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NOTES

Cellular Elongation Under the Influence of Antibacterial Agents: Way to Differentiate Coccobacilli from Cocci

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Representatives of *Moraxella*, *Acinetobacter*, and various other groups of short, gram-negative bacilli are readily distinguished from *Neisseria* by microscopic observation of filaments produced by the rods during growth in the presence of low concentrations of penicillins or sulfadiazine. Wet mounts of bacteria from routine antibiotic susceptibility test cultures are satisfactory for examination of morphology.

Deciding whether a gram-negative organism is rod shaped or coccal is usually simple. However, the coccobacillary forms of some clinical isolates may be confused with diplococci. DeBord (3) noted that organisms which might be mistaken for the gonococcus were found in 30% of the specimens from a group of health clinic patients. He proposed the name *Mimeae* for a new tribe in which he classified certain species of gram-negative bacteria that are pleomorphic rods but commonly mimic the diplococcal form of neisseriae. This artificial grouping of diverse bacteria based merely on morphological resemblance has been rejected after years of controversy and study; some of the species named by DeBord are now classified as species of *Acinetobacter* or *Moraxella* (5).

In spite of the numerous publications, these bacteria remain a source of difficulty for the clinical laboratory. One must be continually alert to their possible presence in clinical specimens when making a preliminary diagnosis from the microscopic examination of Gram-stained preparations of spinal fluid (1), joint fluid (4), or discharge from urogenital tract infections (3, 6). Furthermore, failure to recognize pairs of coccobacillary forms as rods when examining recently isolated cultures of *Moraxella osloensis* or *M. nonliquefaciens* may lead to an erroneous diagnosis of *Neisseria catarrhalis* or some other species of *Neisseria* which does not produce acid from carbohydrates. Figure 1a shows the appearance of such a culture which was received as a species of *Neisseria*.

A simple way to differentiate many of these gram-negative coccobacilli from true cocci takes advantage of the long-recognized tendency of rod-shaped bacteria to elongate when growing in the presence of certain antibacterial drugs. Low concentrations of penicillins or sulfonamides appear to block septum formation more strongly than synthesis of lateral cell wall components. As a result, bacteria which normally undergo division to yield very short rods (Fig. 1a, c, f) may fail to divide while continuing to grow, thereby forming long filaments (Fig. 1b, d, g). Microscopic observations of such "snakes" may be made on routine antibiotic susceptibility test cultures after incubation for 18 to 24 h. Bacteria are removed from the medium near the outer margins of zones of growth inhibition surrounding disks containing penicillin G, ampicillin, or sulfadiazine, for example. The cells are suspended in a small loopful of sterile broth or other isotonic fluid on a microscope slide and covered with a no. 1 cover glass large enough to prevent the fluid from flowing to the edges when pressed down. This wet mount is observed under the microscope using the oil immersion objective and reduced illumination. The preparation of a wet mount is simpler than a Gram stain and has the additional advantage that the bacteria, rendered pleomorphic and fragile in response to the antibacterial drug, will not be ruptured by drying and heat fixation. However, if a stained preparation is preferred, the use of crystal violet alone is recommended because the staining qualities of the bacteria also are adversely affected by the drug.

No tendency toward filament formation was detected during examinations of recent isolates

