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Difficulties Encountered in Identification of a Nutritionally Deficient Streptococcus on the Basis of Its Failure To Revert to Streptococcal Morphology

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Difficulties were encountered in the recognition of a nutritionally deficient streptococcus which continued to display aberrant morphologic forms (especially bulbous swellings and filament formation) despite provision of growth factors. With isolates displaying diverse morphologic entities not characteristic of a given species, e.g., *Streptobacillus moniliformis* or *Erysipelothrix rhusiopathiae*, nutritionally deficient streptococci should be considered.

Nutritionally deficient streptococci (NDS) have been recovered from a variety of clinical sources (i.e., blood, abscesses, oral ulcers, and urethral samples) in which they have been accorded an etiologic role (1, 5, 8).

Variously described as cell wall-deficient, L-form, thiol-requiring, satelliting, pyridoxal-dependent, and nutritionally deficient streptococci (1, 5, 8), species composing this group have been determined to be present in clinical specimens mainly by their growth factor requirements subsequent to initial detection (4).

Although bizarre microscopic morphology characterizes NDS, such occurrences in conjunction with restricted growth on routine bacteriologic media and reversion to more typical streptococcal morphology upon the provision of sulfhydryl group-containing growth factors aid in identification. In the absence, however, of cellular morphologic reversion, and despite repeated subculture in the presence of the requisite growth factor, true identification of the isolate as an NDS may be hampered. We document herein difficulties encountered in the identification of an NDS blood isolate because its pronounced and persistent morphologic aberrations (especially bulbous swelling and filament formation) and rough colony morphology on chocolate agar were suggestive of either *Streptobacillus moniliformis* or *Erysipelothrix rhusiopathiae*.

The isolate was detected in blood cultures of a 56-year-old female patient with mitral valve endocarditis through the use of the BacTec system (Becton Dickinson and Company, Sparks, Md.). Initial smears showed gram-variable pleomorphic bacillary forms with ovoid swellings irregularly spaced along the bacillus. Aliquots of the blood culture were inoculated onto 5% sheep blood agar and onto chocolate agar (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C in 5% CO_2. On the latter medium, small, flat colonies with a dull texture and irregular borders developed after 48 h of incubation. Growth on blood agar was barely perceptible. Smears of colonies growing on chocolate agar showed an impressive array of gram-variable morphologic forms ranging from disjointed filaments to shorter bacillary forms with distinct bulbous swellings which distorted the bacterial cell (Fig. 1). Occasional pairs of cocci were also noted. Furthermore, as the smear background was also noteworthy for the presence of palely staining gram-negative "ghost" forms, the range of morphologic presentation was suggestive of *S. moniliformis* (8).

Pursuing this identification, we inoculated the isolate into thioglycolate broth supplemented with fetal calf serum, which, after 24 h of incubation, resulted in floccular growth beginning

TABLE 1. Characteristics of blood isolate leading to recognition as an NDS most closely resembling *S. anginosus-S. constellatus*

Test	Result
Growth	
Chocolate agar	Rough colonies with irregular edges
Blood agar	Slight growth
Heart infusion agar	Negative
Heart infusion agar plus pyridoxal disk	Small (1.0 mm) colonies around disk
6.5% NaCl broth	No growth
5% Sucrose agar plus pyridoxal	Nonadherent mucoid colonies
Esculin hydrolysis	Positive
Arginine dihydrolase	Positive
Catalase	Negative
Fermentation of[a]:	
Glucose	Positive (no gas)
Lactose	Negative
Mannitol	Negative
Salicin	Positive
Sorbitol	Negative
Sucrose	Positive
Trehalose	Positive
Xylose	Negative
Glycerol	Negative
Inulin	Negative
Vancomycin (30-μg disk)	Susceptible
Nitrate reduction	Negative
Enzyme production	
Neuraminidase	Negative
β-Fucosidase	Negative
β-Glucosidase	Negative
β-*N*-Acetylglucosaminidase	Negative
α-Glucosidase	Negative
Pyrrolidonyl-arylamidase	Positive (weak)
Hyaluronidase (plate)	Negative

[a] In broth supplemented with pyridoxal and rabbit serum.

