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Isolation of *Simonsiella* sp. from a Neonate

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A member of the genus *Simonsiella*, presumptively identified as *S. muelleri*, was isolated from a gastric aspirate taken from a neonate 15 min postpartum. The neonate showed a dental cyst and early eruption of teeth, confirmed by mandibular X ray. The morphological features, cultural characteristics, and antimicrobial susceptibility of the isolate are presented.

Members of the genera *Simonsiella* and *Alysiella* are gram-negative bacteria with unusual morphology and motility. They are commonly described as filamentous with gliding motility (4). Their normal habitat is the oral cavity of a wide range of warm-blooded vertebrates (4, 7). The ecological role of these organisms is unclear. These bacteria have been considered members of the normal flora (4; D. A. Kuhn, D. A. Gregory, J. Pangborn, and M. Mandel, J. Dent. Res **53**[Special issue]:108, 1974) and reported as isolates from erosive lesions of the oral cavity (1), but often are not even mentioned as part of the oral microflora (11). A recent survey, in which simonsiellas were detected in normal mouths of 32% of 212 human subjects between the ages of 4 and 80 years, would indicate that they are part of the normal oral flora (2).

In this paper we report the isolation and characterization of *Simonsiella* sp. from a neonate.

CASE REPORT

A baby girl was born to a 36-year-old mother at approximately 36 weeks of gestation after a 24-h period of ruptured membranes. There was a history of slight bleeding during the first few weeks of pregnancy and again approximately 1 month before delivery. No gross anomalies were detected in the neonate immediately after delivery, except for a dental cyst in the lower mandibular area. A mandible X ray was done because of the dental cyst, and it revealed early eruption of the teeth. Because of the history of prolonged ruptured membranes, septic work-up, including a gastric aspirate, was done on the baby.

MATERIALS AND METHODS

Cultural microbiology. A gastric aspirate was collected approximately 15 min postpartum by De Lee suction tube (Argyl, Div. Sherwood Medical, St. Louis, Mo.) through the oropharynx, examined by direct smear, and plated on 5% sheep blood agar, MacConkey agar, and chocolate agar (Difco Laboratories, Detroit, Mich.). The sheep blood agar plates were made in triplicate for aerobic and anaerobic incubation and incubation in 5 to 10% CO_2 to facilitate early characterization of isolates. All plates were incubated at 36°C for 18 h. Isolates from the plates were subjected to routing staining, biochemical tests, and determination of antimicrobial susceptibility.

Hugh-Liefson O-F medium, Christensen urea agar, Simmons citrate medium, and cystine tryptic agar with added 1% carbohydrate were supplied by Vista Laboratories, Edmonton, Alberta, for the determination of oxidation-fermentation of glucose, urease activity, citratase activity and carbohydrate utilization, respectively.

TSI medium was obtained from BBL (Microbiology Systems, Cockeysville, Md.). Growth in the presence of sodium chloride was determined in Trypticase soy broth (BBL) plus 1.5 to 6.5% NaCl.

Streptococci were identified on the basis of the following: gram stain, catalase test, reaction on bile esculin agar (Difco), growth in Trypticase soy broth plus 1.5 to 6.5% NaCl, hemolysis, the CAMP test (1a), and Lancefield grouping (Streptex systems, Wellcome Diagnostics, Dartford, United Kingdom).

Antimicrobic susceptibility was determined by agar diffusion on Mueller Hinton medium (Difco) with antimicrobial disks (BBL).

Microscopy. Because of initial difficulties in identification of the gram-negative isolate by biochemical tests, extensive microscopic examinations were carried out.

