THE USE OF AMPICILLIN AS A SUBSTITUTE FOR THE CONVENTIONAL BACTERIAL INHIBITORS IN MYCOPLASMA MEDIA

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Introduction

Contamination ensues as a major problem in the isolation and identification of mycoplasmas. This is due to the relatively slow growth of mycoplasma compared to that of bacteria contaminating the culture. Usually penicillin, the common antibiotic which acts on Gram positive bacteria, and thallium acetate which inhibits the growth of Gram negative organisms, are added simultaneously as bacterial inhibitors to solid and liquid media used for primary isolation of mycoplasmas. (Erno & Stipkovits 1973). Thallium acetate at certain concentrations was demontsrated to be inhibitory to Acholeplasma and Ureaplasma (Kunze 1972, Shepard 1967). The purpose of this study is to determine the effect of ampicillin, one of the B-lactam broad spectrum antibiotics, on mycoplasmas and bacteria which usually contaminate the growth of mycoplasmas. The possibility that this product replaces the conventional antibacterial inhibitors used in mycoplasma media is discussed.

Materials & Methods

Materials: Mycoplasma species; these consisted of the following strains: M.mycoides subsp. capri (PG3), M.mycoides subsp. mycoides (UG1), M. arginini (KVC1), and Acholeplasma laidlawii (HRV) "AMRV-C 1453 FAO/WHO Aarhus Denmark". Except for the first species all the other strains were isolated and identified in the Sudan and have already been subjected to the action of ampicillin (Harbi et al. 1981).

Bacteria species : E. coli, Staphylococcus aureus and Streptococcus pyogenes.

Inoculates of the bacterial cultures were generously provided by the Department of Bacteriology at the exponential phase of growth.

Medium: PPLO serum broth and agar (Difco) were systematically used throughout the experiment. Other ingirdients conventionally added in such media are referred to elsewhere (Mohamed Ali 1977).

Methods

Sensitivity testing of the minimum inhibitory concentration (MIC) was determined for ampicillin using the tube method (Audrey et al. 1965). The following concentrations were used: 1 mg/ml, 0.1 mg/ml. 0.01 mg/ml,and 0.001 mg/ml. The dilutions were done serially in the PPLO broth medium. One loupful of bacterial culture was added to the diluted ampicillin and incubated in atmospheric air at 37°C. After 18-24 hours tubes were examined for growth and results registered. To confirm the presence of growth the cultures mentioned above were streaked on the agar to check for the appearnace of colonies. Control tubes containing PPLO broth but without antibiotics also received loupful of bacterial cultures. With mycoplasmas the procedure was done as described before (Harbi et al . 1981). The only difference is that the highest concentration here, was 1 mg/ml for the sake of comparison with that of bacteria.

Results

Ampicillin had no effect on mycoplasmas even at a concentration of 1 mg/ml (Table II). Actually in a recent report (Harbi et al. 1981) it was demonstrated that all the cultures of mycoplasmas resisted ampicillin up to a concentration of 5 mg/ml. However the action of ampicillin on the different bacteria examined in this experiment was remarkable. Data are registrered in table I. Tubes inoculated with bacteria in media without antibiotics and kept as controls showed heavy bacterial growth.

Discussion

Not infrequently bacteria occur as contaminants in primary isolation of mycoplasmas. They may occur in association with mycoplasmas when collected from tissue or supervene during culturing. To inhibit such a growth of bacteria, pericillin and thallium acetate are routinely added to media for primary isolation of mycoplasmas. Their simultaneous effect against Gram +ve and Gram -ve bacteria at certain concentrations has already been determined and found support (Erno & Stipkovits 1973). However thallium acetate which acts specifically against Gram -ve organisms was also found to impair the growth of Acholeplasma and Ureaplasma at certain concentrations (Kunze 1972, Shepard 1967) . It should be borne in mind that mycoplasmas are Gram -ve organisms (Whittlestone 1974). Hence the use of

ampicillin, the broad spectrum penicillin, would be justifiable to replace the above mentioned bacterial inhibitors. Table I shows that the Staph. aureus and Str. pyogenes which are both Gram +ve are inhibited at 0.1 mg/ml of ampicillin, while E. coli a famous Gram -ve organism still grows at this concentration. All the strains of mycoplasmas already proved to resist a concentration of 5 mg/ml (Harbi et al., 1981) were found to grow luxuriantly at 1 mg/ml. It is evident then that 1 mg/ml of ampicillin used in the media for the isolation of mycoplasmas demonstrates a reasonable threshold which supports the growth of mycoplasmas and inhibits the growth of bacteria.

Study of other groups of bacteria usually known to contaminate cultures of mycoplasmas like Pseudomonas aeruginosa and Proteus rettgeri appears to be crucial, and further trials with this antibiotic merit consideration.

Summary

The effect of the broad spectrum penicillin "ampicillin" on the growth of mycoplasmas and on bacteria which would possibly contaminate this growth is discussed. It is presumed that this antibiotic would be recommended to replace the conventional Gram +ve and Gram -ve bacterial inhibitors usually used in media for isolation of mycoplasmas. Preference of ampicillin as an alternative to those inhibitors appears to be of obvious concern.

Acknowledgement

The authors are grateful to the Director Veterinary Research Administration for encouragement. The assistance of the technical staff of the Department of Mycoplasma is highly appreciated. This paper is published with the kind permission of the P.U.S. Animal Resources MAFNR.

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Table I

Growth of bacteris in certain concentrations of ampicillin

Species designation	Concentration 1 mg/ml	0.1 mg ml	0.01 mg ml	0.001 mg ml
E.coli	—	+	+	+
Staph. aureus	—	—	+	+
Str. Pyogenes	—	—	—	+

Table II

Growth of some species of mycoplasmas in certain concentrations of ampicillin

Species designation	Concentration 1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg ml
M. mycoides Subsp Capri	+	+	+	+
M. mycoides Subsp mycoides	+	+	+	+
M. arginini	+	+	+	+
A. laidlawii	+	+	+	+

– No growth
+ Evident growth