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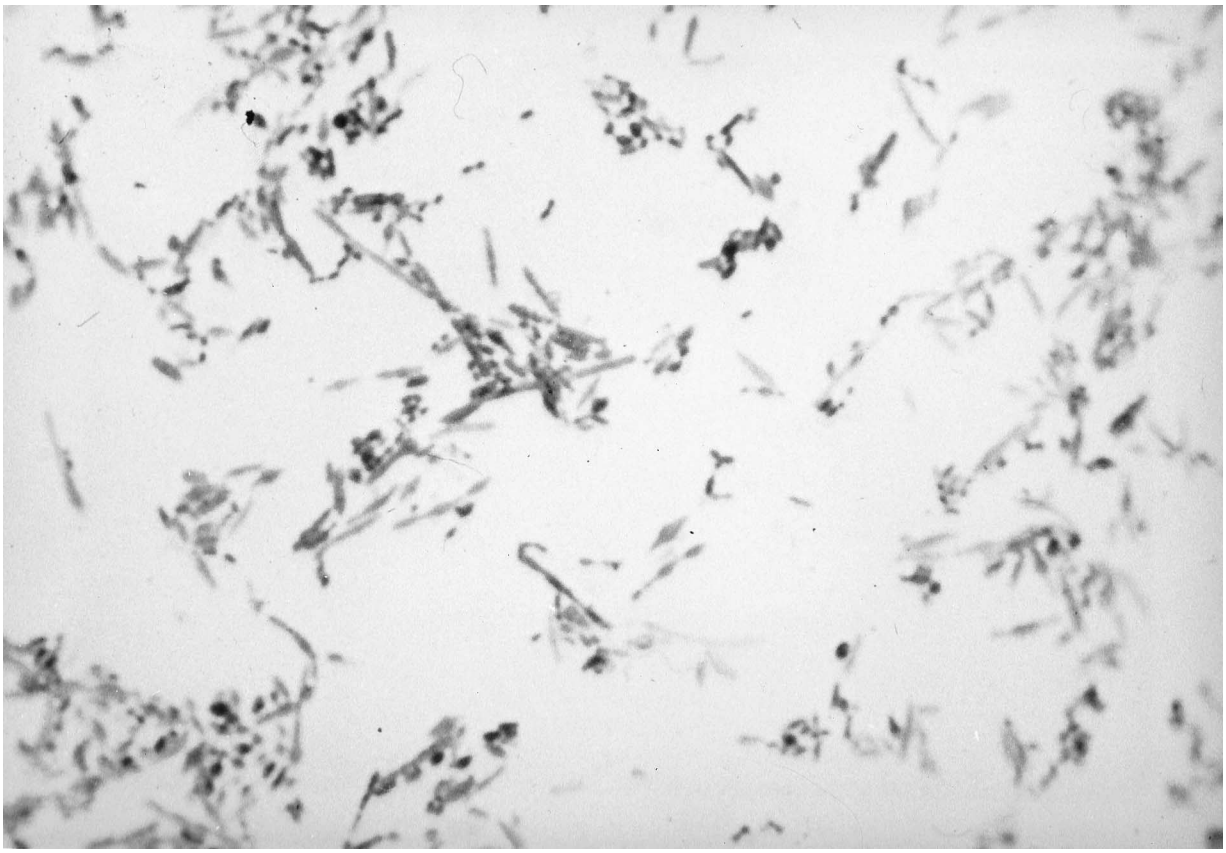

FIG. 1. Gram stain of growth on chocolate agar showing gram-variable filaments with ovoid swellings and occasional cocci in pairs.

at 10 cm below the broth surface and extending downward with some streaking. Phase-contrast microscopy and Gram-stained smears of the broth were remarkable for the presence of bacillary forms in short chains with spore-like swelling admixed with palely staining ovoid cells. This morphologic presentation continued through several days of incubation and was reproducible after subculture of the thioglycolate-serum medium to chocolate agar.

