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CHECK UP FOR ANTIMICROBIAL ACTIVITY OF AMINOGLYCOSIDE ANTIBIOTICS AFTER MEMBRANE FILTRATION

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ABSTRACT

The testing of antibiotics for sterility is an essential stage in the manufacturing process. Before sterility testing the antibiotic must either be removed or be inactivated. The conventional methods for sterility testing of antibiotics use the technique of membrane filtration for separation of contaminating microorganisms from the inhibitory effects of antibiotics prior to cultivation. The results suggest the possibility of adsorption of antibiotic onto the filter and its subsequent release into the nutrient medium. This problem may be partially resolved by the use of a filter with

hydrophobic edges. Washing with 300 ml peptone water does not generally eliminate the antibacterial activity. The filter must be washed well to ensure complete removal of the antibiotic and can also be washed with an inactivating agent if available.

INTRODUCTION

According to contemporary normative documents, sterility control of antibiotics for parenteral application is carried out by membrane filtration method.[1,2] The problem with the control of these medicines is the elimination of their antimicrobial activity before their testing for sterility. The membrane filtration is offered as a method which eliminates the antimicrobial activity. Some authors point at alternative methods for elimination of this activity.[3,4,5, 6,7,8,9] The reason for searching of such methods is the assumption for availability of residual antibiotic activity because of adsorption by the membrane filter or antimicrobial activity of the filter itself.[10]

The aim of this work was to check the degree at which antimicrobial activity of some aminoglycoside antibiotics could be eliminated by means of membrane filters.

MATERIALS AND METHODS

Bacterial strains - Proteus mirabilis 229, a clinical isolate from uroculture,Escherichia coli C_{1a} Nal lac + r-m-(SFM-Institut Pasteur), E.coli ATCC 25922, Klebsiella pneumoniae 7/ 48 (Bulgarian Type Culture Collection). Antibiotics- Gentamicin /Gm/ and Ampicillin /Amp/(Pharmachim, Bulgaria), Netilmicin /Net/ (Anilabo, France), Amikacin /Akn/(Bristol, Italiana). Media - fluid thyoglycolate and soybean casein digest broth, Muller Hinton agar and broth. Membrane filtration - apparatus "Sterifil" 47 mm as a filter holder and filters with 0,22 μm and a hydrophobic edge GSEP 047 AO -Millipore. Minimum inhibitory concentration (MIC) - MIC was found by antibiotic dilution in twofold steps in Muller Hinton broth and inoculated with approximately 1000 microbial cells from diluted overnight cultures. All inoculations were incubated at 37^0 C for 18 h.

Procedures I - A bacterial suspension of 18-24 hours broth culture up to standard 0.5 McFarland was prepared. Two ml from 10^{-2} dilution were added to 17 ml Muller Hinton broth. Then 1 ml from the necessary antibiotic concentration was added and cultivated for 1 h. 30 min at 37^0 C. Following filtration the filter was immediately inoculated in 20 ml Muller Hinton broth. The number of colony forming units (CFU) was determined at 3 h 30, 5 h

30, 7 h 30. After filtration, 0,5 ml of the broth was diluted by tenfold serial dilutions, and 0,25 μl of each dilutions was inoculated on Muller Hinton agar. After cultivation at 37^0 C for 24 h the number of CFU was determined as an average of all drops with countable growth.

II - The membrane filtration was carried out according to USP XXII, and the antibiotic was contaminated immediately before filtration. After filtration the filter was inoculated in 100 ml thioglycolate medium. The number of CFU was determined at 0, 3, 6, 12 and 24h . The controls were prepared in the same way but without any antibiotic.

III - The membrane filtration was carried out according to USP XXII, and the filters were inoculated on Muller Hinton agar. A preliminary inoculation was done pouring 1.6 ml bacterial suspension with 5.10^2 cells. Zones of inhibition around the filter, measured in mm, were determined after cultivation 24 h at 37^0 C.

