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Experience with a Novel Selective Medium for Isolation of *Actinomyces* spp. from Medical and Dental Specimens

R. LEWIS,[1]* D. McKENZIE,[2] J. BAGG,[2] AND A. DICKIE[1]

Department of Bacteriology, Victoria Infirmary, Langside, Glasgow G42 9TY,[1] and Oral Microbiology Unit, Department of Oral Sciences, Glasgow Dental Hospital and School, Glasgow G2 3JZ,[2] United Kingdom

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A selective medium containing mupirocin and metronidazole was evaluated for the isolation of *Actinomyces* spp. from clinical material. The study was a one-year prospective comparison of the method with existing methods, which use nonselective media, at a general hospital and a dental hospital. Significantly more *Actinomyces* spp. were isolated on the selective medium than on nonselective media from both dental specimens and intrauterine contraceptive devices. However, differentiating between *Actinomyces* spp. and related non-sporulating gram-positive rods remains a slow and sometimes uncertain process which can introduce lengthy delays in reporting.

Actinomycosis appears to be a rare disease in the United Kingdom at present. Since there is no statutory notification scheme in force, the exact incidence is uncertain, but in 1992 only 30 cases of isolation of the principal causative organism, *Actinomyces israelii*, were reported by laboratories in England and Wales to the Communicable Diseases Surveillance Centre, Central Public Health Laboratory, London (3a). Most of the isolates were from either the mouth or the female genital tract, sites where the profuse commensal flora can make isolation and recognition of *Actinomyces* spp. difficult. The successful use of metronidazole as a selective agent in the isolation of *A. israelii* from the female genital tract has been described (17). However, little is known of its value for other species and sites, and the gram-positive facultative flora of the genital tract is not inhibited by it. Conversely, media designed for the isolation of microaerophilic *Actinomyces* spp. from dental plaque, while inhibiting growth of staphylococci and streptococci, are of uncertain value for the isolation of *A. israelii* (3, 12).

We report here a prospective comparison of a method using selective medium containing mupirocin and metronidazole with an in-house method, based on nonselective media, for both dental and medical specimens requiring culture for *Actinomyces* spp.

MATERIALS AND METHODS

Specimens examined. Specimens were received at two separate laboratories during the period March 1992 to April 1993. At the Victoria Infirmary, Glasgow, all intrauterine contraceptive devices (IUCDs) received for culture in the Bacteriology Laboratory, as well as all other appropriate specimens in which culture for *Actinomyces* spp. appeared indicated from the clinical details, were included in the study.

In the Microbiology Laboratory of the Glasgow Dental Hospital and School, aspirates and swabs from purulent orofacial infections were included. Although aspirates are the specimens of choice, these are sometimes difficult to collect. Half of the specimens received for the present study were swabs. Aspirates were received in the syringe, whereas swabs were generally received dry, transported directly from clinical areas within the hospital. Delay between collection and processing did not exceed 1 h.

At the Victoria Infirmary, semisolid storage medium, VMG IV (16), was made available to some of the Family Planning Clinics for use with IUCDs. All swabs were received in Stuart's transport medium (Medical Wire & Equipment, Corley, Wiltshire, United Kingdom), and all other IUCDs were received in plain sterile universal containers.

Culture media. At the Victoria Infirmary, the following routine culture media were used. Columbia blood agar (CBA) was prepared from dehydrated Columbia agar (Life Technologies, Paisley, Scotland) with 5% sterile defibrinated horse blood. Gentamicin blood agar consisted of CBA containing 14 mg of gentamicin per liter. MacConkey agar (Oxoid CM7; Unipath, Basingstoke, United Kingdom), de Man, Rogosa, and Sharpe (MRS) agar (Oxoid CM361; Unipath), and Brewer's modified thioglycolate medium (BBL 11716; Becton Dickinson, Cowley, Oxford, United Kingdom) were prepared from dehydrated media according to the manufacturer's instructions.

Mupirocin-metronidazole blood agar (MMBA) consisted of CBA with the addition of 128 mg of mupirocin per liter (Smithkline Beecham Pharmaceuticals, Welwyn Garden City, Herts, United Kingdom) and 2.5 mg of metronidazole per liter (Adatabs; Mast Ltd., Bootle, Merseyside, United Kingdom). Batches of medium were considered suitable for use if they supported the growth of *A. israelii* NCTC 4860 (National Collection of Type Cultures, Colindale, England) after 3 days of anaerobic incubation while preventing the growth of *Enterococcus faecalis* NCTC 775 and of *Bacteroides fragilis* (wild-type strain). A shelf life of one week from quality control was assigned.

