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

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MYCOPLASMA: COMMON CONTAMINANTS

- SMALLEST FREE-LIVING, SELF-REPLICATING ORGANISM CURRENTLY IDENTIFIED
- NEED TO CONSUME CHOLESTEROL, AMINO ACIDS, FATTY ACIDS, VITAMINS AND OTHER CATABOLITES
- THEY LACK A CELL WALL AND ARE THEREFORE DEFORMABLE UNDER PRESSURE (CONTAINS NO PEPTIDO GLYCAN WALL)
- THEY CONTAIN PROTEIN, RNA, DNA, AND ENZYMES
- MYCOPLASMA ARE EXTRACELLULAR PARASITES USUALLY ATTACHED TO THE EXTERNAL SURFACE OF A CELL MEMBRANE
- OCCURRING EXTRA CELLULARLY, ONLY IN RARE CASES INTRACELLULARLY
- PASS CELLULOSE AND POLYVINYL FILTER WITH 0,45 MM PORE WIDTH

SPECIES OF MYCOPLASMA

There are over 190 species of mycoplasma, but only 20 distinct species of human, bovine and porcine origin have been identified in cell culture. Of those twenty, eight species account for approximately 95% of all mycoplasma contamination in cell culture, including *M . arginini* (bovine), *M. fermentans* (human), *M. hominis* (human), *M. hyorhinis* (porcine), *M. orale* (human), *M. pirum* (human), *M. salivarium* (human), and *Acholeplasma laidlawii* (bovine).

Mycoplasmas are frequent contaminants of cell cultures and bioprocessing fluids.

CONTAMINATION

contaminate cell cultures, virus stocks, and other cell-derived biologicals

They can contaminate a variety of eukaryotic cells in culture, leading to detrimental host effects that include changes in growth, morphology, metabolism, protein synthesis, and virus replication

They compete effectively with tissue-culture cells resulting in profound damage to cell metabolism and function. In the worst-case, contamination leads to diminished cell growth and eventually to the loss of the culture

Effects


Inhibition of cell metabolism
• Induction of chromosomal abnormalities
• Disruption of DNA and RNA synthesis
• Changes in virus and antibody production
• Interference with growth rate of cells
• Depletion of arginine
• Activation of B cells
• Altered gene expression in cells

mycoplasma infections can be traced to one of two sources

contaminated animal-derived materials

poor aseptic techniques

POSSIBLE SOURCES OF MYCOPLASMA CONTAMINATION ARE:

Infected incoming cells (cross-contamination)

Cell culture media, serum or trypsin

Laboratory personnel are also a key source of contamination, as human mycoplasmas (*M. orale*, *M. fermentans* and *M. salivarium*)

Laboratory equipment, benches and flow hoods have also been identified as sources of contamination

(droplet transfer)

THE AIM OF THE STUDY

to elaborate present knowledge and experience on the strategies employed by Mycoplasmas while interacting with human tissue culture cells, especially lymphocytes

In this study we wanted to evaluate the genotoxicity of ionizing radiation on health workers, using the CBMN assay and determine it's health risk and risk of cancer

MATERIAL

- VENOUS BLOOD SAMPLE (3 ML) WAS COLLECTED IN HEPARINIZED TUBES FROM EACH INDIVIDUAL OF BOTH EXPOSED AND CONTROL GROUPS
- WE PREPARE THE CELL CULTURES OF LYMPHOCYTES FROM PERIPHERAL BLOOD OF 12 SUBJECTS IN EXPOSED GROUP AND 12 SUBJECTS IN CONTROL GROUP
- USED MICRONUCLEUS ASSEY FOR DETECTION OF MICRONUCLEI IN BINUCLEAR LYMPHOCYTES