RESULTS

For check-up of antimicrobial activity elimination of aminoglycoside antibiotics after membrane filtration, high-sensitive bacterial strains were used. Fig. 1 presents the results after cultivation of 1 h 30 min of P.mirabilis 229 with concentrations of Netilmicin which correspond to MIC, 4 times MIC and 10 times MIC and following filtration

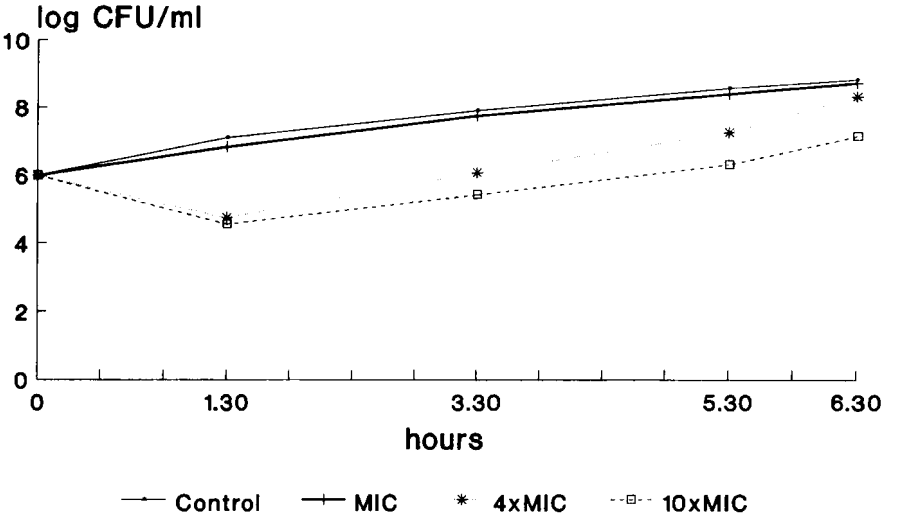

FIGURE 1

Antimicrobial action of different Netilmicin concentrations after incubation for 1 h 30 min with P. mirabilis 229 (MIC 1μg/ml) followed by membrane filtration.

without filter washing. Differences were fixed in the number of CFU/ml to the 7th hour, the difference was not statistically reliable for MIC and 4 times MIC ($p < 0.05$), and was reliable for 10 times MIC ($p < 0.001$). Figure 2 shows the result of the filtration of 20 ampoules (0.8 g) Gentamicin through one membrane filter. The contamination with 10^6 cells P. mirabilis 229 was done before filtration. Immediately after the filtration and to the 6th hour of filter inoculation no substantial differences in the number of CFU were fixed between the controls without antibiotic and filtrated tests with Gentamicin,

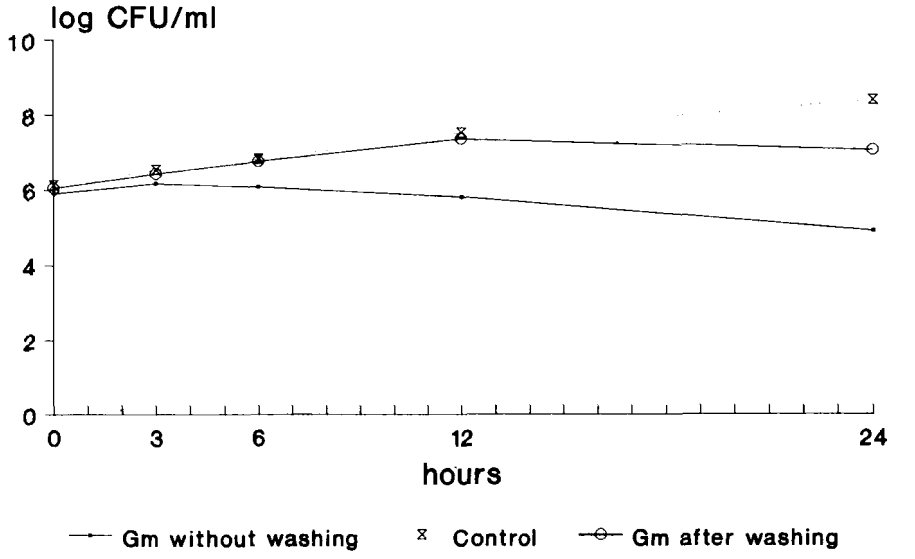

FIGURE 2

Check up for antimicrobial action of Gentamicin (0,8g) contaminated with P. mirabilis 229 (MIC 1μg/ml) for 24 hours after membrane filtration.

regardless of whether the filter was being washed or not washed. The cultures with filters were observed in the course of 14 days. Figure 3 presents the number of CFU in different days after the filtration of 0.8 g Gentamicin through one membrane filter. At the first day there appeared differences in the number of CFU comparing to the controls. Without filter washing, microorganisms were not found after the 3rd day. The number of CFU decreased with 4 logarithms to the 9th day when filter was

