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Short Communication

Experimental observations on the bacteriological controls of the antibiotics — I. Antibacterial activity of membranes employed in bacteriological assays*

F. NEGRETTI

Institute of Pharmacological Sciences, Laboratory of Microbiology and Hygiene, University of Milan, Italy

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Introduction

The method currently available for carrying out bacteriological controls of antibiotics is the technique of membrane filtration. The European Pharmacopoeia recommends washing the filter three times with peptone water (100 ml). The author has employed this technique for many groups of antibiotics, but has never shown microbial contamination. Because some workers [1–3] reported adsorption to membranes, it was suspected that it was possible that antibacterial activity of the membranes employed for the filtration of antibiotics might interfere with the growth of the micro-organisms.

The following experiments have been conducted: detection of zones of inhibition induced by membranes; evaluation of the possible inhibitory activity of membranes on suspensions of filtered sensitive micro-organisms; and detection of the possible antimicrobial activity of media inoculated with the membranes.

Experimental

Membranes

0.22-μm membranes have been used with a hydrophobic surface of various diameters (3–6 mm); two brands (Millipore, Sartorius) were used.

Antibiotics

The assays were applied to the following antibiotics: oxacillin (1 g), amoxycillin (875 mg) + clavulanic acid (125 mg), ceftriaxone (1 g), rolitetracycline (275 mg),

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chloramphenicol (250 mg), tobramycin (100 mg), erythromycin (500 mg), vancomycin (500 mg), rifamycin SV (90 mg), aztreonam (1 g) and ofloxacin (300 mg).

Micro-organisms

The possible antibacterial activity of membranes was tested by the following micro-organisms: *Escherichia coli* (ATCC 25922); *Staphylococcus aureus* (ATCC 25923). The concentration of the suspensions employed for filtration through membranes previously used for antibiotics was about 100 CFU.

Media

The following media were used: Mueller Hinton agar (assay "a"); fluid thioglycollate and soybean casein digest broth (assay "b"); fluid thioglycollate, soybean casein digest broth and soybean casein digest agar (assay "c").

Procedure

Assay "a". The membrane, after filtration of the antibiotic sample and washing with peptone water, was transferred to the surface of Mueller Hinton agar plates inoculated with a sensitive organism. After incubation at 37°C for 16–18 h, the inhibition zones were measured. If the membrane showed antibacterial activity, assays "b" and "c" were performed.

Assay "b". The filtration of another portion of the same antibiotic sample and washing was executed with an analogous membrane and then 1 ml of a suspension of organisms was filtered. The membrane used for antibiotic filtration and the sensitive organism was then transferred: (1) to soybean casein digest agar plates. After incubation at 37°C for three days, the bacterial count was evaluated. A bacterial count was also made on a membrane used for simple filtration of the same number of organisms; (2) to fluid thioglycollate medium (100 ml). The growth was examined after three days at 37°C. Growth from a membrane treated with the bacterial suspension was also examined.

Assay "c". Two membranes, after filtration of the antibiotic sample and washing with 300 ml of peptone water, were transferred, one to 100 ml of fluid thioglycollate and the other to 100 ml of soybean casein digest broth. After incubation at 37°C for 24 h, one

Table 1
Inhibition zones induced by membranes used for antibiotics filtration

N	Antibiotic	Inhibition zones (mm) *Staph. aureus*	*E. coli*
1	Oxacillin	13.5	—
2	Amoxycillin + clavulanic acid	2	—
3	Ceftriaxone	2	—
4	Rolitetracycline	7.5	6
5	Chloramphenicol	—	9.5
6	Tobramycin	—	5.5
7	Erythromycin	13.5	—
8	Vancomycin	16	—
9	Rifamycin SV	9	—
10	Aztreonam	—	14
11	Ofloxacin	10.5	—

Table 2
Inhibition of growth of membranes used for antibiotics filtration

N	Antibiotic	*Staph. aureus* Suspension CFU	Soybean casein digest agar CFU	%	Fluid thiog. growth	*E. coli* Suspension CFU	Soybean casein digest agar CFU	%	Fluid thiog. growth
1	Oxacillin	105	3	−97	—				
2	Amoxycillin + clavulanic acid	102	0	−100	+				
3	Ceftriaxone	110	21	−81	—				
4	Rolitetracycline					86	0	−100	++
5	Chloramphenicol					114	0	−100	++
6	Tobramycin					98	0	−100	+
7	Erythromycin	107	0	−100	+				
8	Vancomycin	116	0	−100	—				
9	Rifamycin SV	95	0	−100	++				
10	Aztreonam					104	1	−99	—
11	Ofloxacin	102	0	−100	+				

— = absence of antibacterial activity.
+ = moderate antibacterial activity.
++ = considerable antibacterial activity.

Table 3
Antibacterial activity of media inoculated with membranes employed for antibiotics filtration

N	Antibiotic	Inhibition *Staph. aureus*	*E. coli*
1	Oxacillin	—	
2	Amoxycillin + clavulanic acid	+	
3	Ceftriaxone	—	
4	Rolitetracycline	++	+
5	Chloramphenicol		+
6	Tobramycin	+	+
7	Erythromycin	—	
8	Vancomycin	—	
9	Rifamycin SV	++	
10	Aztreonam		++
11	Ofloxacin	—	

— = absence of antibacterial activity.
+ = moderate antibacterial activity.
++ = considerable antibacterial activity.

drop of the medium was transferred to the surface of soybean casein digest agar plates inoculated with the sensitive organism. After 16–18 h at 37°C the inhibition zone was measured.

Results

The results are detailed in the tables (Table 1: assay "a"; Table 2: assay "b"; Table 3: assay "c") and can be summarized as follows. (1) The membranes employed for filtration of antibiotics show, in general, an antibacterial activity. (2) The membranes used for antibiotics filtration produce variable inhibition zones (2–16 mm). These membranes frequently interfered with microbial growth (diminution of CFU of 81–100%).

Discussion and Conclusions

The results confirm the adsorption of antibiotics on the membranes employed for filtration and demonstrate clearly by various assays the antibacterial activity of such membranes. After washing with 300 ml of peptone water this activity remains partially and impedes the growth of contaminant micro-organisms.

The control of sterility and contamination of the antibiotics examined under these conditions is not advisable; thus it is necessary either to modify the procedures of bacteriological control of the antibiotics or to change the chemical characteristics of the membranes.

The author is investigating some modifications to the procedure of bacteriological control of antibiotics and it is hoped to eliminate or at least to reduce the antibacterial activity of the membranes employed for filtration and washed with 300 ml of peptone water according to the recommendations of the Pharmacopoeia.

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