Hydrogen Sulfide-Producing Variants of *Escherichia coli*

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Seventeen strains of H$_2$S-producing variants of *Escherichia coli* were isolated from specimens submitted for microbiological study (ten from stool, five from urine, and two from postmortem material). Production of H$_2$S was unstable in several strains; however, other than their production of H$_2$S, all strains closely resembled typical *E. coli* in their biochemical reactions. In vitro susceptibilities of the H$_2$S-producing variants to antimicrobics closely resembled those of typical *E. coli* in this laboratory.

The biochemical and serological characteristics of hydrogen sulfide-producing (H$_2$S-producing) variants of *Escherichia coli* have been recently described in detail by Darland and Davis (1). Between 1970 and 1973, 17 such strains were isolated from clinical material at the Mayo Clinic. The clinical, biochemical, and antimicrobial susceptibility characteristics of these strains are reported here.

**MATERIALS AND METHODS**

Initial screening of gram-negative bacilli isolated from clinical specimens is accomplished in this laboratory by inoculation of a portion of an isolated colony into triple sugar-iron agar, lysine-iron agar, Simmons’ citrate agar, Christensen’s urea agar, ornithine-motility semisolid agar, and indole test broth (13). Additional confirmatory tests are those described by Edwards and Ewing (3), and differentiation of the *Enterobacteriaceae* is based on the revised classification and nomenclature of Ewing (4). Isolates of H$_2$S-producing variants of *E. coli* conform to the reactions described by Darland and Davis (1).

Antimicrobial susceptibility testing of the isolates was performed by the agar-dilution procedure with an inocula-replicating apparatus as described by Steers et al. (10) and an expanded dilution scale as described by Washington (12). The inoculum was adjusted so that $10^4$ colony-forming units were delivered onto the agar surface.

The records of the patients from whom H$_2$S-producing variants of *E. coli* were isolated were reviewed in an attempt to establish whether or not these isolates were clinically or epidemiologically significant.

**RESULTS**

The colonies ranged in size from 2 to 3.2 mm after 24 h of incubation. They appeared as glistening, grayish-white, convex colonies on 5% sheep blood agar after 24 h. On eosin-methylene blue agar the colonies were opaque and slightly purple with smooth edges; occasionally they had an undulate edge. A green metallic sheen became apparent at 24 to 48 h in the region of heavier inoculum.

The biochemical reactions of the 17 H$_2$S-producing variants of *E. coli* are listed in Table 1. Twelve strains produced an acidic slant and butt in triple sugar-iron agar; five produced an alkaline slant and acidic butt in this medium. None of the strains utilized citrate or malonate, hydrolyzed urea, deaminated lysine or phenylalanine, grew in KCN or on cetrime, were Voges-Proskauer positive, liquified gelatin, or fermented inositol or adonitol. All produced H$_2$S in both triple sugar-iron agar and lysine-iron agar, reduced nitrate, produced indole, were methyl red-positive, and fermented dextrose, arabinose, maltose, xylose, mannitol, and trehalose.

Antimicrobial susceptibilities are listed in Table 2. All of the strains were susceptible to gentamicin, polymyxin B, kanamycin, streptomycin, chloramphenicol, nalidixic acid, and nitrofurantoin. Ampicillin, cephalothin, carbenicillin, and tetracycline exhibited moderate activity against these strains.

Ten of the isolates were from stool cultures, of which four were from patients with signs and symptoms of acute gastroenteritis and from whom no salmonellae or shigellae were isolated. None of these strains was tested for enterotoxin production or invasive properties. No clinical significance could be ascribed to the other stool isolates. Five isolates were from urine: in one case, in small numbers in mixed culture; in three cases, there was significant bacteriuria associated with signs and symptoms of urinary tract infection; and, in one case, the patient had
TABLE 1. Biochemical reactions of H₂S-positive
E. coli