There were some slight differences in the routine media used at the Glasgow Dental Hospital, as follows. The Columbia agar contained 7.5% sterile defibrinated horse blood and was supplemented with 10 ml per liter of vitamin K-hemin solution (Life Technologies). Rogosa medium (Difco, West Molesey, Surrey, United Kingdom) was used for detection of lactobacilli. Fastidious anaerobe broth (FAB) (Lab M, Bury, Lancashire, United Kingdom) was used as the fluid enrichment medium.

Culture methods. At the Victoria Infirmary, IUCDs and pus were inoculated directly onto solid media and streaked out into four quadrants with a bacteriological loop as follows: (i) CBA and MacConkey agar, incubated in air plus 5% CO_2 and examined at 24 and 48 h; (ii) gentamicin blood agar, with metronidazole (5-μg disc), incubated anaerobically and examined at 48 h; (iii) CBA and MMBA, incubated anaerobically and examined after 7 days. Finally, the swab or device was placed in Brewer's medium (20 ml) and incubated anaerobically for 5 to 7 days. All anaerobic incubation was carried out in an anaerobic cabinet (Don Whitley Mark 3 Anaerobic Workstation).

The culture methods employed at the Glasgow Dental Hospital and School were identical, except that a MacConkey agar plate was not incubated routinely for the dental specimens and FAB was used instead of Brewer's medium.

None of the specimens received contained macroscopically visible "sulfur granules," and direct Gram staining of specimens was not performed.

Isolation and identification of organisms. Organisms growing at 24 and 48 h were investigated in accordance with existing laboratory routine. Briefly, pyogenic organisms such as staphylococci and beta-hemolytic streptococci were identified to the species level. Other organisms were identified usually to the genus level, except for non-spore-forming obligate anaerobes, which were identified on the basis of the Gram stain result (2). On day 7 all colony types on the anaerobically incubated MMBA and CBA plates were Gram stained. However, if growth on the CBA was judged to be too profuse and mixed for Gram staining to be feasible, then the MMBA plate alone was investigated.

Gram-positive rods (GPR), regardless of exact morphology, were subcultured to CBA incubated in air plus 5% CO_2, to CBA incubated anaerobically, and to MRS agar incubated anaerobically. Plates were examined after 5 to 7 days of incubation. Catalase-negative organisms producing creamy white colonies on MRS agar which were larger than colonies on the other two media were reported as *Lactobacillus* spp. All other catalase-negative GPR were tested for their enzyme profile with API-ZYM (Biomérieux Ltd., Basingstoke, Hants, United Kingdom) and then referred to the Glasgow Dental Hospital for further identi-

* Corresponding author. Phone: 0141-201-5602. Fax: 0141-649-2206.

TABLE 1. Specimens entered into the study

Specimen type	No. of specimens			
	Victoria Infirmary	Glasgow Dental Hospital	Excluded subsequently[a]	Total (n = 226)
IUCD	143	0	10	133
Pus from dental abscess	14	64	3	75
Other	20[b]	0	2	18

[a] Exclusions were due to violations of culture protocol (7 specimens), loss of strains before identification (6 specimens), and duplicate specimens (2 specimens).

[b] Parotid and submandibular abscesses (10 specimens), tonsillar pus (6 specimens), mandibular bone (2 specimens), cervical lymph node (1 specimen), pus from lacrimal duct (1 specimen).

fication. Catalase-positive GPR producing better growth in air plus 5% CO_2 than anaerobically were not examined further and are described in the Results section as aerobic coryneforms. Other catalase-positive GPR were referred to the Glasgow Dental Hospital for identification. Organisms giving other results upon Gram staining were identified, usually to the genus level only, by routine methods (2).

The Brewer's medium was also examined macroscopically at 5 to 7 days and subcultured, if growth was visible, to MMBA, which was then incubated anaerobically for a further 5 to 7 days and processed as described above.

