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Characterization and Differentiation of 59 Strains of *Moraxella urethralis* from Clinical Specimens

P. S. RILEY, D. G. HOLLIS, AND R. E. WEAVER
Center for Disease Control, Atlanta, Georgia 30333

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The biochemical characteristics of 59 strains of *Moraxella urethralis* from clinical specimens, primarily from urine and the female genital tract, were studied. The characteristics included (i) the inability to acidify carbohydrate substrates, (ii) the ability to produce phenylalanine deaminase, (iii) the ability to reduce nitrite, (iv) the lack of urease activity, and (v) the ability of most strains to alkalinize citrate. A means of differentiating *M. urethralis* from *Moraxella osloensis* and *Moraxella phenylpyruvica* was determined.

Species of *Moraxella* have been reported as possible etiological agents in human disease (1, 2, 7, 8, 13, 17, 18). These gram-negative, oxidase-positive, nonmotile rods are routinely encountered in the clinical microbiology laboratory. Of the presently recognized species of *Moraxella*, *M. osloensis*, *M. phenylpyruvica*, *M. kingii*, *M. nonliquefaciens*, and *M. lacunata* are isolated from clinical specimens.

Recently, Lautrop et al. (12) described another group of *Moraxella*-like bacteria and indicated that they would probably propose the name *M. urethralis* if further study indicates that it is a new species. These microorganisms were isolated primarily from urine samples and from specimens from the female genital tract. The description recorded for *M. urethralis* is compatible with DeBord's observations on *Mima polymorpha* var. *oxidans* (3).

During the past 20 years, the Special Bacteriology Section (SBS) at the Center for Disease Control has received over 50 bacterial cultures with characteristics identical to those described for *M. urethralis*. Preliminary studies of these strains indicated that differentiating *M. urethralis*, *M. osloensis*, and *M. phenylpyruvica* could be a problem in the clinical laboratory. The present investigation was undertaken to determine the characteristics most useful for identifying *M. urethralis*.

MATERIALS AND METHODS

Bacteria. The bacterial cultures used were obtained from the stock collection maintained by the SBS. The following cultures were used: *M. osloensis*- ATCC 19976, SBS#- A608, 9893, 8134, 8292, B8198; *M. phenylpyruvica*- ATCC 23333, SBS#- 9413, A1232, A390, A1019; and *M. urethralis*- ATCC 17960 (*M. polymorpha* var. *oxidans*) described by Lautrop et al. (12). Fifty-nine isolates from various clinical sources were used (Table 1). A culture of the amino acid auxotroph, *M. osloensis* trpE55, was kindly supplied by E. Juni.

Media and techniques. The media preparation and the procedures employed in determining cultural and biochemical characteristics have been described (4, 10, 16). The broth used to assay nitrite reduction consisted of heart infusion broth (Difco), 25 g; KNO_2, 1 g; and distilled water, 1,000 ml. Phenylalanine deaminase was assayed by the method of Ewing et al. (5). A slant of the phenylalanine agar was inoculated heavily with growth from a heart infusion agar slant culture. After 20 to 24 h of incubation at 35 C, 4 to 5 drops of 10% ferric chloride were allowed to run down the surface of the slant. Development of a green color in the agar slant beneath the cellular growth indicated the production of phenylpyruvic acid. The transformation assay was performed according to Juni's procedure (9).

RESULTS

After 24 h of incubation on 5% rabbit blood agar plates, the colonies of *M. urethralis* were approximately 0.5 mm in diameter, circular, convex, glossy, and butyrous. Colonies of *M. osloensis* varied in diameter from 0.5 to 1 mm, and the colonies of *M. phenylpyruvica* measured nearly 0.5 mm. A noticeable difference in the colonial opacity of the three species was observed (Fig. 1). Colonies of *M. urethralis* were nearly opaque, whereas colonies of *M. osloensis*

TABLE 1. *Sources of 59 strains of M. urethralis*

Source	No. of isolates
Urine	36
Female genital tract	16
Penis	3
Leg wound	2
Kidney	1
Mastoid	1