| Substrate or test* | No. (+) | No. (%) | No. (%) |
|-------------------|---------|---------|---------|
| H₂S in TSIA       | 17      | 0       | 100     |
| H₂S in LIA        | 17      | 0       | 100     |
| Citrate           | 0       | 17      | 0       |
| Urea              | 0       | 17      | 0       |
| Motility          | 14      | 3       | 82      |
| Indole            | 17      | 0       | 100     |
| MR                | 17      | 0       | 100     |
| V-P               | 0       | 17      | 0       |
| Lysine decarboxylase | 16     | 1       | 94      |
| Arginine dihydrolase | 2      | 4      | 35      |
| Ornithine decarboxylase | 11     | 2      | 74      |
| Phenylalanine deaminase | 0      | 17      | 0       |
| Malonate          | 0       | 17      | 0       |
| KCN               | 0       | 17      | 0       |
| Gelatin           | 0       | 17      | 0       |
| Growth on MacConkey agar | 17  | 0       | 100     |
| Esculin           | 5       | 5       | 7       |
| SS agar           | 7       | 3       | 7       |
| Cetrimide         | 0       | 17      | 0       |
| Arabinose         | 17      | 0       | 100     |
| Raffinose         | 6       | 11      | 35      |
| Rhamnose          | 14      | 1       | 2       |
| Sucrose           | 5       | 12      | 29      |
| Maltose           | 17      | 0       | 100     |
| Xylose            | 17      | 0       | 100     |
| Adonitol          | 0       | 17      | 0       |
| Inositol          | 0       | 17      | 0       |
| Sorbitol          | 16      | 0       | 1       |
| Dextrose          | 17      | 0       | 100     |
| Gas from dextrose | 17      | 0       | 100     |
| Dulcitol          | 14      | 0       | 1       |
| Mannitol          | 17      | 0       | 100     |
| Lactose           | 13      | 0       | 4       |
| Salicin           | 7       | 3       | 7       |
| Trehalose         | 17      | 0       | 100     |
| Acetate           | 15      | 0       | 2       |
| Nitrate           | 17      | 0       | 100     |

*TSIA, Triple sugar-iron agar; LIA, lysine-iron agar; MR, methyl red; V-P, Voges-Proskauer; SS agar, salmonella-shigella agar.

aA chronic indwelling urethral catheter, and repeated cultures were obtained with H₂S-producing E. coli and Proteus rettgeri in the range of 10⁴ to 10⁵ colonies per milliliter. The organism was isolated from tissues obtained at autopsy in two cases: from an ulcerated lesion of the breast in a fatal case of leukemia and from a kidney encased in tumor in a case of retroperitoneal malignant histiocytoma.

**DISCUSSION**

The biochemical and serological characteristics of more than 200 H₂S-positive variants of E. coli were recently reported by Darland and Davis (1). Because of significant differences in some reactions and the distribution of O and H antigens between the variants and biochemically typical E. coli, these investigators thought that the variants represented a rather limited subgroup of E. coli. Some, but not all, of these differences were noted in our isolates and corroborate Darland and Davis’s contention. These investigators noted pronounced differences in antimicrobial susceptibility between H₂S-positive E. coli and biochemically typical E. coli. Many of the variants were resistant to tetracycline, sulfadiazine, streptomycin, and ampicillin, whereas few of their typical E. coli strains were. Our H₂S-producing variants were slightly more resistant than our typical E. coli to ampicillin (59 and 71%, respectively, inhibited by 5 μg/ml) and tetracycline (59 and 70%, respectively, inhibited by 5 μg/ml) but were otherwise very similar in their susceptibilities to the other antimicrobics tested. Close linkage between the gene responsible for H₂S production and tetracycline resistance has been reported (11).

Instability of H₂S production, a characteristic noted in several of our strains, has been reported by other investigators (1, 5, 8, 9). This instability may limit recognition of the variant in clinical material.

The clinical significance of H₂S-producing

| Antimicrobial     | Strains tested (no.) | Cumulative % susceptible at each μg/ml |
|-------------------|----------------------|----------------------------------------|
|                   |                      | 1  | 5  | 10 | 20 | 50  | 100 | 200 |
| Ampicillin        | 17                   | 0  | 59 | 65 | 65 | —   | 65  | 65  |
| Cephalothin       | 17                   | 12 | 47 | 65 | 76 | —   | 100 |     |
| Carbenicillin     | 17                   | 6  | 12 | —  | —  | 65  | 65  | 65  |
| Streptomycin      | 8                    | 0  | 63 | 75 | 100|     |     |     |
| Tetracycline      | 17                   | 29 | 59 | 59 | 65 |     |     |     |
| Kanamycin         | 17                   | 6  | 88 | 100|    |     |     |     |
| Nalidixic acid    | 17                   |    |    |    |    |     |     |     |
| Polymyxin B       | 8                    | 13 | 100|     |    |     |     |     |
| Chloramphenicol   | 17                   | 0  | 53 | 94 | 100|     |     |     |
| Nitrofurantoin    | 17                   |    |    |    |    |     |     |     |
| Gentamicin        | 17                   | 100|     |    |    |     |     |     |
variants of E. coli remains unclear. The ability of the organism to produce H₂S appears to be mediated by extrachromosomal deoxyribonucleic acid (6), as are hemolysins, colicins, and plasmids for drug resistance and enterotoxin. It is conceivable, therefore, that a plasmid that mediates drug resistance or enterotoxin production can become linked with H₂S production. Enterotoxigenicity by strains of E. coli (2) and other Enterobacteriaceae (7) is now well known, as is the high rate of transfer (6). Unfortunately, none of our isolates were tested for this property, so that their role in producing gastroenteritis could not be assessed. That they are capable of causing significant bacteriuria seems clear. Their role in producing terminal infections in the two patients who died is not at all clear, because the clinical significance of postmortem bacteriology is highly questionable (14).

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