Mycoplasma in the Upper Gastrointestinal Tracts of Southern Indian Control Subjects and Patients with Tropical Sprue


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ABSTRACT

Bhat, P., Boultet, E. A., Rajan, D., Shanthakumari, S., Kapadia, C. R., and Baker, S. J.: Mycoplasma in the upper gastrointestinal tracts of southern Indian control subjects and patients with tropical sprue. Am. J. Clin. Pathol. 59: 825–828, 1973. The mycoplasmal flora of the upper gastrointestinal tract of 28 southern Indian control subjects and 23 patients with tropical sprue have been studied using samples of jejunal secretions, jejunal biopsies, and saliva. Although 45% of the specimens of saliva showed mycoplasma organisms, with M. salivarium as the most frequently encountered species, no mycoplasma organisms were recovered from the jejunal secretions or jejunal biopsies of either group. The results indicate that if mycoplasma organisms have a role in the etiology of endemic tropical sprue, the species involved must be one which has thus far not been detected by conventional technics.

Ever since the discovery by Chanoock and co-workers that the agent of primary atypical pneumonia was a mycoplasma organism, the pathogenicity of mycoplasma found in various sites in human beings has been the subject of much interest. Lev and co-workers recovered mycoplasma from jejunal secretions and from jejunal biopsies of some patients with tropical sprue. The present study was undertaken, therefore, to define the mycoplasmal flora of the upper gastrointestinal tract of control subjects and patients with tropical sprue.

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Materials and Methods

Twenty-eight control subjects without evidence of tropical sprue and 23 patients with tropical sprue were studied. Prior informed consent was obtained from all subjects. All belonged to the poorer socioeconomic group which lives on a largely vegetarian diet similar to that described by Rao and Rao. They were admitted to a metabolic ward and were put on a standard diet containing 50 to 60 Gm. of fat per day. Complete gastroenterologic and hematologic studies were done as described previously. As soon as possible following admission, they were intubated in the fasting state with a sterile radiopaque polyvinyl tube and intestinal contents were aspirated from the first loop of jejunum. In 12 subjects with tropical sprue and in 12 control subjects, a jejunal biopsy was also obtained from the same site with a Crosby
capsule attached in parallel with the tube. From six patients with tropical sprue, samples of gastric and ileal secretions were also obtained. Specimens of saliva were collected from most of the subjects at the conclusion of the intubation.

The specimens were placed in sterile screw-capped bottles and immediately placed in ice and transported to the laboratory. The jejunal biopsies were transferred to small pieces of sterile aluminum foil and weighed immediately on a torsion balance. Following this, the tissue was washed thoroughly in 2 ml of sterile saline solution and then homogenized in 2 ml of sterile saline solution with a teflon tissue grinder in order to obtain a uniform suspension.

Standard pleuropneumonia-like organism (PPLO) agar and PPLO broth media were prepared as described by Chanock and associates,8 with the modification of Taylor-Robinson and associates,9 so as to contain 1,000 units of penicillin per ml in the medium. The same ingredients were used to prepare biphasic medium (agar medium with a broth overlay in screw-capped tubes) according to the method of Grayston and co-workers.10

The ability of mycoplasma to grow in the presence of human jejunal secretions or homogenate of jejunal biopsies was tested as follows. Twofold serial dilution from 1:5 to 1:160 of ten samples of jejunal secretions and ten biopsy homogenates were prepared in PPLO broth. Then 0.05 ml amounts of the original specimens and of each dilution were spread on PPLO agar plates. Broth tubes and plates were then inoculated with one drop of standard reference cultures of M. orale I (M. pharyngis), M. orale 2, and M. salivarium. Broths and plates were incubated and subcultured as described below.

An aliquot (0.05 ml) of each of the undiluted specimens was transferred to a biphasic broth tube and to an agar plate. A broths and spread uniformly with a bent sterile glass rod. The inoculated media were incubated at 37 C. in an anaerobic jar in an atmosphere of 95% nitrogen and 5% carbon dioxide. Subcultures from the biphasic broth tubes were made onto agar plates at weekly intervals for 3 weeks. These, as well as the primary plates, were examined weekly for 3 weeks by means of low power microscopy. The isolated strains of mycoplasma were identified by the growth inhibition test,8 using antiserum1,12 impregnated wet disks to identify the following species of mycoplasma: M. fermentans, M. hominis 1, M. hominis 2 (M. arthritidis), M. orale 1 (M. pharyngis), M. orale 2, M. pneumoniae, M. pulmonis, and M. salivarium.