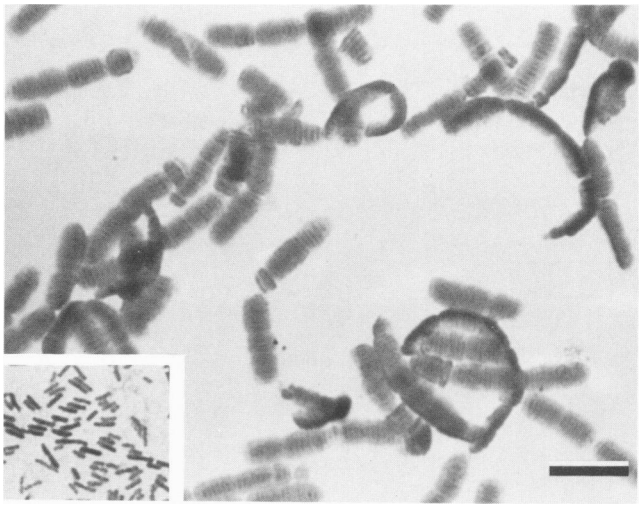

FIG. 1. Light micrograph of *Simonsiella* sp. stained with methylene blue and photographed with the 100× oil immersion objective of the microscope. The darker-staining, curved forms are the organisms viewed from the side. The insert shows cells of *E. coli* photographed at the same magnification for comparison. Bar, 10 μm.

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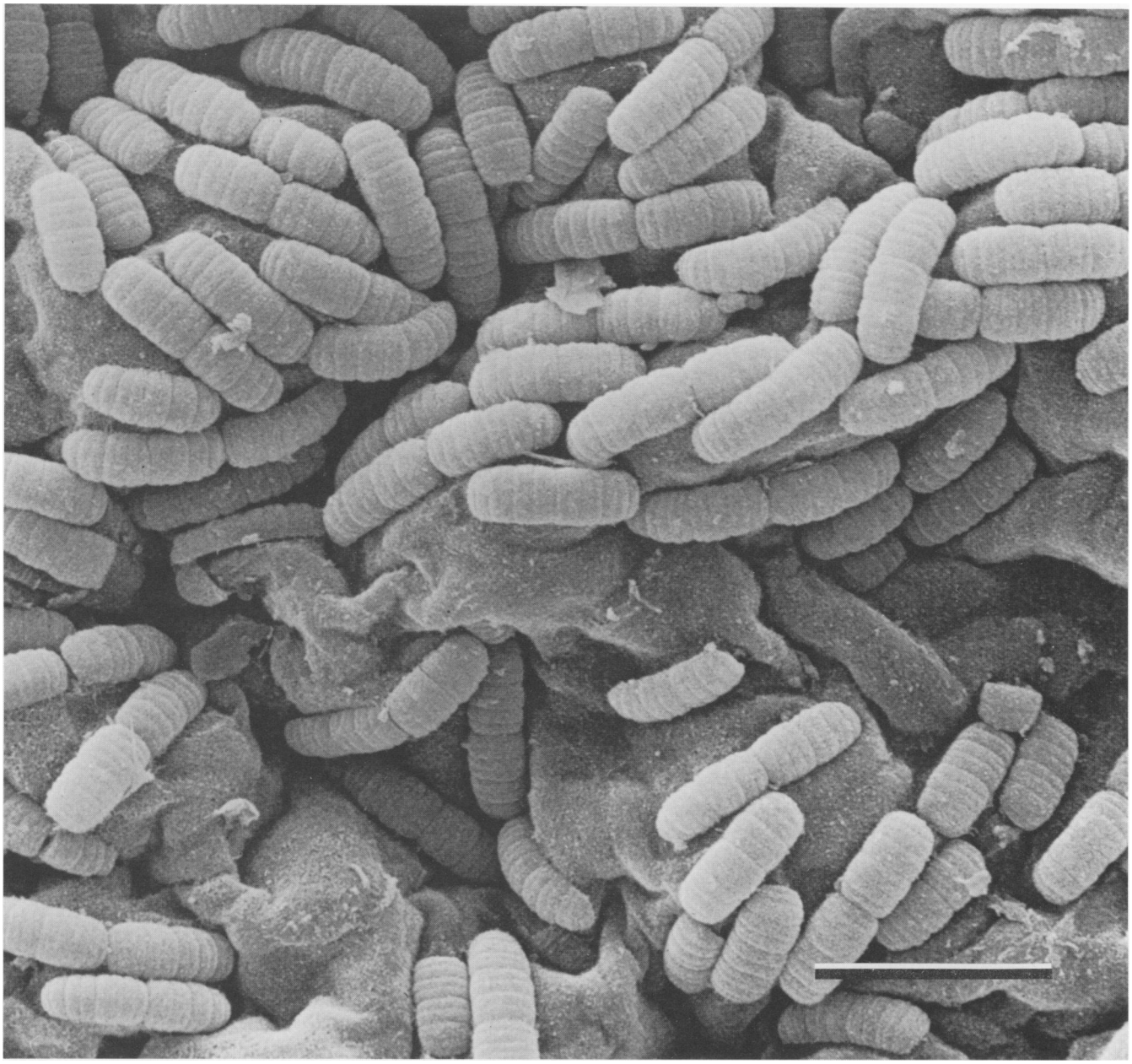

