Nosocomial Urinary Tract Infection with a Slowly Growing, Fastidious *Escherichia coli*

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*Escherichia coli* recovered from a nosocomially acquired urinary tract infection required 48 h of incubation on blood agar and did not grow on other routine clinical laboratory media. This bacterium dissociated readily into three colony types, all of which were confirmed as *E. coli* by DNA hybridization studies. Preliminary studies indicate a prolonged lag phase that could not be corrected by the addition of a variety of peptones and yeast extracts. Better growth was achieved by the addition of 10% horse serum.

*Escherichia coli* ranks very high among bacteria isolated from clinical specimens (1). It can synthesize most, if not all, of its required constituents from a single organic compound and a few minerals, and it can adapt to environmental challenges with exceptional alacrity (5). In the human biosphere, it is a minority constituent of the normal colonic microbiota, but its variants may be involved in intestinal and extraintestinal disease production (3). It is, therefore, accepted that this bacterium can be isolated on simple agar media in the clinical laboratory. This report summarizes our recent experience with a variant of *E. coli* that grew reluctantly in enriched media and hardly at all on the commonly used MacConkey and eosin-methylene blue (EMB) agars (BBL Microbiology Systems, Cockeysville, Md.).

A 68-year-old obese female presented to the emergency room with acute chest pain related to triple vessel disease. Her past medical history was significant for diabetes, angina, diverticulosis, arthritis, hypothyroidism, chronic urinary tract infections, a kidney stone, and a benign colon tumor removed 15 years before this admission. The patient was admitted to the cardiac intensive care unit after cardiac catheterization and scheduled for triple bypass surgery. Coincidentally, cystoscopy revealed a stone in her left kidney. Her admission urine culture, performed on the AutoMicrobic System (Vitek Systems, Inc., Hazelwood, Mo.), was negative; the 5% sheep blood agar (BA; BBL) control showed no growth after 18 h of incubation at 35°C; urinalysis did not indicate pus cells or bacteria. Just before scheduled surgery, the patient became febrile and developed a urinary tract infection with hematuria, but no organisms were isolated from a urine sample. On the insistence of the physician of record, a Gram stain performed on an uncentrifuged fresh specimen showed numerous granulocytes with a very large number of intra- and extracellular gram-negative rods. The same specimen was used to inoculate 5% BA, chocolate agar, MacConkey agar, and EMB agar, all quality controlled and prepared in-house from BBL products, and to inoculate liver broth (2). The automated procedure was repeated and the results were negative again after 18 h of incubation. All agars and the broth were negative at that time. After 48 h of incubation, tiny hemolytic colonies were discerned on BA only. After 72 h of incubation at 35°C, three distinct colony types were apparent: (i) beta-hemolytic mucoid colonies, 2 to 3 mm in diameter (variant A); (ii) beta-hemolytic non-mucoid colonies, 2 to 3 mm in diameter (variant B); and (iii) beta-hemolytic small colonies, 0.5 to 1 mm in diameter (variant C). On treatment, the patient's temperature promptly returned to normal. Her urine yielded a small number (15 CFU/ml) of the same colony types on day 3 of therapy. Surgery was performed on hospital day 10. The patient made an uneventful recovery and was discharged 27 days after admission.

All media except BA and liver broth failed to support growth of the bacteria; 72 h of incubation was required for easy detection in these media. Chocolate agar, enriched with IsoViteX (BBL), required the addition of 10% horse serum to permit growth. EMB, MacConkey, and Mueller-Hinton agars failed to support the bacteria. Repeated subcultures on BA (eight passages) resulted in a decrease in the inordinate lag phase to 24 h. The three distinct colony types were manifested in subcultures of each colony morphotype. Identification of the isolate as *E. coli* was possible with the faster-growing strains. Standard media were used (4); in addition, Enterotube (Hoffmann-LaRoche Inc., Nutley, N.J.), API 20E (Analytab Products, Plainview, N.Y.), and AMS Gram-Negative Identification Card (Vitek) analyses were performed. All identified the three colony types as *E. coli*, although the Enterotube required 72 h of incubation. *E. coli* ATCC 25922 served as a control for these and all experiments mentioned below. Subcultures of each colony type showed 100% identity by DNA hybridization, and these organisms reacted with the standard *E. coli* at a homology level of 85%. Molecular analyses were performed through the courtesy of Don J. Brenner of the Centers for Disease Control, Atlanta, Ga. Diffusion antibiotic susceptibility assays were performed on Mueller-Hinton agar supplemented with 5% BA (BBL); bacterial growth on unsupplemented agars and in various Micro-MIC panels was too scant for evaluation. The *E. coli* variants were susceptible to ampicillin, cefazolin, ticarcillin-clavulanic acid (Timentin), chloramphenicol, gentamicin, tobramycin, amikacin, ticarcillin, cefoxitin, cefuroxime, trimethoprim-sulfamethoxazole, sul-fisoxazole, imipenem, and ciprofloxacin.

