Hydrogen Sulfide-Negative Variant of *Citrobacter*

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The characteristics of 25 hydrogen sulfide-negative strains of *Citrobacter* were studied. The majority of isolates were from the respiratory tract and usually were of indeterminate clinical significance. All strains were highly susceptible to polymyxin B, gentamicin, kanamycin, nalidixic acid, and nitrofurantoin.

Variants of the *Citrobacter* group which fail to produce hydrogen sulfide (H₂S) have been described by Leclerc and Buttiaux (5) and by Davis and Ewing (1). The recent isolation of 25 such strains has prompted us to examine their biochemical and clinical characteristics and their susceptibilities to various antimicrobial compounds.

**MATERIALS AND METHODS**

Except for one from a specimen of spoiled cabbage, all strains were isolated from clinical material submitted to the general bacteriology laboratory of the Mayo Clinic. Initial screening of gram-negative bacilli was carried out with triple sugar-iron (TSI) agar, lysine-iron agar (LIA), Christensen's urea, Simmons' citrate, indole test broth, and ornithine decarboxylase (Moeller base) in 0.3% agar (2). All products except indole test broth were purchased from BioQuest, Cockeysville, Md. All strains were further studied with tests described by Edwards and Ewing (3); nomenclature and biochemical reactions are based on the taxonomic system of Ewing (4). Minimum inhibitory concentrations (MIC) were determined by the agar-dilution technique by using the Steers et al. inocula-replicating apparatus (7) with an expanded dilution scale as described in an earlier report from this laboratory (8).

Clinical data were obtained by review of each patient's clinical records. Each case was designated as representing primary, secondary, or indeterminate infection, based on criteria previously specified (9).

**RESULTS**

**Bacteriological.** Biochemical reactions of the H₂S-negative *Citrobacter* strains are listed in Table 1. With respect to the TSI agar reaction, 23 strains produced an acidic slant and an acidic butt, and two strains produced an alkaline slant and an acidic butt. All strains produced gas in TSI agar. All strains produced an alkaline slant and acidic butt in LIA. All but five strains yielded black colonies with a metallic sheen on eosin-methylene blue agar. All strains were methyl red-positive and Voges-Proskauer-negative. None decarboxylated lysine or deaminated lysine or phenylalanine. All but one grew in KCN broth within 48 hr. Eighty per cent of the isolates yielded a positive urease reaction within 4 days, and all utilized citrate within 4 days. None of the isolates liquefied gelatin or fermented adonitol or inositol. Sixteen and 24% of the isolates failed to decarboxylate arginine and ornithine, respectively, within 4 days.

**Antibiotic susceptibility.** MIC values of the 25 isolates are listed in Table 2. Only one isolate was inhibited by 20 μg of cephalothin per ml, whereas 80% of the isolates were inhibited by 10 μg of carbenicillin per ml. All isolates were highly susceptible to polymyxin B, kanamycin, gentamicin, nalidixic acid, and nitrofurantoin. The susceptibility to chloramphenicol, tetracycline, and streptomycin was intermediate. Ampicillin inhibited only 24% at 20 μg/ml but inhibited 92% at 200 μg/ml.

**Clinical data.** All but three isolates were found in mixed cultures, and all were clinically evaluated as being of secondary or indeterminate significance (Table 3). The greatest number of isolates were from the respiratory tract, usually in small numbers in a mixed culture. In eight cases, H₂S-negative *Citrobacter* was isolated from the urine; in all but three of these, it was encountered in insignificant numbers. Two cultures from postoperative wounds and one from a gangrenous toe yielded H₂S-negative *Citrobacter*, all in mixed culture.

