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MYCOPLASMA AS CONTAMINANT OF CELL CULTURE WITH SPECIAL REFERENCE TO SOURCE OF INFECTION

BY

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INTRODUCTION

Mycoplasma as contaminant of tissue culture represents a potential disaster due to its profound influence on viral propagation, the sensitivity of the serological examination, also producing severe cytopathic effects and representing a source of infection when used as a vaccine (Stalheim, 1975).

Theerfore, spcific assays were performed for the detection of cell culture as contamination as well as a quality control program for prevenion of Mycoplasma infection of cell cultures and quarantine of infection. El- Ebeedy et al. (1987), in Egypt strated the first step in this field of study, succeeded in the isolation and treatment of human mycoplasmas from contaminated cell cultures.

The present study was planned to detect mycoplasmal contamination of tissue cultures source and origin of contamination and suggesting method of control.

MATERIAL AND METHODS

1. Samples:

Nine samples of tissue cultures of different types, seven media of tissue culture propagation and 5 lots of serum samples from different sources.

2. Culture media used for the isolation and culture procedures were done as described by Erno and Stipkovits (1973).

3. Genus determination was performed according to Erno and Stipkovits (1973).

4. Biochemical characterization was applied using dextrose fermentaiton test media, arginine deamination media as described by Sabry (1968).

5. Serological identification was done using growth inhibition test (GIT) according to Clyde (1964).

The refeence antisera used were (NIH) U.S.A.

6. Antimicrobial agent and their concentraitons were recorded in

Table 2 and were interpreted according to Clark and Berle (1978).

7. Antibiotic sensitivity test was done: A) using metabolic inhibition test. (MIT) according to Senterfit (1983) to determine the minimum inhibitory conentration.

B) Using disk growth inhibition test (GIT) (Clyde, 1964).

8) Eight laboratory personnel were allowed to talk infront of agar plates held 2 inches from the mouth, also throat swabs were taken.

antigenically related to M. hominis.

Three Mycoplasmas were isolated from 7 culture media, hey were arginine + ve film and spot + ve and were identified as M. salivarium.

Five lots of bovine serum revealed 3 Mycoplasma isolates 2 of them were arginine positive glucose-ve and wre antigenically identified as M. arginini and one was glucose + ve tetrazolium +ve and was identified as A. laidlawii by GIT.

Table(1): Mycoplasma isolated from cell culture.

	Kind	Source	No.Exam	No. +ve. and M.species			
				M.hom.	M.saliva	M.arg.	A.laid
Cell line	Vero	S.V.EL.	9	4	-	-	
	HRT	Agoza and AHRI					
	CER						
Culture Media	BGM	AHRI	7	-	3		
	BFK						
Bovine sera	Gibco	AHRI	5	-	-	2	1

S.V.El-Agoza = Serum and Vaccine Laboratories El-Agoza
AHRI = Animal Health Research Institute.

RESULTS

From table 1, it is clear that out of 9 cell cultures, 4 mycoplasmas were isolated, they were arginine positive film and spot -ve and were

The results of antibiotics sensitivity testing of Mycoplasmas (Table 2) showed that they were highly sensitive to spectinomycin, streptomycin, lincomycin, vibramycin, and chloramphenicole while

Table (2): Mycoplasma sensitivity to antibiotic.

Antibiotic designation	GIT Inhibition zone in mms.				MIT			
	M.hom	M.Saliva	M.arg.	M.laid.	M.hom	M.Saliva	M.arg.	M.laid.
Spectinomycin µg/ml.	7	7	7	7	1024	1024	512	512
Lincomycin 20 µg/ml.	6	6	6	6	512	256	512	512
Chloramphinicol 30 µg/ml.	6	6	6	6	512	512	512	512
Streptomycin 15 µg/ml.	6.5	7	7	7	512	1024	1024	1024
Vibramycin 20 µg/ml.	6	6	6	6.5	512	512	512	512
Sulphonamide 20 µg/ml.	-	-	2	2	-	-	16	32
Erythromycin 5 µg/ml.	3	3	3	4	4	4	8	8
Apramycin 15 µg/ml.	2	1	2	2	16	32	32	32

apramycin and erythromycin were less effective and sulphonamides wre ineffective for human Mycoplasmas, on the contrary, they were effective for bovine mycoplasmas.

The examination of laboratory personnel revealed the isolation of *M. salivarium* from 6 out of 10 persons when the plates were held opened in front of their mouth and from 7 out of 10 throat swabs.