At the Glasgow Dental Hospital, the primary CBA and MMBA plates were examined after 7 days of incubation. Colonies of different morphological types from the CBA plates and MMBA plates were subcultured to fresh CBA plates and incubated anaerobically for a further 5 to 7 days. The FAB medium was subcultured only if no growth was present on solid media.

Identification of *Actinomyces* spp. All potential *Actinomyces* spp. were subcultured onto eight fresh CBA plates. Following anaerobic incubation at 37°C for 48 h, growth was harvested from two plates for inoculation to an API 20A identification strip (Biomérieux) and from two plates for inoculation to Minitek Anaerobe II identification plates (Becton-Dickinson). Growth from the remaining four plates was used for disruption followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein profiling (9). In brief, cells were harvested from the plates and suspended in 1 ml of phosphate-buffered saline. This suspension was disrupted in a disintegrator (The Mickle Laboratory Engineering Co. Ltd., Gomshall, Surrey, United Kingdom) for 30 min, and the cellular material was deposited by centrifugation in an MSE Super Minor Centrifuge (Fisons, Crawley, Sussex, United Kingdom) (3,000 rpm; 15 min). The supernatant fluid was removed, and electrophoresis was performed in polyacrylamide slab gels with an adaptation of the discontinuous buffer system of Laemmli (13). The protein profiles were then compared with those of known standard strains. In addition, 20 ml of FAB was inoculated for subsequent determination of metabolic end products by isotachophoresis (5). Isotachophoresis is a technique which can be used for the qualitative and quantitative analysis of ions in bacterial fermentations. The method uses the principle of electrophoresis in a discontinuous electrolyte system (i.e., two different electrolytes), and the ions are separated according to the differences in their effective mobilities. It has the advantages that very small samples (<5 μl) can be analyzed and no pretreatment or derivatization of the sample is required.

Final verification was based upon the pooled results from these identification methods.

TABLE 2. Number of isolates of individual *Actinomyces* spp. from study specimens

Species	No. of isolates from:		
	Dental specimens, Glasgow Dental Hospital (n = 61)	Dental and other specimens, Victoria Infirmary (n = 32)	IUCDs, Victoria Infirmary (n = 133)
A. viscosus	18	3	4
A. odontolyticus	14	8	15
A. israelii	6	2	9
A. naeslundii	6	5	3
A. meyeri	1	0	5
Actinomyces spp.	1	1	5
Total	46	19	41

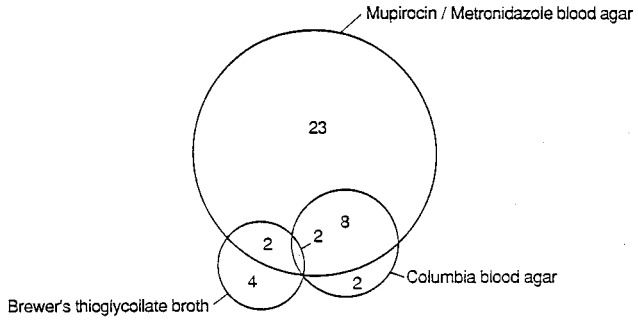

FIG. 1. Comparative isolation of 41 *Actinomyces* strains from IUCDs on MMBA and two other media.

RESULTS

Of the 241 specimens entered into the study, 226 yielded evaluable results (Table 1). *Actinomyces* spp. were isolated from 35 IUCDs (26%), 10 dental specimens at Victoria Infirmary (71%), 34 dental specimens at the Glasgow Dental Hospital (53%), and 5 other specimens at the Victoria Infirmary (24%). Thus, dental specimens yielded *Actinomyces* spp. more than twice as frequently as IUCDs or specimens from other sites, although most of the latter were from sites related anatomically to the oral cavity (Table 1). A total of 106 strains, isolated from 84 specimens, were identified as belonging to the genus *Actinomyces*, with a wide range of species being isolated from all sites (Table 2). A small number (7) of these strains could not be allocated to a known species with certainty by the methods described; these strains are included as *Actinomyces* spp. IUCDs received in transport medium yielded *Actinomyces* spp. slightly more often (16/52) than those received dry (18/79), but this difference was not statistically significant (χ^2 = 1.04; $0.5 > P > 0.1$).

