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The culture of sterile urine for detection of anaerobic bacteria—not necessary for standard evaluation

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EXAMINATION of urine specimens for aerobic bacteria is a well-established procedure in the clinical microbiology laboratory. The method used at the Cleveland Clinic is as follows. Catheter or clean catch-voided urine specimens are cultured with minimal delay. A MacConkey agar plate is used for the isolation and preliminary identification of gram-negative bacteria. A colony count is prepared by uniformly streaking a sheep-blood agar plate with 0.001 ml of well-mixed, undiluted urine delivered by a calibrated platinum loop. This plate also serves for isolation of gram-positive bacteria. The plates are incubated aerobically at 37 C overnight. Those cultures demonstrating aerobic pathogenic bacteria are identified by means of appropriate differential media.[1] In standard sterile urine cultures, a search for anaerobic bacteria is not usually undertaken. Our study was designed to determine whether or not anaerobic bacteria are important factors in urinary tract infection; if they are, to identify them would be mandatory.

Materials and methods

In addition to culturing urine specimens by the previously described method, 0.5 ml of undiluted, well-mixed urine was placed in 7.5 ml of freshly prepared thioglycollate medium, incubated at 37 C, and examined daily for macroscopic and microscopic growth. All specimens that grew aerobic pathogens by the routine method, as well as those that did not demonstrate growth in the thioglycollate medium at the end of 72 hours were discarded and studied no further. Each of the remaining specimens was then transferred to two blood agar plates. Each plate was incubated for 48 hours at 37 C, one aerobically and one anaerobically, utilizing the BBL Gaspack.† Bacteria growing both aerobically and anaerobically were identified and discarded. Those bacteria growing on *anaerobic subculture only* were considered to be anaerobes. The bacteria growing on anaerobic subculture only were transferred to an aerobic blood agar plate incubated at 37 C, a blood agar plate incubated at 37 C in 10 percent carbon

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dioxide, and a tube of brain-heart infusion broth incubated at 37 C. An absence of bacterial growth after 48 hours under these aerobic conditions was evidence that these bacteria were strictly anaerobic.

Bacterial identification.[2, 3] Gram-negative, pleomorphic rods, growing anaerobically on blood agar, producing small, slightly raised transparent colonies were regarded as Bacteroides species. This group of organisms was not further identified.

Nonsporulating, small, gram-positive, catalase-negative rods producing poor growth in thioglycollate medium, and sparse, pinpoint colonies or no growth on anaerobic blood agar plates or blood agar plates incubated in 10 percent carbon dioxide were classified as Lactobacillus species.

Gram-positive cocci growing anaerobically as small translucent colonies were identified as Peptococcus or Peptostreptococcus on the basis of the morphologic pattern on the gram stain, and the presence or the absence of catalase.

Large, gram-positive rods growing anaerobically were considered to be Clostridia. The species were identified by means of further appropriate tests.

Results

Two hundred random urine specimens (*Fig. 1*) were cultured anaerobically. The specimens were obtained both from inpatients and outpatients. Of these 200 specimens, 93 grew aerobic pathogens when cultured by the routine method

