PRESENT KNOWLEDGE AND EXPERIENCE ON THE STRATEGIES EMPLOYED BY MYCOPLASMA CONTAMINATION OF THE HUMAN CELL CULTURES

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Abstract

Introduction: *Mycoplasma* species often contaminate cell cultures and other cell-derived biological substances, leading to detrimental effects on the host that include changes in growth, morphology, metabolism and protein synthesis. In cell cultures, mycoplasma are extracellular parasites, usually attached to the external surface of a cell membrane. Many researchers use a mixture of penicillin and streptomycin in the cell culture to prevent contamination.

Material and methods: We prepared cell cultures of lymphocytes from peripheral blood of 12 subjects and used micronucleus, assay which is the standard method, for detection of micronuclei in binuclear lymphocytes.

Results: Use of standard antibiotics does not protect cell cultures against *mycoplasma* contamination. Penicillin has no effect on *mycoplasma* since *mycoplasma* lack cell wall. Streptomycin inhibits about half the *mycoplasma* strains but is ineffective against others. In fact, *mycoplasma* is generally resistant to most antibiotic mixtures commonly used in cell culture. We didn't find any *mycoplasma* contamination in the cell culture where penicillin-streptomycin mixture was absent, but confirmed infection in the culture containing mixture of antibiotics.

Conclusion: Antibiotics and mixture of antibiotics like penicillin-streptomycin mixture does not protect the cell culture against *mycoplasma* contamination. Hence, contamination can spread rapidly to other cell lines through aerosol droplet dispersion.

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Introduction

Most cell culture contaminations result from the accidental introduction of fungi or bacteria, sometimes along with *mycoplasma*. *Mycoplasma* is a genus of bacteria that is a frequent contaminant in cell cultures. The presence of *Mycoplasma* in a cell culture can change cell physiology, thus preventing their appropriate use in experiments. As a result of their small size, *Mycoplasma* infections are very common in cell cultures as they can pass through filters used to prevent contamination and can spread to other cultures in a laboratory. Up to 87% of cell lines may be contaminated by mycoplasma and this infection can affect virtually any function and activity of eukaryotic cells leading to experimental defects and unreliable results (Uphoff & Drexler, 2003, 2005; Somasundaram, Nicklas, & Matzku, 1992; Drexler et al., 1994).

Mycoplasma are the smallest independent, self-replicating organisms identified so far. They lack a true cell wall and are therefore deformable under pressure. They contain proteins, RNA, DNA, and enzymes. *Mycoplasma* species often contaminate virus cell-derived biological substances. In cell cultures, *Mycoplasma* develop as extracellular parasites and attach themselves to the surface of the cultured cell's membrane. They can contaminate a variety of eukaryotic cells in culture, leading to detrimental host effects that include modifications in cell morphology, growth and metabolism of the host cell. They can also influence protein synthesis and virus replication. Because *Mycoplasma* lack a cell wall, antibiotics (such as penicillin) that interfere with formation of cell walls are ineffective against them when used at standard concentrations (Nikfarjam and Farzaneh, 2012; Pooja, Rajeev, Sunil, & Kumkum, 2013). *Mycoplasma* originating from human, bovine, and porcine sources are the most prevalent, the most common isolates of which are: *Acholeplasma laidlawii*, *Mycoplasma*

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arginini, Mycoplasma fermentans, Mycoplasma hyorhinis, and Mycoplasma orale (Priebe and Bromm, 2005).

Research Goal

This study aims at elaborating the present knowledge and experience on managing *Mycoplasma* infections while interacting with human tissue culture cells, especially lymphocytes like blood cells.

Material and Methods

We prepared the lymphocyte cell cultures from peripheral blood of 12 subjects and used the standard method, which is micronucleus assay for detection of micronuclei in binuclear lymphocytes. Venous blood sample (3 ml) was collected in heparinized tubes by trained nurses from each individual of both exposed and control groups. Blood culture protocol was carried out according to Fenech (2000). Blood cultures from the same subject were divided into two groups. In the first set of cultures we added penicillin-streptomycin mixture (100 units/mL penicillin and 100 µg/mL streptomycin). In the second, we didn't add antibiotic mixture in order to observe whether the penicillin-streptomycin mixtre, which is added to many cell cultures, is necessary for cultivation. The slides were stained by 2% alkaline Giemsa stain for 8 minutes, washed in distilled water and examined by Leica DM4500 P light microscope (×40 and×100).

Results

Results showed that in cultures where antibiotic mixture had been added, there was a greater *Mycoplasma* contamination compared to those cultures where antibiotic mixture was absent. Thus, we concluded that antibiotics are not necessary for prevention of *Mycoplasma.* Rather, *Mycoplasma* contamination can be prevented by maintaining good aseptic conditions and lab practices which include better techniques, avoiding accidents, cleaning up the work area and surrounding environment before and after handling culture and checking for contamination regularly. Figure 1 (a, b, c, d, e) in Appendix shows mononuclear and binuclear lymphocytes with *Mycoplasma* contamination in the blood culture (different sizes of cycle formation, dark cytoplasm and large cell wall).

Other three major sources leading to mycoplasma contamination of cell cultures are:

- addition of previously infected cells to the culture,
- addition of medium ingredients from a contaminated cell culture, and
- contamination by laboratory personnel.

Contamination can also spread rapidly to other cell lines through aerosol droplet dispersion, especially in storage.