Results

Reference strains were not inhibited even by the undiluted jejunal secretions or the jejunal tissue homogenates.

Mycoplasma strains were recovered from samples of saliva obtained from 11 (30.3%) of 38 control subjects and ten (55.6%) of 18 patients with tropical sprue. The difference between the isolation rates for the two groups was not statistically significant. Four of the isolates, two from each group, were lost on subculture and could not be typed. In eight control subjects and in seven patients, M. salivarium was isolated. M. orale 1 (M. pharyngis) was isolated from one control subject and one patient with tropical sprue.

Of the samples of gastric juice obtained from six patients with tropical sprue, mycoplasma was recovered in only one. The same strain (M. salivarium) was isolated from saliva from this patient also. No

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† Supplied by Dr. D. Taylor-Robinson, Common Cold Unit, Harvard Hospital, Coombe Road, Salisbury, Wills, England.
‡ Hyperimmune rabbit antiserum (lyophilized) for growth inhibition test. Microbiological Associates, Bethesda, Md.
mycoplasma was recovered from the samples of jejunal or ileal secretions or the jejunal biopsy homogenates.

Discussion

In recent years great interest has been focused on the possible role of mycoplasma in human disease. In order to attribute significance to their presence, it is necessary to define the normal mycoplasmal flora of the various regions in the human body under different conditions. Although work in this connection in man has been done in relation to the respiratory, genital tracts and specimens from other sites, including feces, we have been unable to find published studies of the mycoplasmal flora of the small intestine.

The isolation rate of 45% from the specimens of the saliva in this study is similar to that reported from studies in other countries in which the isolation rates ranged from 40 to 70%.

As in this study, Mycoplasma salivarium has been the most frequently encountered species.

Crawford and Kraybill have shown that mycoplasma inhabiting the human pharynx are often isolated as heterogeneous species. The failure to demonstrate mixed mycoplasmal flora in this study may have occurred because only those agar blocks having crowded growth of mycoplasma were studied by means of the growth inhibition test, and no attempt was made to study single colonies. This would probably have resulted in delineating the most predominant type (Kumagai and colleagues).

Mycoplasma was recovered from only one of six specimens of gastric secretions. This was in a patient who had the same strain in his saliva and may represent contamination of the gastric secretion with saliva. However, no mycoplasma was isolated from any of the specimens of jejunal secretions, jejunal biopsy homogenates, or ileal secretions.

It has been reported that homogenates of such mammalian tissues as liver, kidney, and synovial membrane have a mycoplasmicidal factor which increases in activity during incubation at 37 C. Growth of mycoplasma may also be interfered with by the mycoplasmicidal activity of polymorphonuclear leukocytes and the presence of specific antibody. However, no such inhibitory effect could be demonstrated with jejunal secretions or biopsy homogenates in this study.

Most species of mycoplasma which cause epidemics of disease among animals and birds seem to be primarily respiratory tract inhabitants, usually producing long-lasting chronic infection. However, an unusual mycoplasmal infection in sheep and goats which produced septicaemia and enteritis associated with ulcers and necrotic lesions in the small and large intestines, has been described. The only species definitely known to be the cause of major human illness is a respiratory pathogen, although various mycoplasma have at one time or another been suggested as etiologic factors in a large variety of conditions, including ulcerative proctosigmoiditis.

Lev and associates studied jejunal aspirates and jejunal biopsies from patients with tropical sprue and control subjects, and obtained mycoplasma from six of 12 patients, but from only two of 12 control subjects. Unfortunately, the isolates could not be typed before they died out (Herbert, personal communication). The study reported here shows that neither control subjects nor patients with sprue had mycoplasma species which could be cultured by conventional technics, either in their jejunal secretions or in their jejunal mucosa, even though nearly half had mycoplasma in their saliva. Tropical sprue occurs in both endemic and epidemic forms and is probably caused by an unknown infective agent. The patients with sprue all had endemic sprue, since no epidemics were de-
tected during the course of this study. Although the two diseases are clinically indistinguishable, it is possible that investigation of patients with acute epidemic sprue might produce different results.

It may be concluded that in southern India, by means of the technics used in this study, mycoplasma cannot ordinarily be isolated from the jejunal secretions or mucosa of either control subjects or patients with tropical sprue. If mycoplasma organisms have a role in the etiology of endemic tropical sprue, the species involved must be a species which cannot be grown by conventional cultural technics.14

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References