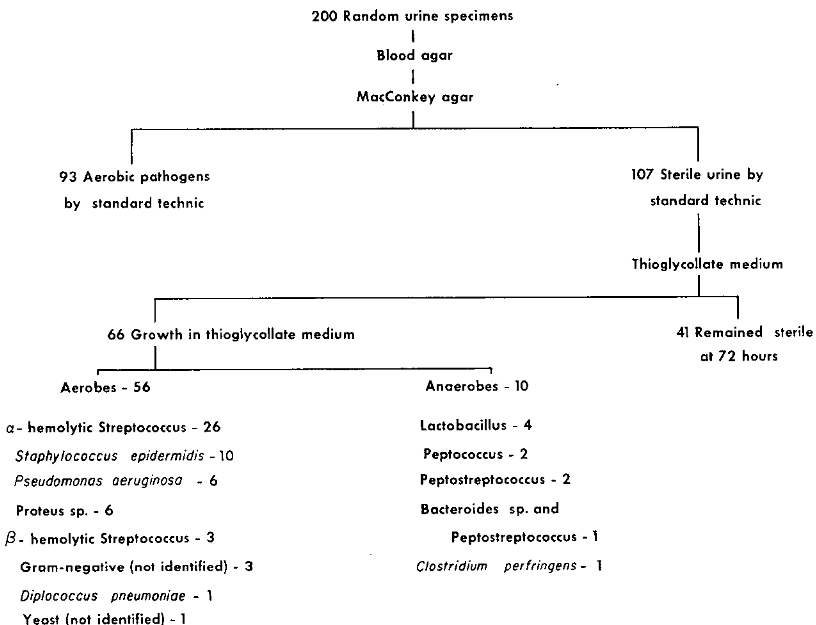

Fig. 1. Results of aerobic and anaerobic culture and subculture of 200 random urine specimens.

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and were discarded from the study. Of the 107 specimens regarded as sterile, 66 (62 per cent) produced growth in thioglycollate medium, and 41 (38 per cent) remained sterile after 72 hours. Aerobic bacteria were obtained from 56 (52 per cent) of the urine specimens regarded as sterile by the standard method. Bacteria identified included: α-hemolytic Streptococcus (26); *Staphylococcus epidermidis* (10); *Pseudomonas aeruginosa* (6); Proteus species (6); β-hemolytic Streptococcus (3); gram-negative, not identified (3); *Diplococcus pneumoniae* (1); and yeast, not identified (1). These 56 aerobically sterile urine specimens grew obviously aerobic pathogens for the following possible reasons. The inoculum size in our study was 0.5 ml, or 500 times greater than that used in the technic for standard colony counts; also the culture was incubated for three days, which allows for great bacterial numbers even when only a few bacteria are present in the original sample.

Anaerobic bacteria were isolated from 10 (9 per cent) of the aerobically sterile urine specimens: Lactobacillus (4), Peptococcus (2), Peptostreptococcus (2), Bacteroides species and Peptostreptococcus (1), and *Clostridium perfringens* (1). The clinical records of the 10 patients whose urine specimens had grown anaerobic bacteria, were reviewed for notes on urinary tract symptoms, urinalysis, renal function studies, and urography (when performed). Findings in nine patients' records were within normal limits. One patient, whose urine specimen grew *Clostridium perfringens*, had a one-year history of pyuria and + to ++ proteinuria, without symptoms of urinary tract infection. She is diabetic and also has systemic lupus erythematosus, which could possibly account for the urinary findings. A second urine culture, one month later, grew 18,000 colonies of Peptostreptococcus; no *Clostridium perfringens* was isolated.

Comment and conclusion

Anaerobic bacteria have been shown to be important in septicemia, bacterial endocarditis, wound abscess, putrid lung abscess, central nervous system abscess, osteomyelitis, peritonitis, acute gaseous cholecystitis, endometritis and pelvic abscess, and superficial skin abscesses.[4] McHenry and associates[5] reported a case of *Clostridium perfringens* septicemia associated with clostridial pyelonephritis in a patient with chronic lymphocytic leukemia. It has been shown that Bacteroides species and anaerobic Streptococcus are occasionally isolated from the urine but are seldom responsible for pyelonephritis[6] in the absence of renal calculi.[7] Headington and Beyerlein[8] cited seven patients with urinary tract symptoms whose urine specimens grew only anaerobic bacteria; the authors were not able to prove that these bacteria were pathogens, because on repeated cultures the same species of anaerobe could not be isolated. There are only a few other reports of anaerobic bacteria causing urinary tract infection.

The fact that the presence of anaerobic bacteria was not significantly correlated with urinary tract infection in the patients of our study, together with the paucity of published reports of such a correlation we believe warrants the conclusion that attempts to isolate and to identify anaerobes in urine specimens as

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a routine clinical procedure is not justifiable. The value of the results is not commensurate with the considerable extra time and effort involved in isolating and identifying anaerobes in routine sterile urine specimens.

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