METHODS

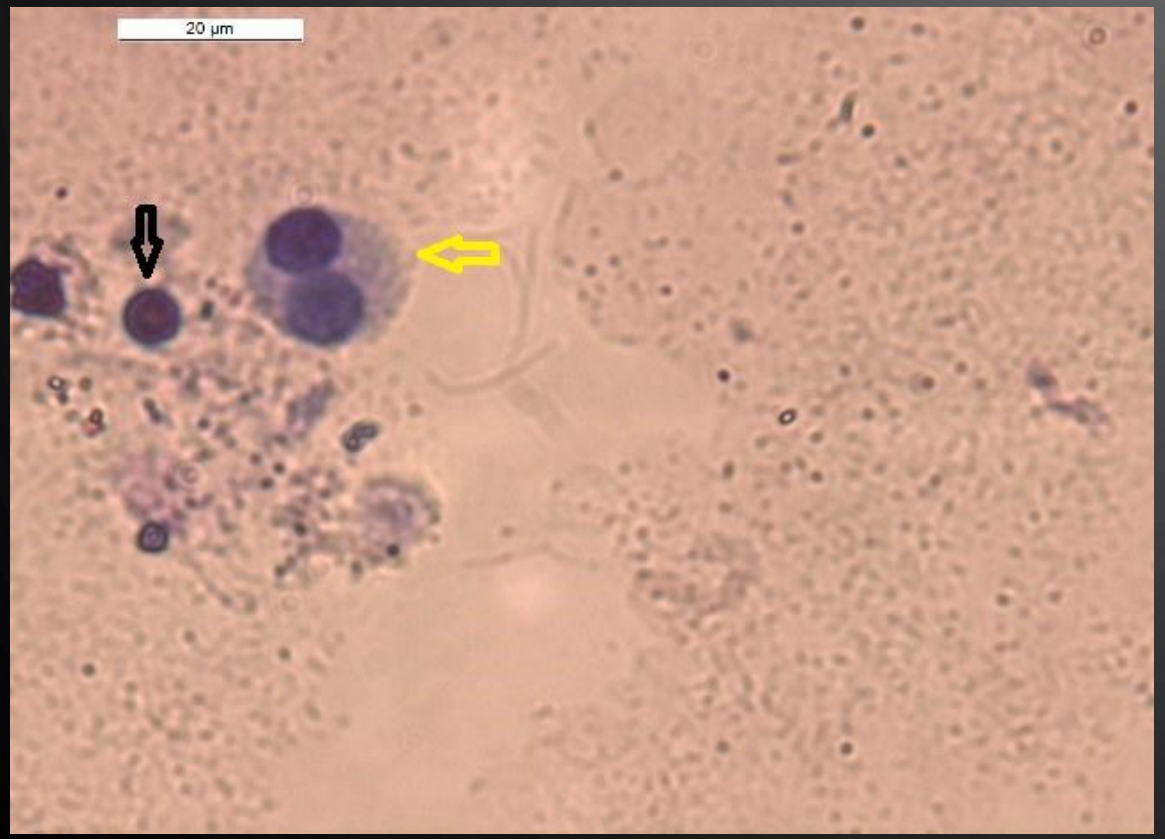
- BLOOD CULTURE PROTOCOL WAS DONE ACCORDING TO FENECH, 2000
- O,5 ML OF BLOOD SAMPLE WAS ADDED TO CULTURE TUBES CONTAINING 4.5 ML OF RPMI 1640 MEDIA ENRICHED WITH 20% FETAL BOVINE SERUM, L-GLUTAMINE AND 0.2 **PHYTOHEMAGLUTININ** 1% PHA.
- THE TUBES WERE INCUBATED FOR 44 HOURS AT 37 °C IN A SLANT POSITION. 3 MG/ML OF **CYTOCHALASIN B** WAS ADDED TO EACH CULTURE TO BLOCK CELL CYTOKINESIS AND THEN THE TUBES WERE REINCUBATED AT 37 °C FOR ANOTHER 28 HOURS.
- HARVESTING OF CELLS BY CENTRIFUGATION AT 1000 RPM FOR 10 MIN. THEN WARM KCL HYPOTONIC SOLUTION WAS ADDED TO EACH TUBE.
- FIXATION WITH SERIES OF CENTRIFUGATION WITH GLACIAL ACID AND METHANOL
- THE SLIDES ARE STAINED BY 2% ALKALINE GIEMSA FOR 8 MINUTES, THEN WASHED IN DISTILLED WATER AND EXAMINED BY LIGHT MICROSCOPE LEICA DM4500 P (×40 AND×100).