**DISCUSSION**

Among the biochemical characteristics listed in the definition of *Citrobacter* by the International *Enterobacteriaceae* Committee (6) is the production of H₂S. Strains failing to produce H₂S were reported in 1965 by Leclerc and Buttiaux (5), although these authors described two subgroups on the basis of the presence or absence of lysine
TABLE 1. Biochemical reactions of 
H₃S-negative Citrobacter

| Test or substrate | Number | Per cent |
|-------------------|--------|----------|
|                        | + (+)  | −    |
|                        | + (+)  | −    |
| Hydrogen sulfide on:  |        |        |
| TSI agar             | 0 0 25 | 0 0 100 |
| LIA                 | 0 0 25 | 0 0 100 |
| Urease              | 16 4 5 | 64 16 20 |
| Indole              | 5 0 20 | 20 0 80 |
| Methyl red (37 C)    | 25 0 0 | 100 0 0 |
| Voges-Proskauer (37 C) | 0 0 25 | 0 0 100 |
| Citrate (Simmons')  | 24 1 0 | 96 4 0 |
| Malonate            | 10 0 15 | 40 0 60 |
| KCN                 | 24 1 0 | 96 4 0 |
| Motility            | 18 0 7 | 72 0 28 |
| Gelatin             | 0 0 25 | 0 0 100 |
| Lysine decarboxylase | 0 0 25 | 0 0 100 |
| Arginine dihydrolase | 14 7 4 | 56 28 16 |
| Ornithine decarboxylase | 19 0 6 | 76 0 24 |
| Phenylalanine deaminase | 18 1 6 | 72 4 24 |
| Acetate             | 0 0 25 | 0 0 100 |
| β-Galactosidase (ONPG) | 25 0 0 | 100 0 0 |
| Lactose             | 23 1 1 | 92 4 0 |
| Sucrose             | 19 0 6 | 76 0 24 |
| Adonitol            | 0 0 25 | 0 0 100 |
| Inositol            | 0 0 25 | 0 0 100 |
| Arabinose           | 24 0 1 | 96 0 4 |
| Raffinose           | 20 0 5 | 80 0 20 |
| Rhamnose            | 20 0 5 | 80 0 20 |
| Dulcitol            | 6 0 19 | 24 0 76 |
| Mannitol            | 24 0 1 | 96 0 4 |
| Salicin             | 14 1 10 | 56 4 40 |

* Symbols: +, positive reaction within 48 hr; (+), delayed positive reaction; −, no reaction.

TABLE 2. Antimicrobial susceptibilities of H₃S-negative Citrobacter

| Agent              | Cumulative MIC (%) at various antibiotic concn (μg/ml) |
|--------------------|------------------------------------------------------|
|                    | 1          | 5          | 10         | 20         | 50         | 100        | 200        | 300        |
| Cephalothin        | 4          | 4          | 4          | 24         |           |            |            |            |
| Ampicillin         | 8          | 20         | 24         | 72         | 92         |            |            |            |
| Carbenicillin      | 24         | 76         | 80         | 80         | 100        |            |            |            |
| Streptomycin       | 0          | 44         | 72         | 76         |            |            |            |            |
| Chloramphenicol    | 28         | 64         | 92         |            |            |            |            |            |
| Tetracycline       | 8          | 52         | 84         |            |            |            |            |            |
| Polymyxin B        | 76         | 92         | 92         |            |            |            |            |            |
| Kanamycin          | 4          | 100        |            |            |            |            |            |            |
| Gentamicin         | 100        |            |            |            |            |            |            |            |
| Nalidixic acid     | 84         | 100        | 100        |            |            |            |            |            |
| Nitrofurantoin     | 84         | 96         | 100        |            |            |            |            |            |

* One of the 25 isolates was from a spoiled cabbage.

decarboxylase. These authors proposed that such strains be grouped under their old species name, 
Citrobacter intermedium. Davis and Ewing (1) described the characteristics of 93 cultures of 
Citrobacter which failed to produce H₂S in TSI agar; however, none of these strains decarboxylated 
lysin, a characteristic included in the definition of Citrobacter (6).

