JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1978, p. 137-138
0095-1137/78/0007-0137$02.00/0

Vol. 7, No. 2
Printed in U.S.A.

Modified Culture Technique for *Corynebacterium diphtheriae* Isolation from Desiccated Swabs

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Received for publication 25 November 1977

Corynebacterium diphtheriae was isolated from pyoderma and ulcerative skin lesions with a modified delayed culture procedure as late as 9 weeks after field collection of silica gel-desiccated swabs. Biotypes *gravis* and *mitis* were identified. Most isolates were nontoxigenic. Todd-Hewitt broth enrichment enhanced recovery of *C. diphtheriae* by 70%.

Bacteriological study of pyoderma-associated organisms in remote areas has been restricted by the availability of laboratory facilities. Recently, sterile silica gel transport medium has been used for delayed culture of skin swabs with consequent recovery of *Streptococcus pyogenes* (9), *Staphylococcus aureus* (5), and *Corynebacterium diphtheriae* (8).

C. diphtheriae has been found in tropical pyderma lesions with a direct plating technique. Successful recovery of this pathogen after it has been stored for 6 to 9 weeks in silica gel-desiccated swabs has not been reported.

We report here the recovery of *C. diphtheriae* from silica gel-desiccated swabs, processed by a modified culture technique 6 to 9 weeks after field collection.

MATERIALS AND METHODS

Thirty-nine people with pyoderma and seven with nonpyoderma ulcerative skin diseases were selected for culture from several Amazonian Indian populations in July and August 1976. From each of the 39, at least one swab was collected from an active pyoderma lesion. From two (5.1%) of this group, a second swab was collected from a second lesion site. Only one swab was collected from each of the seven persons in the nonpyoderma group. No persons had received any recent antibiotic therapy. The conditions of collection were uniform throughout. One physician examined and selected all the culture subjects.

Skin lesions were cultured directly, without prior cleansing. Loosely adhering crust margins permitted sterile calcium alginate (Calgiswab, Colab Laboratories, Inc., Chicago, Ill.) swabs to be inserted into the lesion base or into the purulent drainage without removing the crust. The swab was immediately placed into an aluminum foil packet of sterile silica gel (Carter, Rice, Storrs, and Bennett, East Hartford, Conn.) (9) and sealed. The foil packets were then placed in waterproof bags. During collection and storage, temperatures ranged from 17 to 30°C, except for a few hours during shipment when the swabs were at 9°C.

Swabs were first moistened with a few drops of Todd-Hewitt broth (THB), rubbed gently on the surface of a Pai slant (2), and then placed in a tube of THB with 3% rabbit blood. The Pai slants were incubated at 35°C and examined after 24 and 48 h and 7 days. If growth was visible on a Pai slant, the culture was streaked onto a cystine-tellurite blood agar plate (3) and a Tinsdale plate (4) and incubated at 35°C for 24 to 48 h. The THB cultures (containing the swabs) were incubated at 35°C for 16 to 18 h. All THB cultures were streaked onto a cystine-tellurite blood agar plate and incubated at 35°C for 24 to 48 h. Suspect *C. diphtheriae* colonies were picked from all cystine-tellurite blood agar plates to Pai slants. After the slants were incubated overnight at 35°C, smears were made from their surface growth and stained with Loeffler alkaline methylene blue. If microscopic examination showed cellular morphology typical of *C. diphtheriae*, growth from the subcultured Pai slant was used to inoculate a modified Elek plate (1) for toxigenicity, a Tinsdale plate to confirm halo production, and biochemicals to determine biotype. All isolates that were nontoxigenic on in vitro plates were confirmed by tissue culture (6, 7).

RESULTS

A total of 48 swabs were collected from skin lesions found among the residents of seven Amazon Indian villages. Forty-one (85.4%) of the 48 swabs were from typical pyoderma lesions; in five of the villages, seven swabs were collected from patients with nonpyoderma ulcerative skin lesions.

Seventeen (35.4%) of the 48 swabs were culture positive for *C. diphtheriae* (Table 1). Sixteen (39.0%) of the 41 pyoderma cultures were positive for *C. diphtheriae*, whereas only one (14.3%) of the seven nonpyoderma cultures was positive. *C. diphtheriae* was never the sole pathogenic bacterium isolated. Most (88.2%) of the isolates were not toxigenic. Of these, most (58.8%) were biotype *mitis*; the remainder were biotype *gravis*.