FIG. 2. Scanning electron micrograph of *Simonsiella* sp. remaining on an agar surface after most of the colony has been removed. The concave sides of the organism are in contact with the surface, and the rounded ends are often embedded in the medium. Sculpturing of the agar is likely due to the action of the organisms. Bar, 10 μm.

(i) Light microscopy. The isolate was grown on BSTSY agar (5), a medium containing bovine serum, glucose, tryptic soy broth, and yeast extract. Smears from colonies on this medium were stained with methylene blue for 1 min and photographed through a 100× oil immersion objective.

(ii) Electron microscopy. Overnight cultures on BSTSY agar incubated aerobically at 37°C were used for electron microscopy. Small squares of agar were excised on which isolated colonies were growing. During processing the colonies became detached from the agar, but the agar was processed along with the colonies because we presumed that some of the organisms would still be found on the agar surface, which might possibly provide useful information on surface adhesion. Colonies and agar were fixed overnight at room temperature with 1% osmium tetroxide in cacodylate buffer (pH 6.8). After two 15-min washes in cacodylate buffer the material was dehydrated through a graded series of ethanol up to absolute ethanol. After three changes of absolute ethanol, cultures were stored in this solvent for 3 weeks at 4°C. Colonies and squares of agar were then further processed for scanning electron microscopy by standard techniques, namely, critical point drying followed by mounting on stubs and sputter coating with gold (3). The preparations were examined in a Stereoscan 250 scanning electron microscope (Cambridge Instruments Co., Cambridge, United Kingdom).

