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Antibacterial Activity of Gentamicin Sulfate in Tissue Culture

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Gentamicin is a more effective in vitro bacterial inhibitor than combined penicillin-streptomycin, is nontoxic to tissue culture monolayers, and does not inhibit virus replication.

Bacterial contamination of clinical specimens obtained for virus isolation has long been a problem for virologists. Addition of antibiotics to tissue culture media, particularly the combination of penicillin and streptomycin, has reduced, but not eliminated, this problem. Gentamicin sulfate, a newly introduced aminoglycoside antibiotic, has been shown to inhibit the growth of both gram-positive and gram-negative bacteria in vitro and in vivo (2) and to inhibit several strains of mycoplasma in tissue culture (3). It has been used with success as an additive in commercial mycology media to inhibit growth of bacteria (4) and has been shown to be bactericidal for a wider range of organisms (*Pseudomonas aeruginosa*, *Proteus* sp., and *Streptococcus faecalis*) than penicillin and streptomycin. It does not interfere with the production of cytopathic effect (CPE) by certain echoviruses and polioviruses in tissue culture, is nontoxic to Rhesus monkey kidney (RMK), HeLa, and human amnion cells, and is stable at autoclave temperatures (1).

We have recently had an opportunity to confirm and expand these observations while studying the role of respiratory viruses and other microorganisms in the causation of exacerbations and progression of chronic bronchitis in a group of patients with this disease. One aspect of this investigation has involved the collection of nose and throat swabs and sputum specimens and their inoculation into tissue culture tubes of WI-38, human embryonic kidney, RMK, and HEp-2 cells for virus isolation. Tissue cultures were maintained in Eagle's minimum essential medium (EMEM) containing 2% fetal bovine serum, sodium bicarbonate, 100 units of potassium penicillin G per ml, 100 μg of streptomycin sulfate per ml, and nonessential amino acids and L-glutamine. Bacterial contamination has been a periodic problem.

By using 14 strains of bacteria isolated from these clinical specimens, the effectiveness of gentamicin was compared with the penicillin-streptomycin combination. Gentamicin was dissolved in EMEM and used in a final concentration of 200 μg/ml. Strains of bacteria were grown in Brain Heart Infusion broth for 18 to 36 hr and then inoculated in 0.1- and 0.2-ml amounts into tissue culture tubes of African green monkey kidney (GMK) cells containing EMEM as described above with either no antibiotics, gentamicin, or the penicillin-streptomycin combination. Tubes were observed for the gross appearance of bacterial growth for 7 days and then all tubes were subcultured. All bacterial strains grew in the absence of antibiotics and all were grossly inhibited by gentamicin (Table 1). On subculture, four colonies of *Sarcina* were isolated from gentamicin-treated tubes but this would not have interfered with the reading of the tissue culture tubes for viral CPE. In those tubes treated with the penicillin-streptomycin combination, eight showed gross evidence of bacterial growth (turbidity) after 7 days and could not have been read for viral CPE. One clear set of tubes which had been inoculated with *P. aeruginosa* yielded two colonies on subculture.

From these results, it is clear that gentamicin is superior to penicillin and streptomycin for control of bacterial growth in tissue culture. Casemore indicated its superiority in controlling *P. aeruginosa*, *Proteus sp.*, and *S. faecalis* organisms (1). To this list may now be added *Bacillus cereus*, *Klebsiella pneumoniae*, *Aeromonas*, *Enterobacter*, *Serratia*, *Citrobacter*, and *Alcaligenes faecalis*. Based on this data, we have adopted gentamicin for use in tissue culture and have reduced the incidence of bacterial contamination.

In adopting gentamicin for use in studies involving virus isolations, it is important to estab-

TABLE 1. *Bacterial growth in cell cultures treated with gentamicin or penicillin-streptomycin*

Bacteria	Turbidity[a]			Subculture	
	No antibiotics	Gentamicin[b]	P/S[c]	Gentamicin	P/S
Escherichia coli	+	−	−	−	−
Pseudomonas aeruginosa	+	−	−	−	+
Bacillus cereus	+	−	+	−	+
Klebsiella pneumoniae	+	−	+	−	+
Staphylococcus aureus	+	−	−	−	−
Aeromonas	+	−	+	−	+
Enterobacter	+	−	+	−	+
Serratia	+	−	+	−	+
Citrobacter	+	−	+	−	+
Proteus morganii	+	−	+	−	+
Sarcina lutea	+	−	−	+	−
Alcaligenes faecalis	+	−	+	−	+
Corynebacterium hoffmani	+	−	−	−	−
Bordetella pertussis	+	−	−	−	−

[a] Symbols: +, signifies bacterial growth or turbidity; −, signifies no bacterial growth or turbidity.

[b] Gentamicin was used in a final concentration of 200 μg/ml.

[c] Penicillin was used in a final concentration of 100 units/ml, and streptomycin was used in a final concentration of 100 μg/ml.

lish the fact that this antibiotic does not interfere with virus replication. Casemore indicated that gentamicin did not inhibit the replication of a poliovirus or an echovirus. The serotypes were not identified (1). Labile viruses were not tested. The present study indicates that a wide variety of stable and labile viruses including rubella, echovirus 11, herpes simplex, rhinovirus 39, and mumps are not inhibited by gentamicin (Table 2). It is also necessary to know that gentamicin is nontoxic to tissue culture cells. Casemore indicated that gentamicin was nontoxic to RMK, HeLa, and human amnion cells. Our studies indicate that this antibiotic is also nontoxic for

TABLE 2. *Comparison of virus growth in media containing gentamicin or penicillin-streptomycin (P/S)*

Virus	Cells in which virus grown	Infectivity titer in media containing	
		Gentamicin	P/S
Rubella	GMK	4.0[a]	3.5
Echovirus 11	GMK	6.5	6.5
Herpes simplex	WI-38	6.5	7.0
Rhinovirus 39	WI-38	3.5	3.5
Mumps	RMK	5.5	4.5

[a] Infectivity titer expressed as the $\log_{10}$ TCD_{50} per milliliter of inoculum.

GMK and WI-38 cells at a concentration of 200 μg/ml.

Although insufficient data are available to clearly demonstrate that the use of gentamicin will increase the number of virus isolations in field studies, accumulating data would support this conclusion. Gentamicin appears to be an excellent single antibiotic for use in tissue culture to reduce bacterial contamination.

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