Because of the persistence of the morphologic diversity in thioglycolate broth supplemented with fetal calf serum and also in colonies developing on chocolate agar, recognition as an NDS was delayed. Additionally, because of the marked bacillary aspect noted in colonies growing on chocolate agar, *E. rhusiopathiae* was also being considered. No epidemiological link, however, could be established in the patient's history to account for bacteremia due to either *S. moniliformis* or *E. rhusiopathiae*. On further testing, satelliting growth around a pyridoxal-containing disc and adjacent to a *Staphylococcus aureus* cross streak on a lawn of the isolate on heart infusion agar was obtained. Nevertheless, the described morphologic presentation was still apparent. Biochemically, although our isolate hydrolyzed arginine, it fermented trehalose and most closely resembled *Streptococcus defectivus*, described by Bouvet et al. (1), or, alternatively, *Streptococcus anginosus-S. constellatus* (7) (Table 1).

NDS have frequently been recovered from human clinical sources and have not presented a diagnostic dilemma once their growth factor requirements have been provided and concomitant streptococcal morphologic reversion has taken place (4, 8). Not uncommonly, however, upon their initial recovery in broth media their pleomorphism may be so extreme that they mimic other bacterial species and even fungi (3). In the present instance, however, despite adequate nutritional supplementation, protracted morphologic aberrations obscured ready recognition as an NDS.

Although it has been emphasized that NDS may demonstrate a plethora of forms remote from streptococcal morphology, rapid conversion to streptococcus-like cells is usually achieved upon adequate nutritional supplementation of growth media (2). Zierdt (8), however, has shown that correcting nutritional deficiency does not abolish all abnormalities. Therefore, failure to achieve such conversion, coupled with initial slight growth on chocolate agar or other media in routine use, may lead to the pursuit of other microbial identities, thereby delaying recognition as an NDS and definitive antimicrobial therapy (6). Thus, we strongly suggest that, when presented with an isolate displaying a wide range of morphologic entities not truly characteristic of a given species, one consider NDS.

REFERENCES

1. **Bouvet, A., F. Grimont, and P. D. Grimont.** 1989. *Streptococcus defectivus* sp. nov. and *Streptococcus adjacens* sp. nov., nutritionally variant streptococci from human clinical specimens. Int. J. Syst. Bacteriol. **39:**290–291.
2. **Clark, R. B., R. E. Gordon, E. J. Bottone, and M. Reitano.** 1983. Morphological aberrations of nutritionally deficient streptococci: association with pyridoxal (vitamin B_6) concentration and potential role in antibiotic resistance. Infect. Immun. **42:**414–417.
3. **Dykstra, M. A., S. M. Polly, C. C. Sanders, D. E. Chastain, and W. E. Sanders.** 1983. Vitamin B_6-dependent streptococcus mimicking fungi in a patient with endocarditis. Am. J. Clin. Pathol. **80:**107–110.
4. **McCarthy, L. R., and E. J. Bottone.** 1974. Bacteremia and endocarditis

caused by satelliting streptococci. Am. J. Clin. Pathol. **61:**585–591.
5. **Ruoff, K. L.** 1991. Nutritionally variant streptococci. Clin. Microbiol. Rev. **4:**184–190.
6. **Stein, D. S., and K. E. Nelson.** 1987. Endocarditis due to nutritionally deficient streptococci: therapeutic dilemma. Rev. Infect. Dis. **9:**908–916.
7. **Whiley, R. A., H. Fraser, J. M. Hardie, and D. Beighton.** 1990. Phenotypic differentiation of *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* strains within the "*Streptococcus milleri* group." J. Clin. Microbiol. **28:**1497–1501.
8. **Zierdt, C. H.** 1992. Light-microscopic morphology, ultrastructure, culture and relationship to disease of the nutritional and cell-wall-deficient α-hemolytic streptococci. Diagn. Microbiol. Infect. Dis. **15:**185–194.