

THE FIRST ISOLATE OF *MYCOPLASMA PNEUMONIAE* IN MALAYSIA

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INTRODUCTION

Few clinical laboratories offer diagnostic services for *Mycoplasma pneumoniae* infections. This has led to the under diagnosis of the infection, especially in developing countries, where laboratory facilities are limited. Serological tests are more popular in the diagnosis of these infections and culture has been avoided by many laboratories probably because it takes too long and most bacteriologists are not familiar with the technique. This paper describes the first successful isolation of the organism in our laboratory, the Division of Bacteriology, Institute for Medical Research. It discusses the factors influencing its growth and the usefulness of culture in its diagnosis.

ISOLATION OF THE ORGANISM

The specimen was a nasopharyngeal aspirate collected from a 2½ year old Chinese boy with bronchopneumonia. It was inoculated into a transport medium (Trypticase Soy Broth with 0.5% Bovalbumin and 200 u/ml penicillin) within 2 hours of collection and then transported to our laboratory for processing.

The method of isolation was according to the Center for Disease Control (CDC) Manual on Laboratory Diagnosis of Mycoplasma Infections.¹ 0.1 ml of the inoculated transport media was transferred to a selective diphasic medium which contained the basic mycoplasma medium, 1% glucose and 0.001% methylene blue.

This was incubated aerobically at 35°C. The culture was checked daily for evidence of growth indicated by a colour change from purple or blue to green or yellow and no turbidity. This sample began to change colour in the second week and was immediately subcultured to the Pleuro-Pneumonia-Like-Organism (PPLO) glucose agar and broth for further identification tests. Unfortunately, at the first subculture, this strain did not grow on the PPLO glucose agar and required four passages through the glucose broth before it grew on the agar. It then became possible to stain the organism with the Diene's stain and carry out the haemadsorption test with guinea pig red blood cells (rbc) which are preliminary

tests for identification. *Mycoplasma pneumoniae* is the only human mycoplasma that adsorbs guinea pig rbc, therefore a presumptive diagnosis was made on the basis of this test.² The identification was later confirmed by the indirect immunofluorescent test.

DISCUSSION

The two main factors which contribute to the successful isolation of *Mycoplasma pneumoniae* are a properly and freshly collected sample and a quality controlled media. Sputum, tracheal aspirates or nasopharyngeal aspirates should be transported immediately to the laboratory on wet ice, and throat or nasopharyngeal swabs should be inoculated directly into a transport medium before sending to the laboratory for processing.¹

Artificial media were first introduced in 1962 by Chanock *et al.*³ This consists of a base of seven parts beef heart infusion, supplemented with two parts horse serum and one part yeast extract. Certain batches of serum used may contain antibodies or inhibitors.⁴ Too great a gel strength of the agar may also affect the growth of the organism.¹ To obtain optimum results, only glassware with a high standard of chemical cleanliness or non-toxic plastics should be used.¹ Either glass distilled or very pure deionized water may be used and sterilization of media should be by millipore filter rather than asbestos pads.⁵ Therefore every batch of media prepared should be tested for its ability to support growth with a known culture of *Mycoplasma pneumoniae* before being used.

The importance of culture in the diagnosis of mycoplasma pneumonia is that a single positive specimen from a case with a compatible clinical syndrome can be considered diagnostic, whereas in most serological tests paired sera are required.⁵ Unfortunately this is a rather slow growing organism and it can take as long as four weeks before a definitive diagnosis can be made. Although it is not helpful in influencing the management of an individual case, it is definitely useful for case definition in an epidemiological study where serological studies are not possible owing

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to the problem of obtaining blood and paired sera. Although resistance to erythromycin and the tetracyclines has not been reported it would be useful for further study on antibiotic susceptibility should the need arise.

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