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LETTER

Culturing *Chlamydophila pneumoniae*

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*Chlamydophila pneumoniae (*formerly *Chlamydia pneumoniae)* is an intracellular pathogen responsible for respiratory tract infection. A recent British survey of the microbial aetiology of community-acquired pneumonia (CAP) indicated that *C. pneumonia* is responsible for 13% of CAP and is the second highest bacterial cause of CAP [1]. It has been associated with bronchitis, pharyngitis, sinusitis, myocarditis, endocarditis and coronary artery disease [2].

In order to treat *C. pneumoniae* infections it is first essential to know the efficacy of the available antimicrobial agents against this pathogen. Minimum inhibitory concentration (MIC) tests are used to investigate the *in vitro* capabilities of antimicrobial agents on bacteria. Therefore, MIC testing of *C. pneumoniae* needs to be carried out before an antimicrobial agent is used to treat the infection. Culturing of *C. pneumoniae* must occur before MIC tests can be performed. *C. pneumoniae* is known to be very difficult to culture and is far more difficult than other chlamydial species [2]. Many methods of culturing *C. pneumoniae* have been proposed with differing cell lines, centrifugation conditions and incubation times. There is no standard method for culturing *C. pneumoniae* nor for testing the MIC of antimicrobial agents against it. There is no agreement on optimal culture conditions between different laboratories and even within the same laboratory. For *C. pneumoniae* to be a viable organism in the sense that it may be tested with regularity *in vitro*, a reliable method of culturing is required.

Molecular techniques, particularly PCR, are used to detect *C. pneumoniae* instead of culturing. However, in the case of antimicrobial testing this is not an alternative to culturing. This partly results from the fact that all the possible mechanisms of antibiotic resistance are not yet known. Therefore, testing for antibiotic resistance by investigating the presence or lack of certain genes is not appropriate. The absence of genes associated with resistance does not necessarily mean that the organism is sensitive to the antimicrobial agent. In contrast, the presence of a resistance gene does not necessarily mean that it is resistant. Only phenotypic testing such as MIC tests will indicate if an organism is sensitive or resistant. Thus we return to culturing.

Three different culture methods were investigated for their reproducibility using four different strains. TW 183, ATCC 2023 and AR 39 were collected from the American Type Culture Collection, the fourth strain, D 1, was received from Ninewells Hospital, Dundee. The D 1 strain had been successfully cultured using an in-house culture method in Dundee. All strains were stored at -70°C. HL (source: the Washington Research Foundation) and HEp-2 cells were used.

The first method was that of Sriram *et al* [3], the second was a method used by Roblin *et al* [4] and the third method was an in-house method used by Ninewells Hospital, Dundee, as described. A monolayer of HL cells were grown up in shell vials for 24 or 48 hours prior to inoculation. The growth medium was removed from the shell vials and the cells were rinsed with 1-2 mL of filter sterilised phosphate buffer solution (PBS). Each shell vial was inoculated with 300 µL of inoculum of TW 183, ATCC 2023, AR 39 or D 1. The vials were then centrifuged at 2400 X g and 35°C for 1 hour. The medium was replaced with medium containing 1.3 µg/mL cycloheximide. The vials were incubated at 37°C for 7 days with additional centrifugation on days 3, 4 and 5. On day 7 the

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vials were sonicated in an ultrasonicating water bath. 300 μL of the sonicated medium were added to freshly prepared monolayers. All negative controls were processed before examination of those thought to contain *C. pneumoniae*. Negative controls consisted of a shell vial containing uninoculated cells. The cultures were fixed and stained using direct and indirect antibody tests. No inclusions were found in any of the vials tested. The cells were washed and the growth medium and PBS were filtered in order to eliminate bacterial contamination. Three different methods with four different strains, two different cell lines and two different strains did not result in identification of a single *C. pneumoniae* inclusion. The lack of inclusions suggests that either the *C. pneumoniae* were not viable or the methods used are not easy to reproduce. As the D 1 strain had previously been cultured this strain was viable and so should have resulted in the identification of inclusions.

Thus none of the methods attempted could be reproduced. This lack of reproducibility is a common problem with *C. pneumoniae*, and should be highlighted. Development of new methods will not alter the problem of *C. pneumoniae* culturing. Standardisation of the existing methods so that they are reproducible not only in the same laboratory but also in different laboratories is the only way that data, particularly information about the antibiotic profile of *C. pneumoniae*, may be generated.

Standard, reproducible methods, which are agreed upon by more than one laboratory are needed not only for culturing but also for the investigation of antibiotic resistance in *C. pneumoniae*. A working party of *Chlamydophila* scientists is needed that will create culture guidelines with defined parameters similar to those available for antimicrobial agent testing. Thus accurate and valid information on culturing would be available.

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