Isolation of *Corynebacterium* Group JK from Clinical Specimens with a Semiselective Medium

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A semiselective medium for the detection of multiresistant lipophilic corynebacteria is described. The medium consisted of tryptose agar, supplemented with Tween 80, lecithin, histidine, glycerol, sodium thiosulfate, fosfomicin, ticarcillin, and 5-fluorocytosine. The medium was tentatively called lecithin-Tween medium (LT medium). It promoted mainly the growth of *Corynebacterium* group JK, which has recently been identified as a cause of serious infection in immunocompromised patients. The application of LT medium to 6,859 routine clinical specimens increased the percentage of *Corynebacterium* group JK isolation from 0.1 to 1.0%. Although a total of 72 isolates were found in 65 patients, on the basis of clinical criteria only 2 patients were regarded as having an infection with these bacteria. Consequently, *Corynebacterium* group JK infection can only be diagnosed through a combination of clinical and microbiological findings. In high-risk areas, however, the use of LT medium for surveillance may facilitate the early detection of these potentially dangerous organisms.

Corynebacteria or diphtheroids are often isolated from various clinical specimens. These bacteria are generally disregarded as contaminants. In recent years, there has been evidence that diphtheroids can cause serious and life-threatening infections in immunocompromised hosts. One species of diphtheroids has been identified and classified as *Corynebacterium* group JK (6). A significant feature of this species is its resistance to almost all presently available antibiotics. Infections with *Corynebacterium* group JK have been described in patients with prosthetic valve endocarditis (4), leukemia (3, 8), polytrauma (10), meningitis (S. Hoffmann, H. Ersgaard, T. Justesen, and H. Friis, Eur. J. Microbiol., in press), and other clinical conditions (1, 11). Since no detailed information about the prevalence of these bacteria in clinical specimens was available, we developed a semiselective medium for the isolation of resistant lipophilic corynebacteria. By correlating the clinical data with the microbiological findings, we hoped to provide information about the true incidence of infections with *Corynebacterium* group JK.

MATERIALS AND METHODS

Clinical specimens. A total of 6,859 clinical specimens were included in the study. Table 1 lists the various materials and the percentage of *Corynebacterium* group JK isolation. Of these, 4,947 specimens (72%) came from inpatients. Our medical center has 1,300 acute medical and surgical beds. Patients in the intensive care units and oncological wards were the source of 1,400 (20%) of the specimens. Since this institute also serves as a microbiological laboratory for a population of ca. 400,000, 1,912 (28%) of the specimens were from outpatients (mainly urine and throat swabs).

Definition of *Corynebacterium* group JK infection. The definition of an infection with this bacterium was based on the following criteria (1): (i) isolation of *Corynebacterium* group JK as the only isolate in cultures from significant sites (blood, catheter tips, wound swabs, drain sites); (ii) symptoms and signs of bacterial infection (e.g., pus, fever, delayed wound healing); (iii) persistence of symptoms after the removal of venous catheters, when the bacteria were

isolated from blood or catheter tips; (iv) failure of conventional antibiotic therapy; (v) ability of vancomycin to improve symptoms and to eradicate *Corynebacterium* group JK.

Medium. The following lecithin-Tween (LT) medium was used for the isolation of resistant corynebacteria: tryptose agar (Difco Laboratories, Detroit, Mich.) supplemented with 3.0% Tween 80 (vol/vol; Sigma Chemical Co., St. Louis, Mo.), 0.5% phosphatidyl choline (lecithin) type IX from egg yolk (wt/vol; Sigma), 0.1% histidine, free base (wt/vol; Sigma), 0.5% sodium thiosulfate (wt/vol; E. Merck AG, Darmstadt, Federal Republic of Germany), 0.3% glycerol (vol/vol), 100 µg of ticarcillin per ml (kindly provided by Johann Wülfing-Beecham, Neuss, Federal Republic of Germany), 200 µg of 5-fluorocytosine per ml (Hoffmann-La Roche, Inc., Basel, Switzerland), and 100 µg of fosfomicin per ml (kindly provided by Boehringer, Mannheim, Federal Republic of Germany) containing 30 mM glucose-6-phosphate.

