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Mycoplasma Sp. Detected in the Airways of Normal Healthy Subjects

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Abstract: The lack a rigid cell wall allows direct and intimate contact of the mycoplasma membrane with the cytoplasmic membrane of the host cell. During the fusion process, mycoplasma components are delivered into the host cell and affect the normal functions of the cell. The objective of this study was to determine the frequency of recovery of *Mycoplasma* sp. in throat swabs from healthy subjects. A microbiological cultures and PCR assay was used to detected mycoplasmas in throat swabs. Two hundred ten subjects provided throats swabs, of which 142 (68%) and 68 (32%) were females and males, respectively. Seventy five swabs cultured in broth and fifty one swabs cultured in agar plates were *Mycoplasma* sp. positive. PCR detected *Mycoplasma* sp. far more frequently than culture and these results indicate that mycoplasmas can grow in close interaction with epithelial cells with occurred frequently at mucosal sites in a healthy population.

Key words: *Mycoplasma*, throat swabs, culture and PCR essay, healthy subjects, domestic animals, Mexico

INTRODUCTION

Mollicutes are prokaryotes that are widely distributed as pathogens and commensals of plants and animals. They include eight recognized genera: *Acholeplasma*, *Anaeroplasma*, *Entomoplasma*, *Mesoplasma*, *Spiroplasma*, *Asteroplasma*, *Ureaplasma* and *Mycoplasma*. In humans and domestic animals the majority of Mollicutes isolated belong to the genus *Mycoplasma*, although, *Ureaplasma* and certain *Acholeplasma* sp. may also be present in clinical specimens (Keceli and Miles, 2002). Mycoplasmas represent the smallest self-replicating organisms in both cellular dimensions and genome size that are capable of cell-free existence. The small size and volume of mycoplasma cells allow them to pass through 0.45 μM porosize filters that cellular mass also means that mycoplasmas cannot be detected by light microscopy and they do not produce visible turbidity in liquid growth media. Typical colonies rarely exceed 100 μM in diameter when cultivated on enriched medium such as SP4 agar and require examination under a stereomicroscope to visualize their morphological features (Waites and Talkington, 2004).

A genomic price had been paid to maintain parasitism, so that a significant number of mycoplasmal genes is devoted to adhesins, attachment organelles and variable membrane surface antigens directed towards evasion of the host immune system (Razin, 1997). The lack a rigid cell wall allows direct and intimate contact of the mycoplasma membrane with the cytoplasmic membrane of the host cell. Under appropriate conditions, such contact may lead to cell fusion. During the fusion process, mycoplasma components (potent hydrolytic enzymes, hydrogen peroxide and superoxide radicals) are delivered into the host cell and affect the normal functions of the cell (Rottem, 2003).

Most Mollicutes lives as commensals and many arthropods they may even be considered symbionts. Infections with pathogenic mycoplasmas are rarely of the fulminant type but rather are close to the concept of ideal parasites, usually living in harmony with their host (Razin *et al*., 1998). A recent, some what inciting review discusses the role of mycoplasmas in disease pathogenesis, referring also to the variety of diseases of unknown etiology that have been linked to mycoplasmas (Baseman and Tully, 1997).

Recently, *Mycoplasma* sp. were detected in the airways of humans in absence of symptoms of acute infection and the incidence was greater in asthmatics. Using sensitive PCR-based detection methods, high incidences of *Mycoplasma fermentans* positively have

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been noted in saliva, blood and urine from apparently normal healthy subjects (Shibata *et al*., 1999; Ainswoth *et al*., 2000; Kovacic *et al*., 1996). The purpose of this study was to determine the frequency of recovery of *Mycoplasma* sp. in throat swabs from healthy subjects.

MATERIALS AND METHODS

Subjects and specimens: This study was conducted at the Laboratorio de Micoplasmas del, Centro de Investigaciones Microbiológicas, Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla, México, from September 2008 to July 2009. A total of 210 subjects (142 females and 68 males, age range of 18-24 years), throat swabs from persons without respiratory tract infections were taken and considered in the study.

Throat samples were collected on cotton-tipped swabs and were put in Eaton medium (PPLO-broth, yeast extract 10%, unheated horse serum 20%, glucose 0.5%, phenol red 0.002% and penicillin 1000 μ mL^{-1}) and incubated at 37°C for 30 days or until the phenol red indicator changes color. As soon as the color changes 5 µL broth cultures were seeded on Eaton agar plates and stereomicroscope visualized.

DNA extraction and amplification: DNA was extracted from samples by the method described by Sidhu *et al*. (1995). Primers AR_1 sense and AR_2 anti-sense were used for amplification of a 301 bp fragment from mycoplasmas DNA.

