Unusual *Enterobacteriaceae*: H_2S^+ *Shigella sonnei*, One Authentic and One False Positive Due to Contamination with the Obligate Anaerobe *Eubacterium lentum*

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A mixture of *Shigella sonnei* and *Eubacterium lentum* produced H_2S in triple sugar iron agar; however, neither produced any in pure culture. A second culture of *S. sonnei*, isolated in Japan, is thought to be the first documented H_2S^+ *Shigella*.

Reports of *Escherichia coli* that produce hydrogen sulfide date back over 30 years (4). However, it has been only recently that these unusual strains have been recognized as typical *E. coli* that have acquired plasmids (1, 5-7). In H_2S^+ *E. coli*, these extrachromosomal elements code for an enzyme system that reduces thiosulfate or tetrathionate to H_2S (7). Most strains of *Enterobacteriaceae* produce slight amounts of H_2S from the cysteine found in most peptone media (7); this H_2S can be detected by a lead acetate paper suspended in the tube. However, when we refer to H_2S production, we mean the production of a definite black precipitate in triple sugar iron agar (TSI) and peptone iron agar (PIA). This is due to the presence of the enzyme system that reduces thiosulfate to H_2S ($S_2O_3^{2-} \rightarrow H_2S$), which then reacts with iron salts in the medium to form a black precipitate of FeS .

Shigella is very closely related to *E. coli* on the basis of biochemical reactions, antigens, and genetics, so H_2S^+ *Shigella* could exist in nature. However, previous reports of H_2S^+ *Shigella* are not convincing (4). In this paper we describe two strains of *Shigella sonnei* which were reported to be H_2S^+ . The first isolate of " H_2S^+ *S. sonnei*" was from the case described below.

A girl, 4 years and 10 months old, was admitted to a nursery for mentally retarded children where *S. sonnei* has been endemic. She had a respiratory infection and was treated with ampicillin. On day 5 of treatment she developed diarrhea. Stool specimens were collected and submitted for routine culture, and

the ampicillin therapy was discontinued. The laboratory received the stool specimens on 11 and 12 November 1974. Colonies resembling *Shigella* were noted on xylose lysine desoxycholate plates, and picked to Kligler iron agar modified to detect tryptophane deaminase (MKIA). The *Shigella*-like colony was identified on 13 November as *Shigella sonnei*, both biochemically and serologically. At this stage everything was unremarkable, and the Kligler tube was discarded. The next morning one of us (D.A.) saw what appeared to be a *Salmonella* reaction in the Kligler tube in the discard sack. He remarked casually, "Who had the *Salmonella*?" He was informed that no *Salmonella* were isolated that day and a *Shigella* group D had been reported from the specimen in question. His immediate reaction was "Impossible! It is H_2S positive!" The technologist who had worked with the organism on the previous day assured him that it had been a run-of-the-mill *Shigella* when it was discarded. A few hours later another of us (W.I.T.) came into the laboratory and was shown the tube. His immediate impression was that the organism was *S. typhi* because there was a slight H_2S band and no gas. The lysine decarboxylase test was negative, however, and the organism was nonmotile. The next supposition was that the tube contained a mixed culture. Inoculum from the H_2S^+ tube was used to streak xylose lysine desoxycholate and eosine methylene blue plates, and the following day pure cultures of typical *Shigella* were obtained. However, all of the single colonies were H_2S^- . At this point there was confusion about what had actually happened but the matter was not pursued. By this time, a second stool specimen had been

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received and a typical H_2S^- *S. sonnei* was isolated. A third stool specimen was received from this patient, and the *S. sonnei* colonies picked to MKIA had good H_2S production at 18 h. At this point, our interest was rekindled and we decided to make subcultures of this latest slant and send them on to Center for Disease Control (CDC) via the Illinois State Health Department.

At CDC the culture was streaked on Trypticase soy (BBL), xylose lysine desoxycholate, and MacConkey agars (all incubations were aerobic at $36 \pm 1^\circ C$ unless otherwise stated). Isolated colonies were identified as typical H_2S^- *S. sonnei*; however, inoculations from the area of confluent growth always resulted in H_2S^+ tubes of PIA and TSI. Dilutions of the whole culture made in tubes of PIA showed that about 10,000 colony-forming units were necessary to obtain any blackening. Figure 1 shows that about 99.99% of the population was H_2S^- . An area of blackening was picked from one of these tubes and streaked onto two PIA plates. At this stage luck intervened, and the decision to incubate one of them anaerobically was made. No blackening was observed on the aerobic plate, but several areas of blackening were noted on the anaerobic plate. Two distinct colony types were seen on the anaerobic plate, one large and one much smaller. Neither colony type blackened the medium, but when they were close together, a black band was produced between them (Fig. 1). Each colony type was picked to

FIG. 1. Left, 12 H_2S^+ colonies of *E. lentum* in a PIA tube also containing about 10^5 H_2S^- *S. sonnei*; right, area of blackening (PIA plate, anaerobic incubation) between a colony of *S. sonnei* and one of *E. lentum*.

different PIA slants which were incubated aerobically. The small colony type did not grow aerobically; the large colony type, identified as *S. sonnei*, grew but did not produce H_2S . After about 3 weeks the riddle was solved. The culture was a typical *S. sonnei* contaminated with an obligate anaerobe, subsequently identified as *Eubacterium lentum* at the anaerobe reference laboratory, CDC, according to the procedures of Dowell and Hawkins (2). (*E. lentum* was characterized as follows: obligate anaerobe, gram positive, short-thin rod, no spores produced, asaccharolytic, reduced nitrate, catalase negative, no detectable metabolic products by gas-liquid chromatography.) Finegold et al. (3) showed that over 50% of healthy adults of Japanese ancestry carried *E. lentum* in their gut and the average count was over $10^{10}/g$ of feces. The original patient probably carried *E. lentum* in her gut and many cells of this anaerobe were present under the *S. sonnei* colonies. In two of three pickings, *E. lentum* contaminated *S. sonnei* even though a single colony was touched.