Fosfomicin is a broad-spectrum antibiotic that does not share any similarities with other antibiotics. The antibacterial spectrum includes gram-positive cocci and gram-negative rods. *Klebsiella* spp., *Providencia* spp., and *Morganella morganii* are mostly resistant to fosfomicin. The supplements without antibiotics were prepared as a 20-fold concentrate and intensively stirred at 70°C. The antibiotics were filtered through a disposable sterile filter unit (Millipore Corp., Bedford, Mass.) and added to a cooling medium. All specimens were routinely processed on blood agar (blood agar base, Oxoid no. CM 55 supplemented with 10% sheep blood), Endo agar (Endo agar base, Oxoid no. CM 479), and liver agar broth and anaerobically on Schaedler agar (Schaedler anaerobic agar, Oxoid no. CM 437 supplemented with 10% sheep blood). For some experiments egg yolk medium (Lowenstein-Jensen medium, Oxoid no. PM 1) and nutrient agar (E. Merck, AG) were employed. Antibiotic susceptibility testing was performed on DST agar (Oxoid Ltd., London, England) supplemented with sheep blood by using a standard disk diffusion method.

Biochemical differentiation. *Corynebacteria* were identified as group JK by using the criteria of the Centers for

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TABLE 1. Isolation of *Corynebacterium* group JK from various clinical materials by using LT medium and blood agar

Material	No. of isolates	No. positive on blood agar ^a	No. positive on LT medium ^b
Blood culture	326	2	2
Catheter tips	542	0	6
Cerebrospinal fluid	107	0	0
Swabs	1,304	1	14
Throat swabs	1,104	0	0
Sputum	575	0	0
Urine	2,514	3	50
Other materials	387	0	0
Total	6,859	6	72

^a % Positive, 0.09.^b % Positive, 1.05.

Disease Control (6). *Corynebacterium* group JK is a small, gram-positive rod, catalase positive and oxidase, urea, and nitrate negative. Acid from glucose (57%), maltose (35%), and galactose (59%) was produced only after prolonged incubation (48 to 72 h) and the addition of 2% sterile horse serum. The bacteria usually (85%) hydrolyzed Tween 80 and were resistant to penicillin (99%; see Table 3). All isolates were susceptible to vancomycin (see Table 3) and were ONPG negative (ONPG paper disks; BioMérieux, Nuertingen, Federal Republic of Germany).

RESULTS

Corynebacteria of the group JK grew on blood agar as very small, grey-white colonies without hemolysis. Contamination with other bacteria, i.e., *Staphylococcus* spp., resulted in overgrowth and failure to detect these corynebacteria. The bacteria did not grow in most liquid media during the first 48 h of incubation unless such media were supplemented with a lipid source or at least 1.0% serum. These

corynebacteria did not grow on Endo agar or nutrient agar. However, *Corynebacterium* group JK grew slowly on egg yolk medium (Lowenstein-Jensen). Since every type of routine culture medium tested did not yield satisfactory results, we used LT medium containing several antibiotics. In contrast to other media, the antibiotics suppressed other bacteria effectively, whereas the supplements supported the growth of fastidious resistant corynebacteria. Figure 1 shows the colony formation of *Corynebacterium* group JK on LT medium after 48 h of incubation at 37°C. As can be seen, the bacteria form yellow-white colonies, 2 to 4 mm in diameter. On LT medium, group JK corynebacteria form a halo, which may be caused by lipase production in Tween-containing media (7). This halo formation was seen with 69 isolates (85%) after 48 to 72 h of incubation at 37°C (Fig. 1). Further experiments with a different composition of supplements showed that the medium promoted growth of fastidious corynebacteria only when optimally supplemented. Table 2 presents the data for different compositions of LT medium and other media. All five supplements were necessary for the early detection of the bacteria. Other combinations of supplements tested were unsatisfactory.

The choice of antibiotics focused on an effective suppression of *Enterobacteriaceae*, *Pseudomonas* spp., *Staphylococcus* spp., and fungi. Since data about gentamicin resistance of *Corynebacterium* group JK showed considerable variation (4, 6, 8), aminoglycosides were not employed. The same is also true of cephalosporins. All previously isolated group JK organisms from our laboratory, however, were resistant to fosfomicin and ticarcillin. Consequently, these antibiotics were used together with the antifungal agent 5-fluorocytosine. The antibiotics were shown to be sufficiently active after storage for 4 weeks at 4°C.

Alteration of pH of the medium showed that group JK organisms grow at a pH between 5.6 and 8.6 but that optimal growth is obtained between pH 7.4 and 7.8. Consequently, the pH of the medium was adjusted to 7.5 to 7.6.

The use of LT medium for almost 7,000 clinical specimens showed that the isolation of *Corynebacterium* group JK could be increased from 0.09 to 1.05%, as shown in Table 1.

The antibiotic susceptibility patterns of all 72 isolates and of 78 strains isolated during a 2-year period (1980-1982) before the use of LT medium are given in Table 3. No

FIG. 1. Pure culture of *Corynebacterium* group JK on LT medium after 48 h of incubation at 37°C. Yellow-white colonies, 2 to 4 mm of growth. The double contour of halo around every colony is indicative of lipolytic activity.