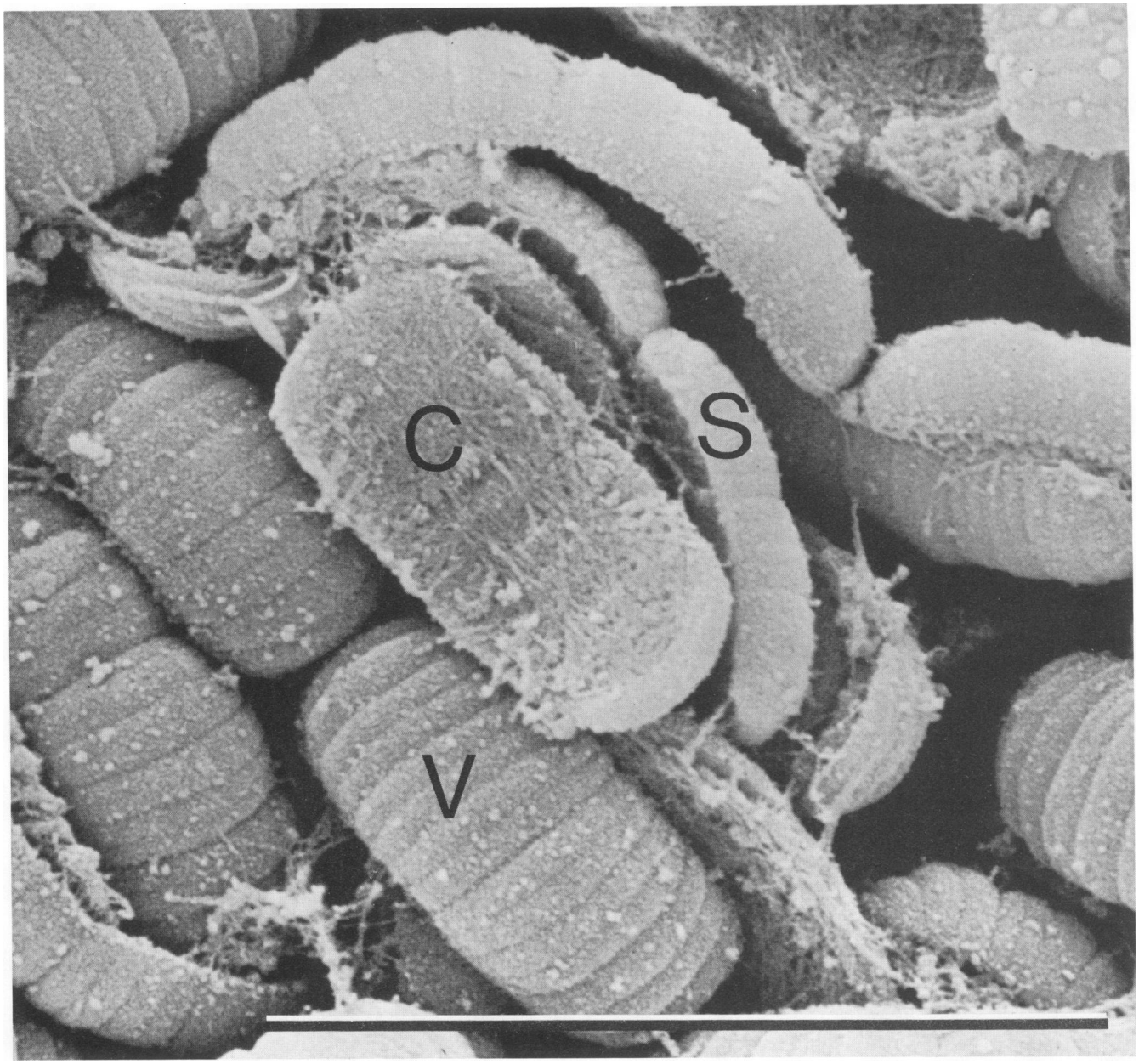

FIG. 3. Scanning electron micrograph of *Simonsiella* sp. showing the different aspects of the organism: V, convex surface; C, concave surface showing fibrillar material normally in contact with the surface to which the organism is attached; S, side view. Bar, 10 μm.

RESULTS

Isolates from the gastric aspirate. Examination of the gastric secretions under a 10× objective revealed 3+ (20 to 25 per field) pus cells; examination under oil immersion revealed 3+ (20 to 25 per field) gram-positive cocci (pairs and chains), and 2+ (10 to 20 per field) gram-negative organisms. Further tests showed that the gram-positive organisms were two different species of *Streptococcus*; one was alpha-hemolytic (not group B or D), and the other was nonhemolytic non-enterococcus group D.