In an earlier report from this laboratory (9), a group of Enterobacteriaceae tentatively called 
"atypical Enterobacter cloacae" was described. None of these strains produced H₂S. All were 
indole- and methyl red-positive and Voges-Proskauer-negative, none had lysine decarboxylase, all 
utilized citrate, and most produced urease. Nearly all had arginine dihydrolase and all pro-
duced ornithine decarboxylase. All fermented arabinose and rhamnose, but none fermented 
raffinose. A group of Enterobacteriaceae closely resembling "atypical E. cloacae" has been 
described by Young et al. (Int. J. Syst. Bacteriol., in press) who proposed establishment of a new 
genus, Levinia, for these organisms. Two species were also proposed, L. malonatica and L. amal-
notatica. That this proposed genus may represent an atypical or unusual variant of Citrobacter has
been suggested by D. A. A. Mossel (personal communication); however, Young et al. (Int. J. Syst. Bacteriol., in press) pointed out significant differences which distinguish the proposed genus *Levinia* from the genus *Citrobacter*. The distinguishing characteristics of *Levinia* include, in addition to the lack of H$_2$S production in TSI agar, the ability to hydrolyze esculin, the inability to utilize citric and α-tartaric acids, the consistent production of arginine dihydrolase and ornithine decarboxylase, the fermentation of adonitol, and the inability to ferment dulcitol or raffinose.

The 25 cultures under consideration in this study biochemically resemble *Citrobacter* closely except for the lack of production of H$_2$S in TSI and LIA media and, therefore, probably represent a variant of *Citrobacter*. Whether the proposed genus, *Levinia*, is accepted or not, it is reasonably clear that these organisms constitute a group with characteristics which distinguish them readily from *Citrobacter* and *E. cloacae*.

The "atypical *E. cloacae*" group was susceptible to cephalothin and cephalaxin (9), in contrast to typical *Enterobacter* species and in contrast to *Citrobacter*. The H$_2$S-negative *Citrobacter* group is resistant to cephalothin and, in this respect, closely resembles our experience with H$_2$S-producing *Citrobacter* (8). The H$_2$S-negative *Citrobacter* group is susceptible to carbenicillin, resembling closely our experience to date with H$_2$S-producing strains of *Citrobacter*. As reported by Slocombe and Sutherland (Antimicrob. Ag. Chemother.—1969, p. 78–85), cephaloridine is destroyed by the β-lactamases produced by *Enterobacter* and *Citrobacter*; however, *Citrobacter* and *Enterobacter* do not significantly inactivate carbenicillin and are indeed susceptible to this agent in low concentrations.

Clinically, the H$_2$S-negative *Citrobacter* isolates represented secondary invaders or, more commonly, were of indeterminate significance. Their frequent respiratory tract origin was similar to that of typical *Citrobacter* species (8). In three patients with chronic obstructive pulmonary disease, H$_2$S-negative *Citrobacter* was isolated from sputum cultures in small numbers and mixed with normal oropharyngeal flora. In one patient referred to this clinic, 2 weeks after endoscopic perforation of the esophagus with secondary massive pneumonitis, mediastinitis, and empyema, the organism was isolated on successive days in pure culture. The remaining isolates from sputum were from four patients with prolonged hospitalizations due to a variety of underlying conditions, including lymphoma, myocardial infarction, head injury with quadriplegia, and hypercoagulability state with pneumococcal pneumonia, respectively. With the exception of the case of massive pneumonitis, in which pure cultures of H$_2$S-negative *Citrobacter* were obtained, it was difficult to ascribe any significance to the finding of this organism in the sputum, either because of the absence of signs and symptoms of lower respiratory infection or because of the concurrent isolation of other gram-negative bacilli, including members of the genera *Klebsiella* and *Enterobacter*, *Pseudomonas aeruginosa*, and *P. maltophilia*. All of the throat culture isolates of H$_2$S-negative *Citrobacter* were from children less than 2 years old with upper respiratory infection of undetermined etiology.

The organism was isolated in significant numbers from three patients with a history of recurrent urinary tract infection, and in one of these patients it was isolated with a significant number of *Escherichia coli*. In the remainder of cases, it was isolated from the urine in insignificant numbers. Cultures from a gangrenous toe of a diabetic patient with peripheral vascular insufficiency yielded H$_2$S-negative *Citrobacter* along with *Proteus mirabilis*, *Klebsiella*, *Serratia marcescens*, *S. epidermidis*, and *Pseudomonas*.

With few exceptions, therefore, it would appear that H$_2$S-negative *Citrobacter* represented a harmless saprophyte requiring no specific therapy. Its role as an opportunist, however, is well illustrated by its recovery in pure culture from the sputum of a patient with massive pneumonitis.

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