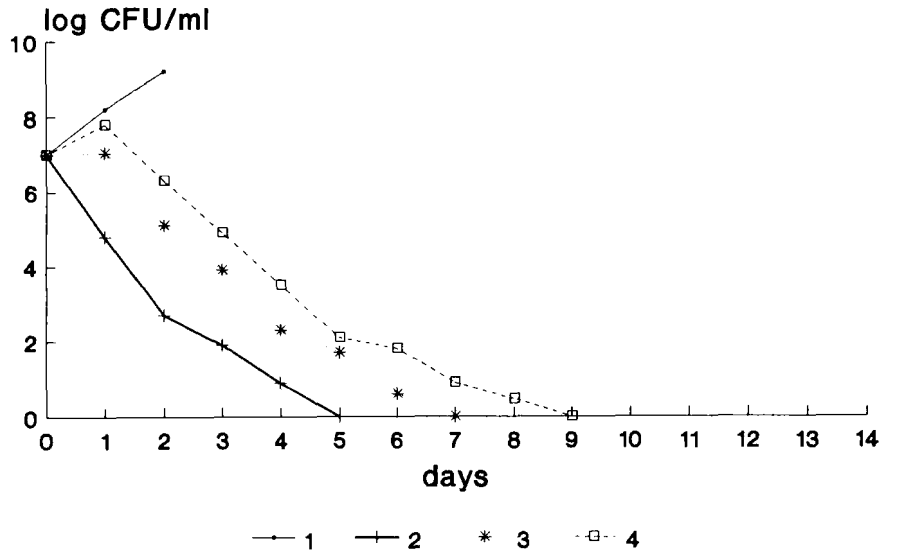

FIGURE 3

Check up for antimicrobial action of Gentamicin (0,8g) contaminated with P. mirabilis 229 (MIC 1 μg/ml) for 14 days after membrane filtration. 1-controls, 2-without filter washing, 3-after filter wasing with 300 ml peptone water, 4-after filter washing with 600 ml peptone water.

washed with 300 ml 1% peptone water and with 600 ml. The check up for antimicrobial activity of the filters after filtration of different antibiotic concentrations was carried out in a solid medium, in which low MIC strains were used. Table 1 presents the results of the measured zones of inhibition.

The fixing of inhibition zones depends on the antibiotic concentration and whether the washing was done after filtration.

TABLE 1

Check up for Antimicrobial Action of Membrane Filters after Filtration of Different Antibiotic Concentrations

No Bacterial Strain	Antibiotic MIC μg/ml	Filtrated concentrations	Inhibition zones(mm) without washing	Inhibition zones(mm) after washing
1.E.coliATCC	Gm(0,5)	10xMIC	-	-
25922		8.10^4xMIC	-	-
	Akn(1)	16.10^7xMIC	2	-
2.E.coliC1a	Gm(0,25)	10xMIC	-	-
Nal		16.10^6xMIC	-	-
lac+r-m	Akn(1)	32.10^7xMIC	3	1
3.K.pneumo-	Amp(0,25)	10^4xMIC	-	-
niae 7148		10^7xMIC	4	1

DISCUSSION

After cultivation of microorganisms for 1 h 30 min with different antibiotic concentrations followed by filtration without filter washing, the number of CFU/ml was influenced only on the 7th hour. We consider this

difference was due to the cultivation. The number of CFU decreased considerably at higher concentrations - 4xMIC and 10xMIC. The number of CFU after the 7th hour has approached those ones of the controls. According to the results obtained we consider that Netilmicin concentrations used by us were eliminated after filtration, despite of the fact that filters were not washed out.

Following filtration of higher antibiotic concentrations as with the sterility control, a decrease in the number of CFU was observed during a 14 days test. Inhibition zones with filter washing were smaller than those ones without filter washing. These results have to be considered with antibiotics sterility control. In such cases a larger quantity of washing solution have to be used in which solution a suitable inhibitor could also be put in.[3,4,11,12] With higher antibiotic doses several filters could be used for division of the dose among them. When using membrane filtration for sterility control of antibiotics it is correct to check the filter for antimicrobial activity after filtration and to carry out a control depending on the results.

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