One of the gram-negative isolates exhibited a peculiar morphology when viewed under oil immersion. It was most commonly described as caterpillarlike or larvaelike, i.e., it appeared transversely striated, and it was much larger than organisms usually found in stained smears from specimens of similar origin. The organism grew well on sheep blood agar, both aerobically and in 5 to 10% CO_2, but failed to grow on MacConkey agar. Colonies were grey and nontranslucent and measured approximately 2 mm in diameter after 18 h of incubation. Colony size diminished somewhat after repeated transfer. No growth occurred in broth containing 1.5 to 6.5% NaCl. Biochemical tests revealed that the organism had the following characteristics: catalase positive; oxidase positive; glucose oxidative; TSI, no change; urease negative; citratase negative; acid from glucose and maltose; no acid from sucrose and lactose. The organism was resistant to clindamycin and susceptible to penicillin, ampicillin, cephalothin, tetracycline, and gentamicin.

At this point the gram-negative isolate could not be

identified. Cultures were sent out to two other laboratories, provincial and national; both were unable to identify the organism.

Microscopy. The microscopy results are shown in Fig. 1, 2, and 3. The transverse striations and the unusual dimensions of this organism can be seen in Fig. 1, where it is compared with *Escherichia coli*. The most obvious feature of the organism from the electron micrographs is its multicellularity. What were originally thought to be rods from the Gram stain or the methylene blue stain are in fact multicellular organisms made up of 10 to 12 individual cells arranged side by side. The organism is not only multicellular, but it also exists in double or multiple units, i.e., the 10- to 12-cell units are held together in twos and threes. Figure 3 clearly shows that the organism has distinct and different surfaces. There are convex and concave surfaces, the latter being covered with threadlike appendages. With this information it was soon possible to narrow the field for identification by reference to the article by Starr and Schmidt (12) and subsequently to confirm the identity as *Simonsiella* sp. from the article by Kuhn (4).

Identification. On the basis of the microscopic and fine structure morphology, we concluded that the isolate was a member of the genus *Simonsiella*, and that on the basis of the results of the biochemical tests it was most likely *S. muelleri* (4).

DISCUSSION

There are three distinct aspects of this study. The first relates to the isolation of this unusual organism from an environment that is normally relatively free from microorganisms. The second relates to the antimicrobial susceptibility of this organism. The third relates to our problem associated with identification of an organism possessing such unique characteristics that presumptive identification solely from a Gram stain would now appear elementary, even for a novice.

It is generally accepted that the mouth of a baby is sterile immediately before birth and that colonization starts during birth and shortly after (6). Species of *Streptococcus*, particularly *S. salivarius*, are the characteristic organisms of the first 48 h after birth (8–10). To our knowledge, this is the first report of a *Simonsiella* sp. isolated from a neonate. We are not suggesting that this isolation is unique; it could be that other workers have simply failed to recognize the organism in question.

The actual source of the organism in this case is not clear. Although the organism was isolated from a gastric aspirate, it could have been associated with the dental cyst and oral eruptions, the ruptured membranes of the mother, or both. One would presume that the presence of an organism in a gastric aspirate from a neonate would be preceded by its occurrence in the oral cavity. In this particular case it is possible that the presence in the oral cavity was a consequence of the ruptured membranes of the mother. Unfortunately by the time the unusual nature of the isolate was recognized the neonate and the mother had been discharged, and no follow-up work was possible. The ruptured membranes, the dental cyst, and the early eruption of teeth could all be significant factors in creating a special ecological niche allowing the organism to colonize the neonate.

The susceptibility of this isolate to penicillin, although not surprising in itself, may serve as a timely reminder that not all gram-negative, oxidase-positive organisms are resistant to this antibiotic.

Identification of the isolate took 3 weeks, yet now presumptive identification could be made from a Gram stain in a few minutes. In fact another isolate has already been identified in this laboratory. Why were so many people totally unaware of this organism? One reason is that the organism is not even mentioned in several texts and reviews of oral microbiology. We suggest that the inclusion of this organism in laboratory exercises for students in medical laboratory sciences, dentistry, dental hygiene and allied disciplines could rectify this situation. We further suggest that students in all courses in introductory microbiology be made aware of the genus *Simonsiella* because of its unique multicellular structure. It would be a significant and fascinating addition to the usual rods, cocci, and spirals.

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