleatum-oralis | S. varius-mortiferus-ridiculosus |
|----------------|-----------------------|------------|---------------------------|--------------------------------|---------------------------------|
| Colistin (10 μg) | S (20–39)<sup>a</sup> | R<sup>b</sup> | S/R (no zone-29)          | S (20–29)                      | S (10–19)                      |
| Erythromycin (60 μg) | S (30–39)              | S (20–39)  | S (20–39)                 | S (10–29)                      | R                              |
| Kanamycin (1,000 μg) | S (>39)               | R          | S (30–39)                 | S (10–29)                      | S (20–29)                      |
| Neomycin (1,000 μg) | S (>39)               | R          | S (10–29)                 | S (10–29)                      | S (10–29)<sup>e</sup>          |
| Penicillin (2 units) | S (>39)               | R          | S (>39)                   | S (30–39)                      | S (20–29)<sup>d</sup>          |
| Rifampin (15 μg)   | S (20–39)             | S (>39)   | S (>39)                   | S (20–39)                      | R                              |

<sup>a</sup> Most frequently observed zone diameters in millimeters in parentheses.

<sup>b</sup> No zone.

<sup>c</sup> Frequently with resistant colonies within the zone of inhibition.

<sup>d</sup> Always resistant colonies or inhibited growth within the zone of inhibition.
TABLE 6. Patterns of susceptibility of strains subsequently identified as gram-positive bacteria

| Antibiotic disc | Clostridium ramosum | Clostridium sp. | Eubacterium limosum | Eubacterium limosum | Eubacterium sp. | Eubacterium sp. | Peptostreptococcus |
|-----------------|---------------------|-----------------|---------------------|---------------------|----------------|----------------|-------------------|
| Colistin (10 μg) | R<sup>a</sup>       | R               | R                   | R                   | R              | R              | R                 |
| Erythromycin (60 μg) | R       | S (42)<sup>b</sup> | R                   | R                   | S (25)         | S (25)         | S (40)            |
| Kanamycin (1,000 μg) | R     | R               | R                   | S (25)              | R              | R              | R                 |
| Neomycin (1,000 μg) | S (13) | S (18)          | S (20)              | S (10)              | S (18)         | S (18)         | S (25)            |
| Penicillin (2 units) | S (19) | S (30)          | R                   | R                   | S (23)         | S (15)         | S (42)            |
| Rifampin (15 μg)  | R       | S (12)          | R                   | R                   | R              | R              | S (35)            |

<sup>a</sup> No zone.
<sup>b</sup> Number in parentheses is the zone diameter in millimeters.

replace those methods recommended for more exacting taxonomic studies.

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