The comparative rates of isolation of *Actinomyces* spp. on MMBA, CBA, and fluid enrichment medium (thioglycolate or FAB) are shown in Fig. 1 to 3. Growth on CBA plates was judged to be too profuse for the plate to be readable in 26 (20%) of the IUCD specimens and in 13 (41%) of the dental and other specimens at the Victoria Infirmary. In only three cases (all IUCDs) was unreadability due to overgrowth by *Proteus* spp., and in none of these was this organism present on the MMBA plate, although it is also true that none of these specimens yielded *Actinomyces* spp. At the Victoria Infirmary,

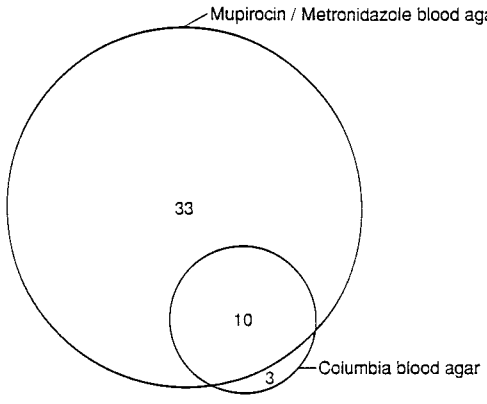

FIG. 2. Comparative isolation of 46 *Actinomyces* strains from dental specimens on MMBA and CBA. (No additional strains were isolated from FAB.)

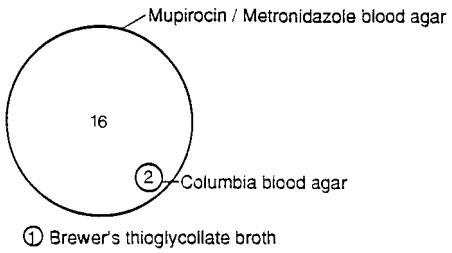

FIG. 3. Comparative isolation of 19 *Actinomyces* strains from dental and other specimens, at the Victoria Infirmary, on MMBA and two other media.

Actinomyces spp. were isolated significantly more often from specimens which gave unreadable CBA plates (19/41) than from specimens that did not (30/108) ($\chi^2 = 4.65$; $0.05 > P > 0.02$).

Apart from *Actinomyces* spp., other organisms commonly isolated on MMBA were lactobacilli, aerobic coryneforms, and propionibacteria. Less common were *Bifidobacterium* spp., *Candida* spp., and *Gardnerella vaginalis*, and there were occasional isolates of *Haemophilus influenzae*, *Capnocytophaga* spp., and *Eikenella corrodens*; the latter three were not generally apparent on the nonselective cultures (Table 3). The major problem among these is with *Bifidobacterium* spp. and *Lactobacillus* spp. Thus, of the 128 strains of catalase-negative GPR referred to the Glasgow Dental Hospital and School from the Victoria Infirmary for formal identification, only 60 were *Actinomyces* spp., while 51 were *Lactobacillus* spp. and 11 were *Bifidobacterium* spp. API ZYM profiles did not distinguish *Actinomyces* spp. from *Lactobacillus* and *Bifidobacterium* spp. (11) and were not helpful as a screening procedure. The organisms isolated in conjunction with *Actinomyces* spp. from the dental specimens are shown in Table 4. The pattern was similar to that for the IUCD and other specimens at the Victoria Infirmary, but *Veillonella* spp. were isolated frequently from the dental specimens although not from the specimens from other sources.

The lengthy isolation and identification process, which included the use of a reference laboratory for final identification, resulted in considerable delays in reporting. Data on turnaround time (the time from receipt of specimen in the laboratory to the issuing of a report) were available for 116 of the 162 specimens from the Victoria Infirmary. Mean turnaround time was 48 days (range, 23 to 109 days) for specimens which yielded *Actinomyces* spp. and 26 days (range, 8 to 77 days) for those which did not.

DISCUSSION

Mupirocin (pseudomonic acid), an antibiotic derived from *Pseudomonas fluorescens*, is currently licensed in the United Kingdom for clinical use as a topical agent in skin infections due to staphylococci and streptococci. Drug MICs against these organisms are typically <1 mg/liter, and similar activity is shown against *Haemophilus* and *Neisseria* spp. MICs for *Enterococcus* spp., *Escherichia coli*, and other enterobacteria are typically in the range of 32 to 128 mg/liter (18). CBA containing mupirocin (128 mg/liter) inhibits the growth of these organisms while supporting that of a range of *Actinomyces* spp. (unpublished data).