TABLE 2. Growth of *Corynebacterium* group JK on different media^a

Agar medium	Supplement	Growth after 24 h	Growth after 48 h
Nutrient	0	0	0
Tryptose	0	0	(+)
Blood	0	+	++
Tryptose	Tween 80	0	+
Tryptose	Tween 80 + lecithin	(+)	++
Tryptose	Tween 80 + lecithin + histidine	+	++
Tryptose	Tween 80 + histidine	0	+
Tryptose	Tween 80 + lecithin + histidine + sodium thiosulfate	+	+++
Tryptose	Tween 80 + lecithin + histidine + sodium thiosulfate + glycerol	++	++++

^a Symbols: 0, no growth; (+), colonies smaller than 0.1 mm; +, colonies 0.1 to 0.3 mm; ++, colonies 0.4 to 0.5 mm; +++, colonies 0.6 to 0.8 mm; +++++, colonies more than 0.8 mm.

TABLE 3. Antibiotic susceptibility of 150 strains of *Corynebacterium* group JK as measured with a standard disk diffusion method

Antibiotic	No. of sensitive strains	% Resistant strains
Penicillin	1	99
Oxacillin	1	99
Ampicillin	2	99
Mezlozillin	7	95
Ticarcillin	0	100
Cephalothin	2	99
Cefazedone	3	98
Moxalactam	2	99
Chloramphenicol	2	99
Metronidazole	0	100
Fosfomicin	0	100
Netilmicin	21	86
Gentamicin	30	80
Amikacin	20	87
Lincomycin	60	60
Erythromycin	116	23
Minocycline	133	11
Vancomycin	150	0

significant differences could be seen in the antibiotic susceptibility of the isolates from LT medium and the earlier isolates.

Using blood agar incubated in a normal atmosphere at 37°C yielded only one *Corynebacterium* group JK from 1,304 swabs; 15 of these organisms were isolated when LT medium was employed. These swabs were taken from the perianal region (abscesses), 3; from the inguinal region (lymph node abscess), 1; from the testes, 1; from the tibia (osteomyelitis), 3; from a pustule, 1; from the middle ear, 2; from the mouth, 2; and from an unidentified wound, 1. Fifty strains of *Corynebacterium* group JK were isolated from urine specimens with LT medium, as compared with three on blood agar alone. Of the 50 isolates, 16 (32%) were found in pure culture. None of these patients, however, had a clinically relevant urinary tract infection at the time of isolation.

Based on the clinical criteria described above, only two patients could be defined as having an infection with *Corynebacterium* group JK. One of these two patients suffered from severe complications after pancreatic surgery; the other, from osteomyelitis. Both patients received prolonged antimicrobial therapy. After vancomycin treatment, the symptoms improved, and *Corynebacterium* group JK was not isolated from subsequent specimens.

DISCUSSION

These experiments were designed to develop a selective medium for resistant lipophilic corynebacteria, which cause serious infections in immunocompromised hosts and in patients with prosthetic valve endocarditis (1, 3, 4, 8, 11). Although the exact taxonomy is still a matter of controversy, *Corynebacterium* group JK seems to be the main representative of these resistant organisms. In the presence of other microorganisms, these bacteria are often overgrown in clinical specimens. Consequently, antibiotic resistance seemed the only attribute of a selective medium. We are aware that this choice may represent a negative selection, since susceptible strains may be suppressed (2, 4, 6).

Previous studies demonstrated that *Corynebacterium*

group JK requires lipid supplements for optimal growth and biochemical activity (6, 8, 9). Therefore, a Tween-containing medium, originally described for disinfectant testing (5), was used as a basis for our experiments. As far as we know, detailed studies about the prevalence of *Corynebacterium* group JK in unselected clinical material have not been performed. The relatively low frequency of *Corynebacterium* group JK isolations may reflect overgrowth by companion organisms in routine cultures. With LT medium, the prevalence of these organisms in clinical material was found to be 1.05%. Most isolates were from urine and the inguinal and gluteal areas. The correlation of *Corynebacterium* group JK isolation with the corresponding clinical data revealed, however, that only 3% of the isolates could be considered as the cause of serious infections. On the basis of previous findings (2–4, 8) and those from our laboratory (1, 10, 11), it may be concluded that the diagnosis of *Corynebacterium* group JK infection can only be based on a combination of microbiological and clinical findings. Thus, the semiselective medium described here may be especially useful in the surveillance of high-risk areas, where immunocompromised patients are being treated.

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