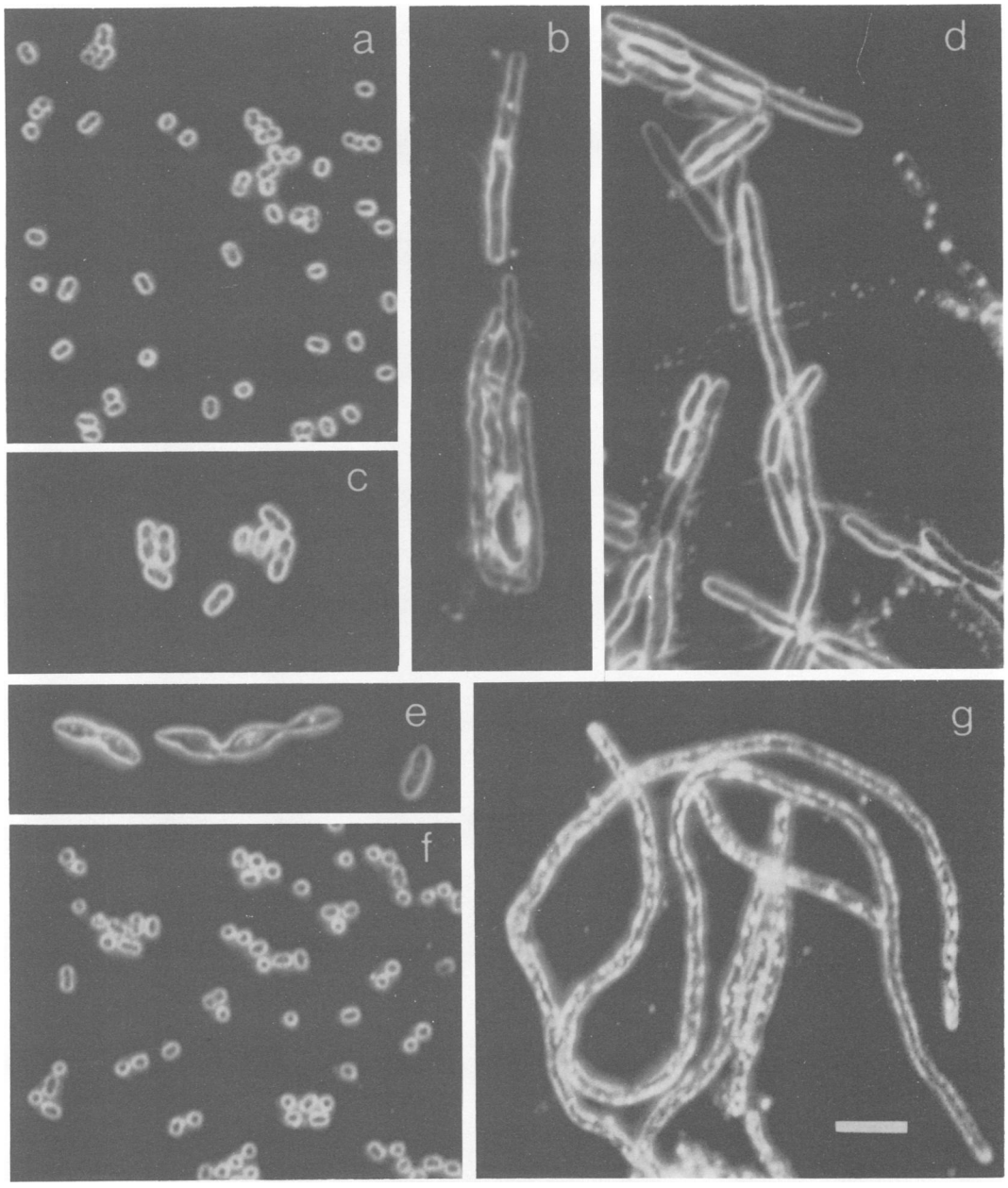

FIG. 1. *Morphology of gram-negative bacilli cultivated for 18 h on Mueller-Hinton agar in the presence or absence of antibacterial drugs. Preparations were made by the agar impression technique (2) and were air-dried, fixed in absolute methanol, and mounted unstained with buffered glycerol (pH 7.2) under a no. 1 cover glass. Photographs were made using a dark-field condenser, oil immersion objective, and a ×10 eyepiece with a Leitz MIKAS microattachment and camera with Tri-X film (described previously; 2). Marker indicates 5 μm. Two strains of Moraxella osloensis were used: strain 20 was cultivated in the absence (a) and presence (b) of penicillin G; strain B-16 was cultivated on drug-free medium (c) and on medium containing ampicillin (d) or sulfadiazine (e). Acinetobacter (Herellea vaginicola) was cultivated in the absence (f) and presence (g) of sulfadiazine.*