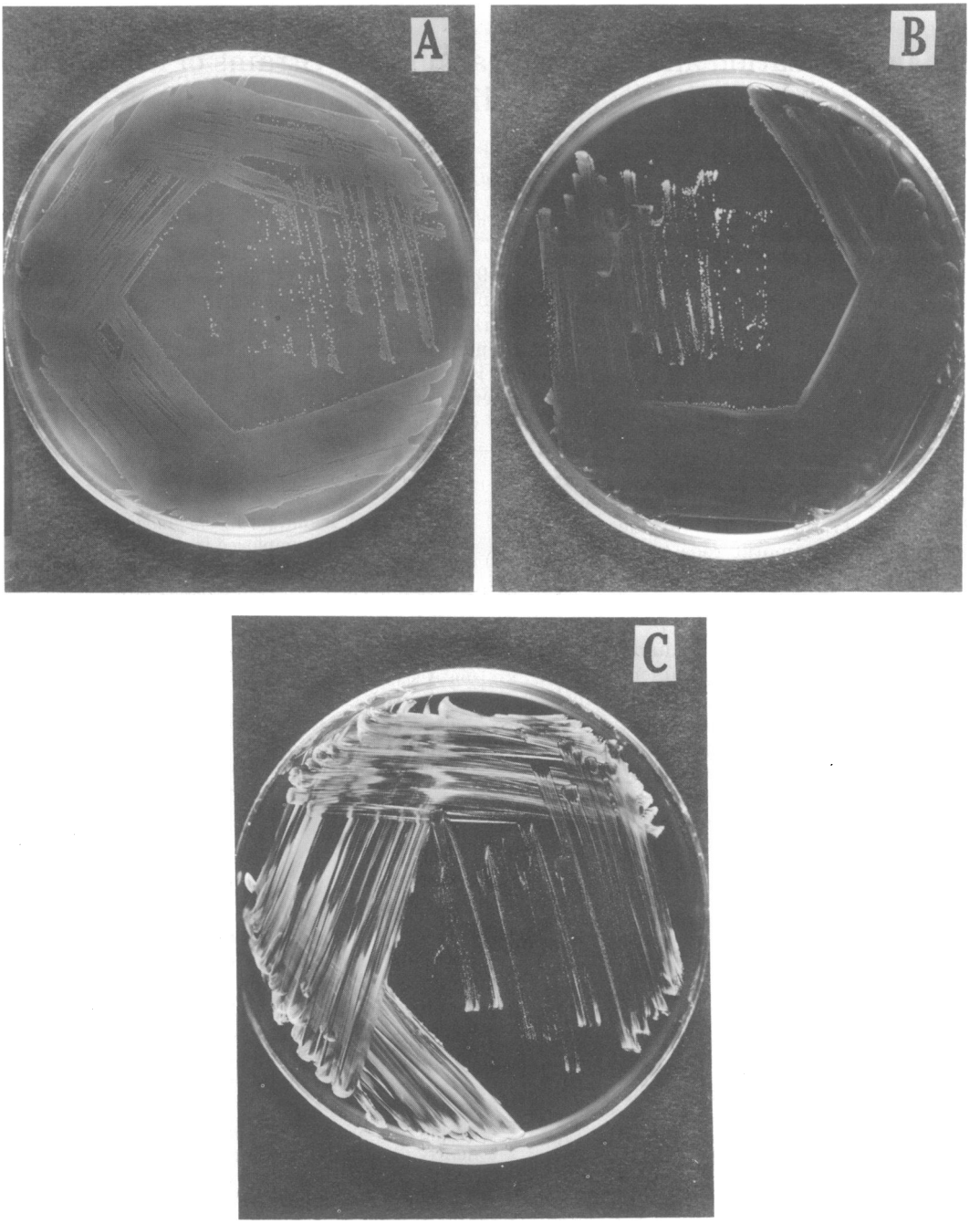

FIG. 1. *Colonial appearance of some Moraxella species when cultivated on 5% rabbit blood agar plates. M. phenylpyruvica, A; M. osloensis, B; and M. urethralis, C. The plates were incubated at 35 C for 48 h (the additional 24 h of incubation enhanced the photographic contrast).*

and *M. phenylpyruvica* were nearly translucent.

Gram stains of 24-h-old cultures of *M. urethralis* revealed small gram-negative coccoid forms, short rods, and an occasional long, slightly pleomorphic rod. Often the cells appeared to be arranged in aggregates. *M. osloensis* strains showed crystal violet retention

and larger coccoid-diplococcoid forms, thick moderate length rods, diplobacilli and 3 to 5 cell chains, a few filaments, and slight pleomorphism. The cells of *M. phenylpyruvica* were similar in size to those of *M. osloensis* and consisted of coccoid and diplococcoid forms, short thick rods, and diplobacilli with some evidence of crytal violet retention, and occasionally long pleomorphic rods and filaments.