- **BLOOD CULTURES FROM THE SAME SUBJECT WERE DIVIDED INTO TWO GROUPS. IN THE FIRST SET OF CULTURES WE ADDED ANTIBIOTIC PENICILLIN-STREPTOMYCIN (100UNITS/ML PENICILLIN AND 100MG/ML STREPTOMYCIN), AND IN THE OTHER ONES WE DIDN'T, IN ORDER TO OBSERVED AND DEFINE WHETHER THE ANTIBIOTIC PENICILLIN-STREPTOMYCIN WHOSE ADDED AT THE CULTIVATION OF CELLS IN MANY STUDIES IS NECESSARY IN THE PROCESS OF CULTIVATION**

RESULTS

Our results showed that in cultures that had been added antibiotic for prevention of mycoplasma contamination (100 units/mL penicillin and 100 mg/mL streptomycin) had a lot more mycoplasmas compared to those cultures that has not added antibiotic penicillin-streptomycin, which we want to emphasize that they are not necessary as prevention of mycoplasma or other words

contamination with mycoplasmas can be prevented by application of good aseptic conditions and techniques, reduce accidents, keep the laboratory clean, clean up the work area and surrounding environment and routenely monitor for contamination.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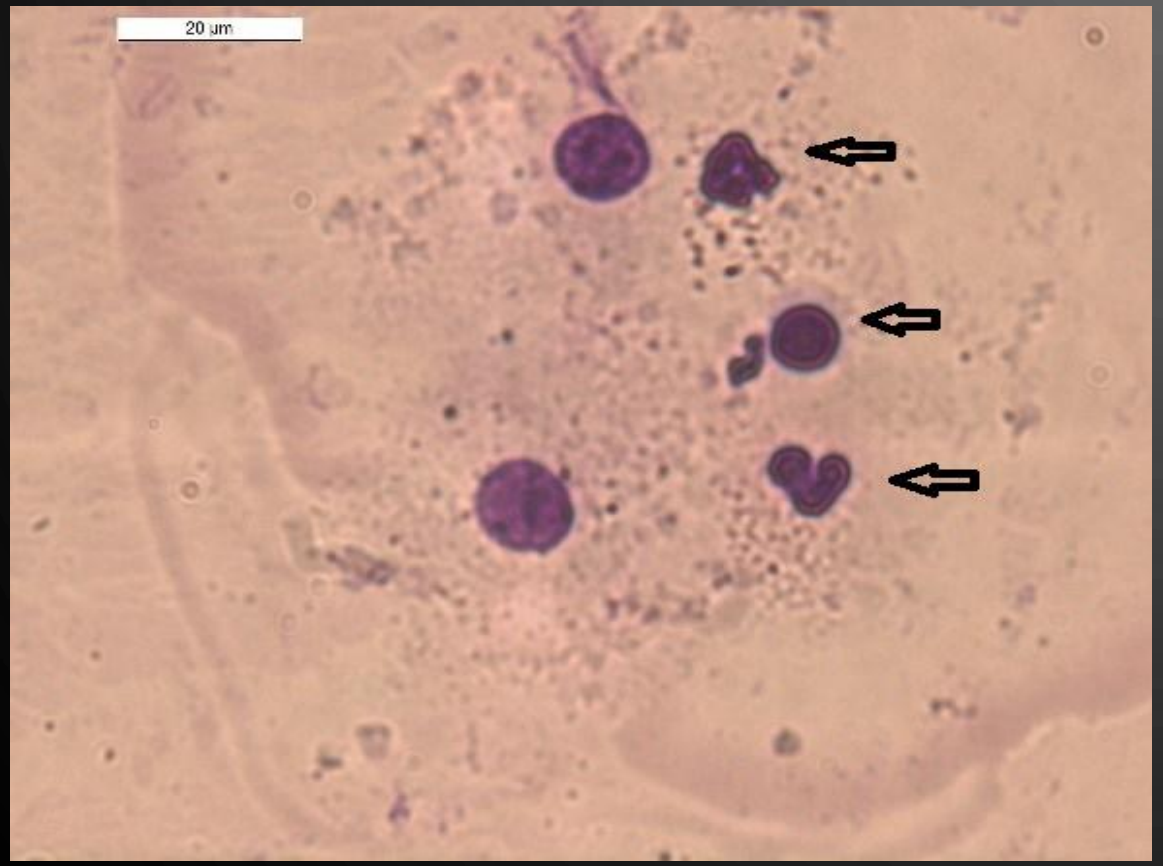
50 µm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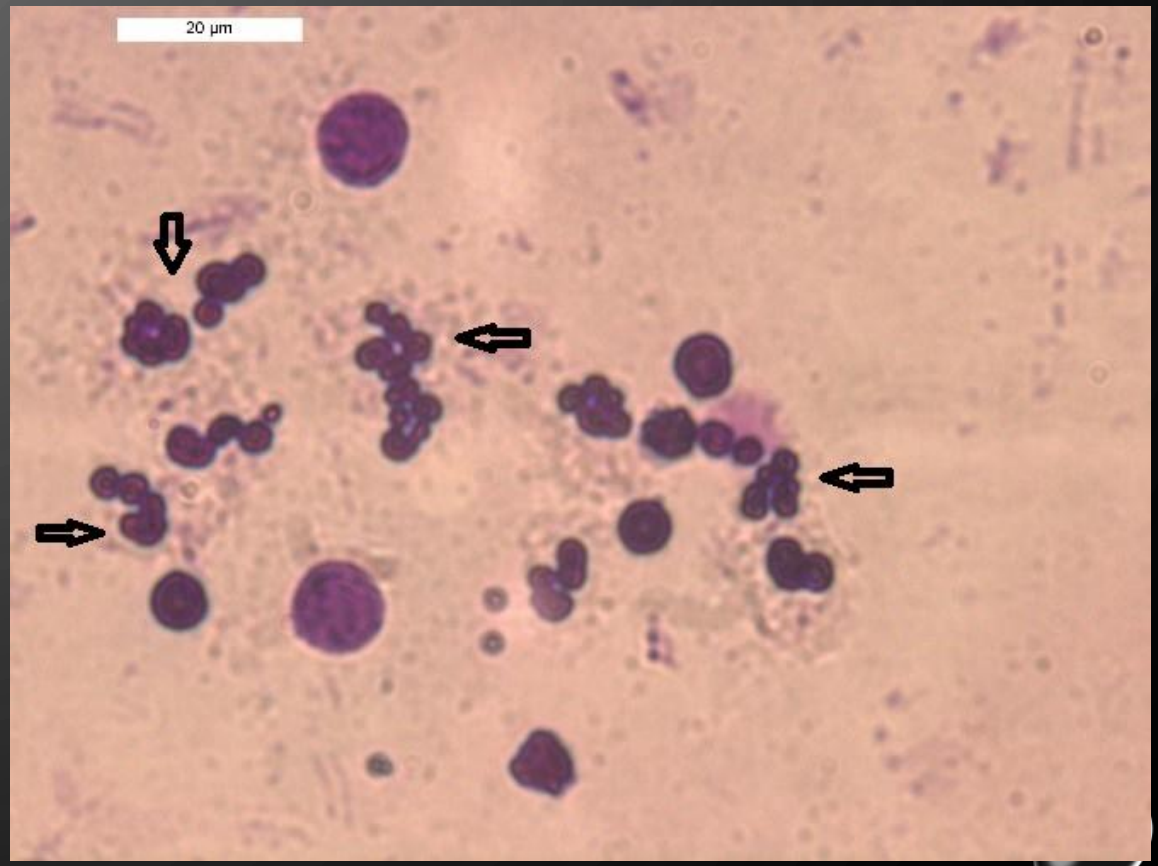
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CONCLUSIONS