Conclusion

Mycoplasma contamination presents a major problem during the cultivation of cell cultures. Their presence can lead to modification of cell morphology, metabolism and rate of growth. Because of their specific structure, *Mycoplasma* growth in cell cultures can't be prevented by the use of standard antibiotics mixtures. Mycoplasma don't pose a cell wall and so penicillin has no effect on their growth whereas streptomycin can prevent the development of only half of the known mycoplasma strains. Other antibiotics, at the concentrations that are routinely used in cell cultures, are generally ineffective. The use of standard antibiotics can sometimes mask the *Mycoplasma* infection by minimizing their growth but cannot eliminate them completely. Most efficient way to prevent *Mycoplasma* is the maintenance of strictly aseptic conditions and techniques. Best way to stop ongoing contamination is the use of specific antibiotics that can fully eradicate the infection.

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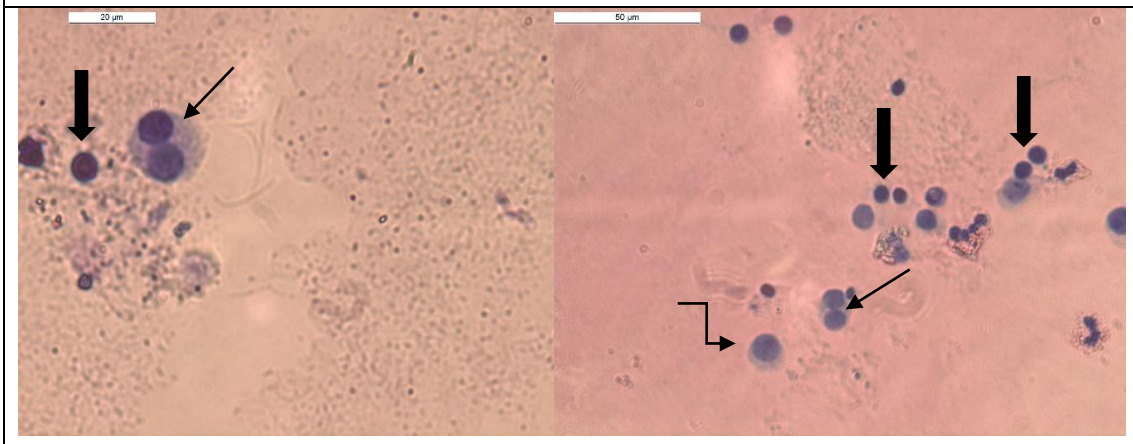
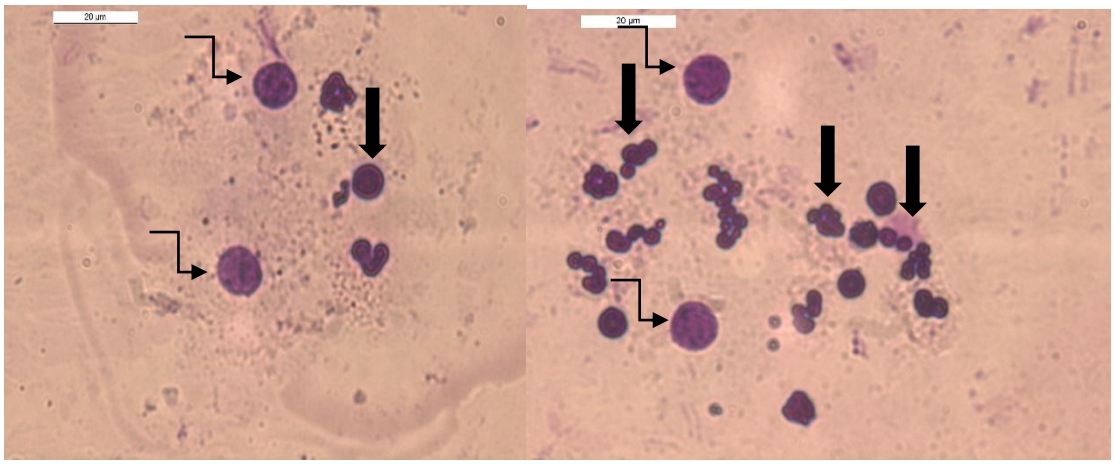
Appendix

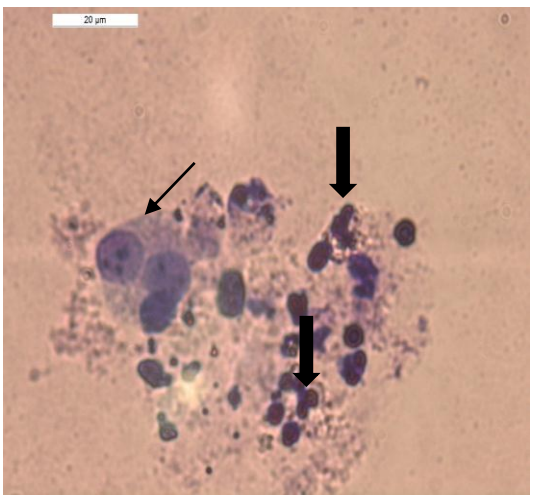
Figure 1: Slides (a, b, c, d, e) with contamination of mononuclear and binuclear lymphocytes with mycoplasma infection



a) **b)**



c) **d)**



e)

↙ - binuclear lymphocites, ⤵ - mononuclear lymphocytes, ↓ - mycoplasma

Observed by microscope Leica DM4500 P (×40 and×100)

Source: Author