DISCUSSION

Mycoplasma infection of cell cultures is considered one of the imporant problems confronting the virologists in regard to its effect on the cell culture including ciliostasis of organ cultures (Stalheim, 1975), amino acids disorders (Sales et al., 1978), increased cellular granularity and destruction affecting cell topography characterized by loss of microvilli and cellular processes, Emerson et al. (1979). Also non viable suspensions of M. hominis inhibit mitosis in cultured lymphocytes due to arginine deaminase (Barile and Leventhal, 1968).

Special attention wa drawn to potential sources of contamination e.g. bovine serum, laboratory personnel and original tissue specimens, they were examined routinely. It was found that from 9 tissue culture samples 4 were M. hominis while 7 culture media revealed 3 M. salivarium.

The source of M. hominis may be the original tissue while M. salivarium was proved to be due to contaminated throats of laboratory, personnel. Examination of 10 throat swabs revealed 7 (70%). M. Salivarium while, examination of agar plates held infront of them while talking revealed 6 60% M. Salivarium.

Examination of 5 lots of bovine sera revealed 2 M. arginini as was previously found by Barile and Kern (1971), its implication in

contamination of tissue culture was proved. A. laidlawii was also isolated from bovine serum the cause may be that filturation of the serum does not necessarilly eliminate every Mycoplasma cell.

Antibiotic sensitivity testing of the isolated mycoplasmas showed that spectinomycin and streptomycin were the drugs of choice and would be benificial as a prophylactic and eliminating antibiotics.

It was also recommended to quarantine the contaminated cultures and to examine new ones routinely as well as regarding the hygienic measures such as prevention of mouth pipetting and talking while working.

SUMMARY

Nine tissue cultures, 7 media for virus propagation and 5 lots of bovine sera as enrichment were brought to the Mycoplasma research department and routinely examined for contamination with Mycoplasma four M. hominis were isolated from cell cultures , 3 M. salivarium were isolated from culture media while bovine serum samples revealed 3 bovine mycoplasmas isolates, 2 of them were M. arginini and one as A. laidlawii.

Antibiotic sensitivity test for Mycoplasma isolates was applied against 8 antimicrobial agents using metabolic inhibition test (MIT) and growth inhibition test (GIT) and spectinomycin proved to be the most effective and was recommended for treatment of contaminated tissure cultures.

A trial was made to investigate the source of contamination.

Out of 10 laboratory personnel 7 (.70%) Mycoplasma salivarium were isolated from throat samples while 6 (60%) were isolated when they were allowed to talk infront of plates held 2 inches from the mouth.

REFERENCES

Barile, M.F. and J. Kern (1971): Isolation of M. arginini from commercial bovine sera and its implication in contaminated cell cul tures. Proc. Soc. Exp. Biol. Med. 138 (2) : 432 - 437.

Barile, M.F. and B.G. Levinthal (1986): Possible mechanism for mycoplasma inhibition of lymphocyte transformation induced by phytohemagglutinin. Nature 751 - 219.

Clyde, W.A. (1964a): Mycoplasma species identification based upon growth inhibition by specific antisera. J., 29, 958 - 965.

Clark, E.G.C. and J. Berle (1978): Isolation and identification of drugs in pharmaceutical body fluid and post-mortem material 4 th ed. London. The Pharmaceutical Press.

El-Ebeedy, A.A., L.M. El-Shabiny, A. Rashwan A.M. Ragab, A. Karim and S. El Nakashly (1987): Mycoplasma contamination of cells cultures . 1. Antibiotic control of Mycoplasma isolated from various cell cultures. J. Egypt. Vet. Med. Ass. 47, No. 1 & 2 135 - 141.

Emerson, M.; B.L. Nicholson, R. Bayer (1979): Effects of Acholeplasma laidlawii and an unidentified mycoplasma on selected fish cell cultures and the replicaiton of fish viruses. T. Fish. Dis., 2 (3), 227-238.

Erno, H. and L. Stipkovits (1973): Bovine mycoplasmas: cultural and biochemical studies Acta. Vet. Scand. 14, 450-463.

Sabry, M.Z. (1968): Characterization and classification of avian mycoplasma. Diss. Abstr. 29 (4 6): 1920 B.

Senterfit, L.B. (1983): Antibiotic sensitivity testing of Mycoplasmas in methods in Mycoplasmology, Vol. II. by J.C. Tully and S. Razin 397 - 399. Academic Press.

Stales, B., D., Barris, K. Hummeler and S. Segal (1978): Amino acid disorders in tissue culture cells, effects of mycoplasma and antibiotics on cystine metabolism. Monogr. Hum. Genet. 9 (1) 131-134.

Stalheim, O.H.V. (1975): Ciliostasis of bovine oviductal organ culture by T. mycoplasmas of bovine origin. Abstr. Ann. Meet. Am. Soc. Microbiol. 75; 280.