Metronidazole at a concentration of 2.5 to 5.0 mg/liter inhibits most of the obligate anaerobes found in clinical material, with only the genera *Actinomyces*, *Bifidobacterium*, and *Propionibacterium* being regularly resistant (1, 4, 6). Traynor et al. (17) described CBA containing 2.5 mg of metronidazole per liter for the isolation of *Actinomyces* spp. from IUCDs, but a time-consuming serial dilution technique of a kind not commonly used for routine specimens was used to remove competing flora and no data on the comparative performance of their medium with unsupplemented CBA were reported. Using this technique, Jarvis (10) isolated *Actinomyces* spp. from genital tract samples in 11.9% of 259 women who had an IUCD.

The combination of mupirocin and metronidazole is logical since, as Tables 3 and 4 illustrate, organisms normally sensitive to one of these antibiotics are usually present together with *Actinomyces* spp. The rate of isolation of *Actinomyces* spp. from the 133 IUCDs examined in this study (26%) suggests that this results in a practical advantage.

Similarly, *Actinomyces* spp. were isolated from up to 71% of specimens from dentofacial infections. This is much higher than the prevalence quoted in previous studies (15) and probably reflects the inhibition of competing microorganisms. This is of particular value in the analysis of aspirates from dental infections, since they are typically polymicrobial, with as many as eight bacterial species being isolated from a single abscess (14). Thus, MMBA has a potentially useful role in the diagnosis of cervicofacial actinomycosis and related purulent infections of the head and neck region. In addition, there has been research interest in the role of *Actinomyces* spp. in dental

TABLE 3. Isolation of organisms in conjunction with *Actinomyces* spp. from specimens examined at the Victoria Infirmary

Coexistent organisms	No. of strains in which organisms coexisted with:					
	A. viscosus (7 strains)	*A. odontolyticus* (23 strains)	*A. israelii* (11 strains)	*A. naeslundii* (8 strains)	*A. meyeri* (8 strains)	*Actinomyces* spp. (6 strains)
Lactobacillus spp.	0	10	8	4	2	6
Bifidobacterium spp.	0	2	0	2	0	0
Anaerobic gram-positive cocci and gram-negative rods	3	13	6	8	2	1
Streptococcus spp.	7	16	4	8	3	5
Staphylococcus spp.	2	4	1	1	1	0
Propionibacterium spp.	1	1	1	1	0	2
Aerobic coryneforms	2	5	3	3	1	0
Coliforms	0	1	1	0	0	0
Candida spp.	1	2	1	0	1	0
Others[a]	0	1	2	2	1	0

[a] *Capnocytophaga* spp., *Haemophilus influenzae*, *Eikenella corrodens*, and *Gardnerella vaginalis.*

TABLE 4. Isolation of organisms in conjunction with *Actinomyces* spp. from dental specimens examined at the Glasgow Dental Hospital and School

Coexistent organisms	No. of strains in which organisms coexisted with:					
	A. viscosus (18 strains)	*A. odontolyticus* (14 strains)	*A. israelii* (6 strains)	*A. naeslundii* (6 strains)	*A. meyeri* (1 strain)	*Actinomyces* spp. (1 strain)
Lactobacillus spp.	2	5	2	3	0	1
Bifidobacterium spp.	0	1	0	0	0	0
Anaerobic gram-positive cocci and gram-negative rods	6	6	2	3	1	1
Streptococcus spp.	13	11	6	3	2	1
Staphylococcus spp.	1	0	0	0	0	0
Veillonella spp.	7	3	1	2	0	0
Others[a]	6	2	2	3	4	1

[a] *Capnocytophaga* spp., *Haemophilus* spp., *Eikenella corrodens*, and *Eubacterium* spp.

caries (7), and a selective medium such as MMBA may prove helpful in such studies to allow analysis of the complex plaque microflora.

The identification of gram-positive, non-spore-forming rods is an expensive, slow, and complex process. The methods used in this study permitted a firm microbiological diagnosis to be made for 99 of the 106 isolates of *Actinomyces* spp. Despite the difficulties in identification, it has been suggested that the most common mistake made with both anaerobic GPR and gram-positive cocci is that the organisms are never recovered at all (8). The use of MMBA is suggested as a means of overcoming this problem for processing clinical specimens from infections in which *Actinomyces* spp. play a significant role.

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