Can *E. lentum* grow under an *S. sonnei* colony on an aerobic plate? The microenvironment under a colony of an aerobe might be reduced enough to allow an obligate anaerobe to multiply, even though the surface environment of the plate is oxidized. All attempts to show multiplication of *E. lentum* under colonies of *S. sonnei* (aerobic incubation) were negative, but *E. lentum* did survive well.

At this point we were concerned that all reports of H_2S^+ *E. coli* might be due to contamination with anaerobes because the behavior of an anaerobe could mimic that of a plasmid. However, all 12 cultures (1) of H_2S^+ *E. coli* that we

FIG. 2. H_2S production (TSI, 24 h) by the authentic strain of *S. sonnei* (9020-75).

tested were pure. Other cultures have been submitted in the past as H_2S^+ but have yielded only H_2S^- single colonies. We suspect that these were contaminated with anaerobes, but unfortunately none were available for retesting. On the basis of our experience in this one instance we feel that research into the interactions of *Enterobacteriaceae* with anaerobes deserves more emphasis. Because neither culture produced H_2S in pure culture (*E. lentum* produced a trace amount in PIA when incubated anaerobically) we suggest the name "syn-

TABLE 1. Biochemical reactions of the two H_2S^+ *Shigella* strains and typical reactions given by 991 other strains of these serotypes

Test	<i>S. sonnei</i> 9020-75	640 other <i>S. sonnei</i>	<i>S. flexneri</i> 4a 2196-69	351 other mannitol ⁺ <i>S. flexneri</i> 4a
Indole	- ^a	0 ^b	+ ^{wk}	49
Methyl red	+	100	+	100
Voges-Proskauer	-	0	-	0
Simmons citrate	-	0	-	9
H_2S on TSI	+	0	+	0
Urea	-	0	-	0
KCN	-	0	-	0
Motility	-	0	-	0
Gelatin (22 C)	-	0	-	0
Lysine	-	0	-	0
Arginine	-	0.5	-	0
Ornithine	+	99.4	-	0
Phenylalanine	-	0	-	0
Malonate	-	0	-	0
D-Glucose, acid	+	100	+	100
D-Glucose, gas	-	0	-	0
Lactose	+ ⁷	2 (88)	-	0
Sucrose	+ ⁶	0.1 (85)	+ ⁶	1 (45)
D-Mannitol	+	99	+	100
Dulcitol	-	0	-	0
Salicin	-	0	-	0
Adonitol	-	0	-	0
i-Inositol (<i>meso</i>)	-	0	-	0
D-Sorbitol	-	1	-	32
L-Arabinose	+	94	+ ⁴	63 (21)
Raffinose	-	3 (82)	+	70
L-Rhamnose	+	77 (21)	-	11
Trehalose	+	100	+	100
Mucate	-	16	-	0
Acetate	-	0	-	8
Jordan tartrate	+	100	-	0
ONPG ^c	+	95	-	0
$NO_3^- \rightarrow NO_2^-$	+	100	+	100
Oxidase	-	0	-	0
H_2S on PIA	+		+ ⁴	
H_2S on lysine iron agar	-		-	

^a Symbols: +, positive within 24 h (48 h for indole, methyl red and Voges-Proskauer); -, negative at 7 days; +ⁿ, n gives the date the reaction became positive; +^{wk}, a weak positive reaction.

^b Gives the percentage of positive within 48 h as given in the CDC publication *Biochemical Reactions of Shigella*, 1971. The percentage of delayed reactions (+ after 48 h) is sometimes given in parentheses.

^c ONPG, *o*-nitrophenyl- β -D-galactopyranoside.

ergistic H_2S production" for this phenomenon.

Recently two of us (H.I. and T.A.) have recovered isolates of H_2S^+ *S. sonnei* (to be described in greater detail elsewhere) from an outbreak of shigellosis in Japan. One of these isolates was confirmed as an H_2S^+ *S. sonnei* (CDC No. 9020-75) by the International Shigella Center at CDC (Fig. 2). Although there have been other reports of H_2S^+ *Shigella* (4), ours may be the first authentic strain of this unusual biotype. We estimate that about 1 in 1,000 clinical isolates of *E. coli* will be an H_2S^+ *E. coli*. Since hospital laboratories isolate perhaps 500 to 1,000 *E. coli* for every *Shigella*, we estimate that a clinical laboratory that processes about 1,000,000 typical *E. coli* might find one H_2S^+ *Shigella*. This is why reports of H_2S^+ *E. coli* are more numerous; however, we are interested in other reports of H_2S^+ *Shigella*.

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ADDENDUM

We have now confirmed a second H_2S^+ *Shigella*. This culture (CDC No 2196-69) was isolated from the stool culture of a patient in Illinois. It was confirmed at the International *Shigella* Center at CDC as an H_2S^+ *Shigella flexneri* 4a (H_2S production was almost identical to that in Fig. 2) that was serologically and biochemically (except for H_2S) typical. The biochemical reactions of the two authentic H_2S^+ *Shigella* cultures are given in Table 1 along with biochemical reactions of H_2S^- isolates of the same species and serotype.

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