The interval between the time of swab collec-

tion and the time of culture ranged from 6.3 to 9.3 weeks, with a median interval of 7.3 weeks. There was no difference in culturability of *C. diphtheriae* by village or by age group. A report of the epidemiological features and bacteriological findings for associated streptococcal and staphylococcal isolates is being prepared.

Ten (58.8%) of the 17 *C. diphtheriae*-positive swabs were recovered from Pai slants (Table 2). The other seven were recovered only from THB. In no instance was an isolation made from the Pai slant and not also from THB.

DISCUSSION

Bacterial pyoderma is the most prevalent dermatological infection in most of the tropical developing countries. Some of the highest prevalences for pyoderma in these countries are among people who live in remote areas with extremes of temperature, humidity, and inaccessibility.

Epidemiological study of the suppurative and nonsuppurative sequelae of pyoderma and thorough characterization of the associated bacterial pathogens have been severely restricted by technical problems associated with attempts to perform direct plating in the field. Field investigations of the role that cutaneous *C. diphtheriae* infection plays in the epidemiology of diphtheria in the tropics have been similarly hampered. The most commonly used transport medium requires refrigeration during shipment, and plates must be prepared within a few days.

Successful delayed culture of streptococcal and staphylococcal isolates from skin lesions was reported using silica gel-desiccated calcium alginate swabs for as long as 4 weeks after collection (5, 9). Recoveries were comparable with immediate and delayed cultures. Our results show that silica gel-desiccated swabs can be used for delayed culture of *C. diphtheriae* up to 9 weeks after field collection. No information is currently available comparing the recovery rates of *C. diphtheriae* with delayed culture methods described and with immediate culture.

It is apparent, however, that the seven additional isolations made only from THB enrichment enhanced by 70% the possible recovery rate with the conventional Pai media.

TABLE 1. *Recovery of C. diphtheriae from desiccated swabs of pyoderma and ulcerative skin lesions*

Lesions	No. of swabs		
	Culture negative	*C. diphtheriae* negative[a]	*C. diphtheriae* positive
Pyoderma	2	23	16
Ulcerative	0	6	1

[a] Other pathogenic species included beta-hemolytic group A and group G streptococci and *S. aureus*.

TABLE 2. *Comparison recovery of C. diphtheriae from silica gel-transported swabs with direct inoculation of Pai slants and THB enrichment*

Recovery from:		No. of swabs	%
Pai	THB		
+	+	10	58.8
+	−	0	
−	+	7	41.2

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation under grant BMS-74 118 23. We are grateful to the National Science Foundation for making available the facilities of the research vessel *Alpha Helix* during July and August of 1976.

LITERATURE CITED

1. **Bickham, S. T., and W. L. Jones.** 1971. Problems in the use of the in vitro toxigenicity test for *Corynebacterium diphtheriae*. Am. J. Clin. Pathol. **57**:244-246.
2. **Herman, G. J., and S. T. Bickham.** 1974. *Corynebacterium*, p. 130-134. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
3. **Frobisher, M.** 1937. Cystine-tellurite agar for *C. diphtheriae*. J. Infect. Dis. **60**:99-105.
4. **Moore, M., and E. I. Parsons.** 1958. A study of a modified Tinsdale's medium for the primary isolation of *Corynebacterium diphtheriae*. J. Infect. Dis. **102**:88-93.
5. **Noble, W. C., D. Presbury, B. L. Conner, J. A. Savin, D. Taplin, and L. Lansdell.** 1974. Prevalence of streptococci and staphylococci in lesions of impetigo. Br. J. Dermatol. **91**:115-116.
6. **Schubert, J. H., S. T. Bickham, and G. L. Wiggins.** 1968. Tissue culture methods for toxigenicity testing of *Corynebacterium diphtheriae*. Appl. Microbiol. **16**:1748-1752.
7. **Schubert, J. H., G. L. Wiggins, and G. C. Taylor.** 1967. Tissue culture method for the titration of diphtheria antitoxin in human sera. Health Lab. Sci. **4**:181-188.
8. **Sinclair, M. C., S. Bickham, and J. H. Schubert.** 1972. Silica gel as a transport medium for *Corynebacterium diphtheriae*. S. Med. J. **65**:1383-1384.
9. **Taplin, D., and L. Lansdell.** 1973. Value of desiccated swabs for streptococcal epidemiology in the field. Appl. Microbiol. **25**:135-138.