Persistent occurrence of ureaplasmas in the oral cavity

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Since Shepard¹⁾ (1954) isolated tiny colony-forming mycoplasmas (T-strain, T-strain mycoplasma, T-mycoplasma or ureaplasma) from the urethra of man, a number of publications have described the organisms in the urogenital tract (Taylor-Robinson et al.²⁾, 1980). However, there have been only a few papers describing ureaplasmas in the oropharynx³⁻⁵⁾. Watanabe et al.^{6,7)} isolated the organisms from the washings of the oral cavity of a female patient suffering from serious gingivitis and also from the saliva of patients afflicted with various oral diseases. These results encouraged us to study whether ureaplasmas were a member of the oral microbial flora and whether ureaplasmas were aetiologically associated with any oral diseases. In the process of the studies, the authors came across a subject from whom ureaplasmas were isolated repeatedly during a period of 15 weeks. In this communication, the authors describe the findings obtained so far, because such a case has never been reported.

Materials and Methods

The subject was a 29-year-old, male patient seeking dental care at Nagasaki University Dental Hospital; systematically healthy, he exhibited slight symptoms of gingivitis and complete dentition.

The liquid medium utilized for isolation and growth of the ureaplasmas consisted of Difco PPLO broth (70%, v/v), horse serum (20%, v/v), 25% yeast extract (10%, v/v), thallium acetate (0.025%, w/v), penicillin G (1,000 U/ml), urea (0.1%, w/v), and phenol red (0.002%, w/v). The reaction was adjusted to pH 7.0. The solid medium

was a liquid medium without phenol red and with urea, the concentration of which was reduced to 0.01% (w/v), and Oxoid agar No. 1 (1%, w/v) was added. The pH was adjusted to 6.5. When used, the medium was plated in glass petri dishes measuring 4.5 cm in diameter.

The mixed saliva was sampled every time the subject visited the clinic. Liquid medium in 4.5 ml amounts, contained in screw-capped glass vials of 5.5 ml capacity, was inoculated with 0.5 ml of saliva samples. Then, the vials were incubated at 37°C aerobically. The growth of ureaplasmas decomposed the urea and caused a colour change from yellow (pH 7.0) to red (pH 8.0). Therefore, when there was a colour change, the cultures were diluted in serial 10-fold steps in a liquid medium. Drops (0.05 ml) of each dilution (1:10 to 1:100,000) were inoculated on agar plates, which were incubated at 37°C for 3 days in an atmosphere of 80% N₂, 10% CO₂ and 10% H₂. After incubation, the agar plates were examined for ureaplasma colonies under a microscope at 100× magnification. A block of solid medium (ca. 5×5 mm) carrying a single colony suspected to be one of the ureaplasmas was cut off and transferred to a liquid medium. Pure cultures were obtained after 5 times of cloning.

The isolated strains were examined for susceptibility to erythromycin (1.0 µg/ml) and thallium acetate (1.0 mg/ml) on solid medium (pH 7.5) and for growth in a liquid medium without horse serum.

Results

Mixed saliva was sampled on 15 different occasions during a period of 15 weeks. All

Table 1 Some properties of strains isolated from saliva

Days after the 1st sampling	Isolated strain number	Colony morphology	Urease activity	Susceptibility to		Growth in medium without horse serum
				thallium acetate (1.0 mg/ml)	erythromycin (1.0 µg/ml)	
0	1-4	Typical	+	+ (1, 4)*	+ (1, 4)*	+ (1, 4)*
1	5-7	Typical	+	+ (5, 6)*	+ (5, 6)*	+ (5, 6)*
2	8-10	Typical	+	+ (8, 10)*	+ (8, 10)*	+ (8, 10)*
4	11-20	Typical	+	+ (18, 20)*	+ (18, 20)*	+ (18, 20)*
7	21-22	Typical	+	+ (21)*	+ (21)*	+ (21)*
10	23-26	Typical	+	+ (23, 26)*	+ (23, 26)*	+ (23, 26)*
11	27-32	Typical	+	+ (27, 31, 32)*	+ (27, 31, 32)*	+ (27, 31, 32)*
14	33-40	Typical	+	+ (33, 35, 37, 39, 40)*	+ (33, 35, 37, 39, 40)*	+ (33, 35, 37, 39, 40)*
15	41-45	Typical	+	+ (41)*	+ (41)*	+ (41)*
17	46-51	Typical	+	+ (46, 48, 49, 50, 51)*	+ (46, 48, 49, 50, 51)*	+ (46, 48, 49, 50, 51)*
24	52-59	Typical	+	+ (52, 59)*	+ (52, 59)*	+ (52, 59)*
33	60-69	Typical	+	+ (60)*	+ (60)*	+ (60)*
39	70-79	Typical	+	+ (70, 71)*	+ (70, 71)*	+ (70, 71)*
82	80-89	Typical	+	+ (80)*	+ (80)*	+ (80)*
104	90-99	Typical	+	+ (90)*	+ (90)*	+ (90)*

* Numbers in parentheses denote the strain number tested.

of the samples yielded ureaplasma colonies; minute (30–60 µm in diameter), round, thin and flat with a very small nipple. Ninety-nine strains were isolated. Of them, 32 strains were examined for susceptibility to erythromycin and thallium acetate and growth in a medium without horse serum. All of the tested strains failed to grow in the medium without horse serum and to revert to bacterial forms, although subcultured 5 times in a medium without thallium acetate or penicillin; but were inhibited in the presence of erythromycin (1.0 µg/ml) and thallium acetate (1.0 mg/ml) (Table 1).

Based on the results, the isolated strains were identified as *Ureaplasma urealyticum*, and the organisms in the oral cavity as a member of the oral microbial flora.

Discussions

Mycoplasmas were originally isolated from animals as an aetiological agent of bovine pleuropneumonia (Nocard et al.⁸⁾, 1898), and about 40 years later from humans for the first time by Dienes et al.⁹⁾ (1937), who

recovered the organisms from an abscessed Bartholin's gland. Since then, efforts exerted for isolation of the organisms from the oral cavity by many investigators had been in vain until Morton¹⁰⁾ (1951) isolated the organisms from saliva for the first time. Nevertheless, Morton's report has been followed successively by numerous publications concerning oral mycoplasmas. At the moment, knowledge regarding ureaplasmas in the oral cavity is limited and the rate of isolation frequency reported so far is very low. However, the findings presented here seem to suggest that ureaplasmas are a common inhabitant of the oral cavity. Through improvement of cultural conditions and techniques for isolation of the organisms from the specimens containing a variety of bacteria in great number, it may be demonstrated that the organisms are distributed widely in the oral cavity.

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