All efforts to grow these bacteria on standard media met with failure even after the faster growth cycle was adopted. Prolonged incubation (48 to 72 h) produced growth in Mueller-Hinton agar and in brain heart infusion broth (BBL); very faint growth was achieved in EMB and MacConkey agars at 25°C. Addition of horse blood to EMB agar did not enhance growth on this medium at 25°C or permit growth at

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35°C. The dyes in EMB agar in the concentrations used for its formulation did not inhibit the organism when incorporated into BA. The bacteria could not grow without horse serum in Trypticase soy broth (BBL) or chocolate agar. Excellent growth was observed in Gilardi medium (4) consisting of, per liter: Casitone (Difco Laboratories, Detroit, Mich.), 10 g; yeast extract (BBL), 8 g; NaCl, 5 g; and agar (BBL), 7 g. Bacto-tryptone (Difco), Bacto-Peptone (Difco), and acid-hydrolyzed casein hydrolysate were substituted for the Casitone in the Gilardi formulation. In addition, medium without yeast extract but containing 7 g Bacto-tryptone (Difco), (BBL), 10 g; yeast extract, 0.2%; KNO3, 0.05 g; CaCl2·2H2O, 0.125 g; CuCl2·2H2O, 0.5 g; K2HPO4, 5.0 g; FeCl3·7H2O, 10 g; Fe(NH4)2(SO4)2·6H2O, 2.5 g; MnCl2·4H2O, 0.05 g; ZnCl2, 0.05 g; CaCl2·2H2O, 5.0 g; FeCl3·6H2O, 0.125 g; CuCl2·2H2O, 0.5 g; K2HPO4, 2 g. The final medium was adjusted to a pH of 7.0, dispensed in 5.0-ml volumes, and autoclaved at 118°C for 15 min. The following were added singly and in all combinations possible: horse serum, 10%; casein, 0.2%; and yeast extract, 1%. The three variants were introduced in 100-μl volumes of 0.5 McFarland density. Incubation was for 24, 48, and 72 h at 35°C. The control E. coli grew in the minimal medium to a density of 0.5 McFarland in 24 h without subsequent increase in biomass. Variants A and C achieved the same degree of growth in the unsupplemented mineral medium in 48 h and did not increase in density, whereas variant B required 72 h to reach this endpoint. The control bacterium achieved better than McFarland 2 densities with horse serum, yeast extract, and all combinations. The variants required the same combination but needed 48 or 72 h of incubation to achieve macroscopic densities. Variant A grew to a McFarland 1 density in 24 h in the presence of horse serum and yeast extract. Further studies of the growth requirements are in progress.

Several months after this observation, a second patient was admitted who eventually acquired a urinary tract infection which yielded similar slowly growing, fastidious E. coli. These preliminary observations indicate the need to further investigate properly obtained urine specimens that are negative on culture despite a persistently excessive number of pus cells. We do not know the frequency of negative cultures in the presence of pus cells in urine specimens and recognize the reluctance to routinely perform Gram stains on urine specimens. Our findings should alert our colleagues to the need to perform Gram stains on specimens from patients with good clinical evidence of urinary tract infections and negative culture findings after 18 to 24 h of incubation if this examination cannot be applied routinely.

LITERATURE CITED