Some of the biochemical characteristics of the *M. urethralis* cultures examined are listed in Table 2. Acid production from carbohydrates was not detected either oxidatively or fermentatively. The majority of the cultures grew on MacConkey agar and on TGY agar at the temperatures tested. Citrate was usually alkalinized and phenylalanine deaminase was produced, but urea hydrolysis was not detected. Nitrite reduction, but not nitrate reduction, was observed.

TABLE 2. *Some biochemical characteristics of M. urethralis*[a]

Test or substrate	Result[b]
Acid production from[c]	
Glucose	−
Xylose	−
Mannitol	−
Lactose	−
Sucrose	−
Maltose	−
Glycerol	−
Aesculin hydrolysis	−
Catalase	+
Oxidase	+
Growth on	
MacConkey agar	+
SS agar	−
Cetrimide agar	−
TGY agar	
25 C	+(4)[d]
35 C	+
42 C	+(7)
Citrate utilization	+(5)
Christensen's urea	−
Nitrate reduction	−
Nitrite reduction	+
Indol	−
Methyl Red	−
Voges Proskauer	−
Gelatinase	−
Peptonization of litmus milk	−
Motility	−
Phenylalanine deaminase	+

[a] The culture of *M. urethralis* described by Lautrop et al. (12) and 59 clinical isolates were tested.

[b] Abbreviations used: SS, Salmonella-Shigella; TGY, tryptone-glucose-yeast extract.

[c] The carbohydrate OF media were prepared by using a modification of King's formula (16).

[d] The numbers in parentheses indicate the number of strains which deviated from the recorded result.

The transformation of *M. osloensis* trpE55 was attempted with crude DNA preparation from each of the cultures used in this study. Only the six strains of *M. osloensis* transformed the auxotroph to prototrophy.

DISCUSSION

Fifty-nine cultures of *M. urethralis* have been identified from clinical isolates which were submitted over the past few years to the SBS. In the original description of *M. urethralis*, the only means of differentiating this bacterial species from *M. osloensis* was the former organism's ability to usually alkalinize citrate and its increased colonial opacity (12). However, Juni (9) observed some strains of *M. osloensis* which alkalinized citrate. Thus, the differentiation of nitrate-negative *M. osloensis* strains from *M. urethralis* was, by definition, dependent upon colonial morphology. For this reason we decided to determine additional characteristics which might be useful in differentiating these species.

Our findings were in agreement with those of Lautrop et al. (12) except that we noted that phenylalanine deaminase was present in *M. urethralis*. Moreover, we also noted that, even though this bacterial species did not reduce nitrate, it reduced nitrite, with an occasional strain producing gas.

The identification of *M. urethralis* in the clinical lab may prove difficult if selected tests are not conducted (Table 3). Confusion may arise unless both colonial morphology and pertinent biochemical characteristics are carefully observed. Either the transformation assay or the nitrite reductase test must be done to avoid

TABLE 3. *Differentiation of some Moraxella species*[a]

Test	*M. osloensis*	*M. phenylpyruvica*	*M. urethralis*
Citrate utilization	−	−	+(5)[b]
Urea hydrolysis	−	+	−
Phenylalanine deaminase	−	+	+
Nitrite reduction	−	−	+
Transformation[c]	+	−	−

[a] Six strains of *M. osloensis*, five strains of *M. phenylpyruvica*, and 59 strains of *M. urethralis* were tested.

[b] The number in parentheses indicates the number of strains which deviated from the recorded result.

[c] Using *M. osloensis* trpE55.

misidentifying *M. urethralis* cultures as *M. osloensis*. Since the transformation assay is not presently employed in clinical laboratories as a routine procedure, the necessity for precise colonial description and the determination of nitrite reductase is apparent. Moreover, the capacity to produce phenylalanine deaminase may also be a source of misidentification. Several bacterial isolates which produced phenylalanine deaminase, but not urease, were initially classified by the SBS as probable urea-negative strains of *M. phenylpyruvica*. Later these microorganisms were determined to be cultures of *M. urethralis*. The difference in colonial morphology, in addition to the reduction of nitrite and urea hydrolysis, easily separates these two species.

Each of the strains employed in this study was isolated from human specimens. The strains of *M. urethralis* were obtained primarily from urine and female genital samples (Table 1), sources which are in accordance with a previous report (12). Whether *M. urethralis* is of pathological significance has not been established. Infections attributed to organisms which have been designated *M. polymorpha* var. *oxidans* may actually be unrecognized strains of *M. urethralis* (6, 11, 14, 15). Presently, we have not accumulated a sufficient number of case histories upon which to base a conclusion.

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