The sequence of AR_1 is 5′ATG RGG RTG CGG CGT ATT AG 3′ and AR_2 is 5′CKG CTG GCA CAT AGT TAG CCRT 3′. PCR was carried out in a total volume 20 µL which includes: PyroStart™ Fast PCR Master Mix 10 µL, primer AR_1 1.3 µL primer AR_2 1.3 µL, template DNA 5 and 2.4 µL water nuclease-free. Amplification was performed in a TC-412 Thermocycler (Techne-USA) with a programme of 5 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 50°C, 1 min at 72°C and a final step of 5 min at 72°C. PCR products were detected by 2% agarose gel electrophoresis with ethidium bromide staining.

Statistical analysis: Values of *Mycoplasma* sp. cfu $m\ L^{-1}$ in throat samples from females and males subjects were compared with the Student′s t-test.

RESULTS AND DISCUSSION

The present study was conducted to determine the frequency of recovery of *Mycoplasma* sp. in throat swabs from healthy subjects. Two hundred ten subjects provided throat swabs of which 142 (68%) and 68 (32%) were female and male genus, respectively. When comparing the number of isolations with respect the genus, appeared the same tendency, there was not statistical difference $p>0.05$ (Table 1). *Mycoplasma* sp. detection by PCR present increased in the positive samples number, culturing mycoplasmas can take 1-4 weeks and can be difficult because of a requirement for special growth conditions. Table 2 shows the general results obtained for the samples evaluated by culture and PCR.

Table 1: Comparative in the isolations number between genus and cultures mediums

Cultures mediums	Subjects	Isolates	Percentage
Broth	142♀	51	36
	68♂	24	35
Agar	142♀	34	24
	68♂	17	25

Table 2: *Mycoplasma* sp. detection between microbiological method and PCR

Cultures mediums	PCR
Broth	
75/210	79/210
Agar	
51/210	54/210

Mcrobiologic diagnosis also has disadvantages, one of which is that *Mycoplasma pneumoniae* can be recovered from the respiratory tract several weeks after acute infection making it difficult to differentiate current from recent infection (Gil *et al*., 1993).

When applied to sample, PCR detected *Mycoplasma* sp. far more frequently than culture. Limited data comparing PCR and culture methods are available. However, PCR was shown to be more sensitive and reproducible than culture for detecting *M. pneumoniae* in throat swabs from experimentally infected hamsters (Bernet *et al*., 1989). One can hypothesize that culture positivity may be partly related to the presence of a large number of organisms in the samples with the culture system we used.

As many as 25% of persons infected with *M. pneumoniae* may experience extrapulmonary complications at variable time periods after onset of or even in the absence of respiratory illness. The presence of *M. pneumoniae* in extrapulmonary sites such as blood, synovial fluid and cerebrospinal fluid, pericardial fluid and skin lesions has been documented by PCR as well as culture, so direct invasion must always be considered (Narita *et al*., 1996). However, the frequency of direct invasion of these sites is unknown because the organism is rarely sought for clinical purposes. It is also important to realize that extrapulmonary complications can be seen before, during or after pulmonary manifestations or can occur in the complete absence of any respiratory symptoms (Cassell and Cole, 1981).

The lack of reliable commercially shown media in the past effectively prevented many clinical laboratories from offering *Mycoplasma* sp. detection by culture, even before alternative techniques such as PCR existed. If culture is attempted, isolation of *Mycoplasma* sp. from nasopharyngeal or throat swabs or lower respiratory tract specimens should be considered clinically significant in most instances but should be correlated with the presence of clinical respiratory disease due to the possibility of asymptomatic carriage (Waites and Talkington, 2004).

Using sensitive PCR-based detection methods, high incidences of *M. fermentans* positivity have noted in several anatomic sites from apparently normal healthy subjects. Data regarding the presence of *M. fermentans* within the human lung or its ability to establish chronic symptomless pulmonary infection, however are severely limited (Gao *et al*., 2004; Rivera *et al*., 2008).

CONCLUSION

In this study, it is interesting to note that mycoplasmal colonization or infections are not necessarily associated with a strong inflammatory response and some mycoplasmas colonize the respiratory and urogenital tracts with no apparent clinical symptoms. It is therefore, tempting to speculate that in addition to triggering the production of proinflammatory cytokines, certain organisms have the capacity to downregulate NF-κB or to induced anti-inflammatory cytokines such as IL-4, IL-10, IL-13 or transforming growth factor-β, contributing to the complex network of synergistic and antagonistic influences induced by mycoplasmas on cells of the immune system. Moreover, mycoplasmas can grow in close interaction with mammalian cells, often silently for a long period of time. However, prolonged interactions with mycoplasmas with seemingly low virulence could through a gradual and progressive course, induce chromosomal instability as well as malignant transformation, promoting tumorous growth of mammalian cells.

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