- USE OF STANDARD ANTIBIOTICS DOES NOT PROTECT CELL CULTURES AGAINST MYCOPLASMA CONTAMINATION
- PENICILLIN HAS NO EFFECT ON MYCOPLASMA SINCE MYCOPLASMA LACK A CELL WALL
- STREPTOMYCIN INHIBITS ABOUT HALF THE MYCOPLASMA STRAINS BUT IS INEFFECTIVE AGAINST MANY OTHERS
- MYCOPLASMAS ARE GENERALLY RESISTANT TO MOST ANTIBIOTIC MIXTURES COMMONLY USED IN CELL CULTURE
- SOME ANTIBIOTICS MIGHT SUPPRESS THEIR GROWTH AND THUS MASK THE PRESENCE OF THE INFECTANTS. BESIDE THE ENFORCEMENT OF STRICTLY STERILE CELL CULTURE TECHNIQUE AND THE DEVELOPMENT OF RESISTANCES, THIS IS ONE REASON NOT TO APPLY ANTIBIOTICS PROPHYLACTICALLY IN ROUTINE CELL CULTURE

IMPORTANCE

Pen/Strep does not provide protection from contamination

Operate free of standards antibiotics

Whenever possible: separate the work benches and incubators for handling contaminated and mycoplasma free materials

Disinfect working surfaces and hands with alcoholic spray before and after working procedures, or with the change of the working material

Thank You!

Have a nice day. ☺