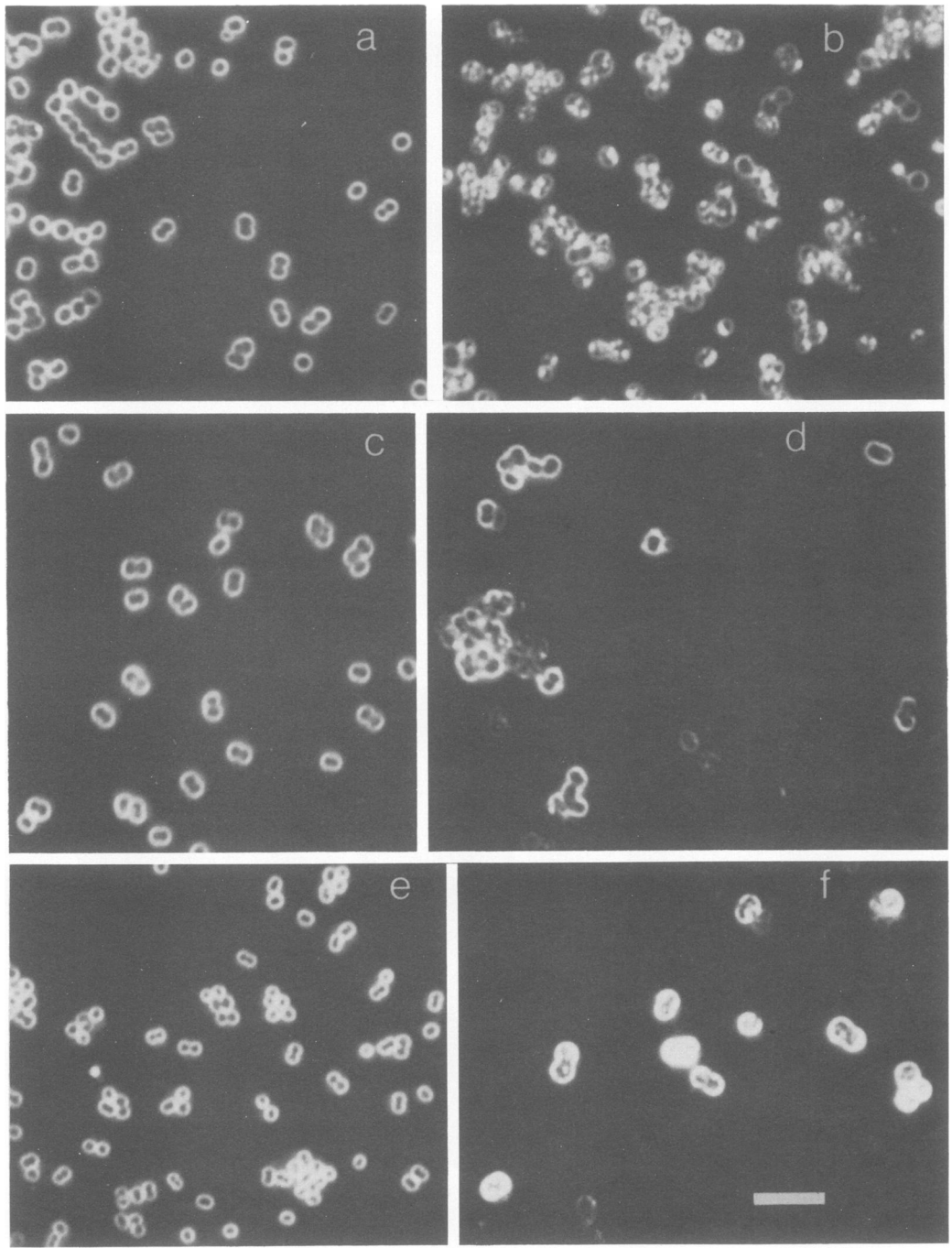

FIG. 2. *Morphology of representatives of Neisseria cultivated for 18 h on Mueller-Hinton agar which contained or lacked penicillins. Preparations were made as described for Fig. 1. Bar indicates 5 μm. N. meningitidis group C was cultivated in the absence (a) or presence (b) of ampicillin. N. sicca was cultivated in the absence (c) or presence (d) of ampicillin. N. canis was cultivated in the absence (e) or presence (f) of penicillin G.*

of *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica*. These cocci typically undergo division in two planes resulting in a transient formation of tetrads (Fig. 2a, e). Under the influence of penicillin G, ampicillin, or sulfadiazine, the neisseriae tend to enlarge, become granulated, and undergo lysis. Some cocci which apparently had failed to divide had the general outline of a large diplococcus, appearing two to three times greater in one dimension than the other (Fig. 2f). Cells more elongated than this were not observed in additional preparations of 16 culture collection strains comprising one or more representatives of *N. sicca* (Fig. 2c, d), *N. perflava*, *N. flava*, *N. subflava*, *N. mucosa*, *N. flavescens*, *N. denitrificans*, *N. animalis*, and *N. canis* (Fig. 2e, f).

Five recently isolated strains of *Acinetobacter* (representatives of the former *Herellea* and *Mima*) which were resistant to penicillin G produced long "snakes" under the influence of sulfadiazine (Fig. 1g). Cultures of *Moraxella osloensis* (*Mima polymorpha* var. *oxidans*), which were susceptible to penicillin G, ampicillin, and sulfadiazine, exhibited filaments in the area of the medium containing low concentrations of penicillin G (Fig. 1b) and ampicillin (Fig. 1d). However, spindle-shaped cells but no filaments were evoked by sulfadiazine (Fig. 1e).

Two other organisms (a heavily capsulated strain of *Klebsiella pneumoniae* and an avirulent strain of *Brucella abortus*) exhibited vacuolation and aberrant morphology but no filaments in response to ampicillin, penicillin G, or sulfadiazine. However, one or more of these drugs evoked conspicuous cellular elongation of all other gram-negative, short bacilli examined. Observation of filament production is rapidly made on appropriate preparations of unstained bacteria and will reduce errors in interpreting the morphology of coccobacilli.

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