DISTURBANCE OF THE TRANSMISSION OF EXTRACHROMOSOMAL RESISTANCE DETERMINANT IN STAPHYLOCOCCI AFTER PRELIMINARY NEOMYCIN TREATMENT

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Preliminary neomycin treatment of donor microorganisms before their introduction into a suppurative inflammatory focus significantly reduces the number of transductants in the purulent exudate. Preliminary neomycin treatment of the recipient microorganisms under the same conditions almost completely blocks the transmission of extrachromosomal determinants of resistance in the staphylocci in the suppurative focus.

The authors have previously shown that introduction of neomycin into a suppurative inflammatory focus in mice reduces the intensity of transmission of extrachromosomal determinants of resistance in staphylococci during spontaneous transduction [4]. In addition, a sharp decrease in the frequency of transduction in vitro was observed when donor and recipient microorganisms grown in the presence of neomycin were used [3].

TABLE 1. Dynamics of Transductant Formation in Suppurative Inflammatory Focus

Day of observation after introduction of culture of microorganism into focus									
3rd	4th	5th	6th	7th	8th	10th	12th	13th	15th
1	210	560	860	720	60				
16	2	28	410	1000	32				
2	10	7	11	1	1				
44	8	2	120	390	2800				
40	100	14	190	200	140				
5	2	1	3	12	21				
7	16	20	22	124	146				
4	1	5	4	6	12				
158	37	10	16	580	547				
2	1	5	4	6	4				
103	173	150	110	123	143				
2	—	1	—	—	12	203	192		
4	—	2	—	—	21				
197	—	164	—	—	15	875	621	13	15
186	—	254	—	—	37	12	6		
1000	—	1000	—	—	—	127	109		
1	—	1	—	—	—	294	1000	1000	328
628	—	329	—	—	86				
1	—	2	—	—	31	64			
237	—	112	—	—	—	1000	637	248	23
1	—	2	—	—	11	124			
9	—	42	—	—	89	139	162	75	9
23	—	59	—	—	21	97	49	5	2

Note: Number of colonies grown on agarized medium containing streptomycin (100 μg/ml) and erythromycin (100 μg/ml) given in Tables 1-3.

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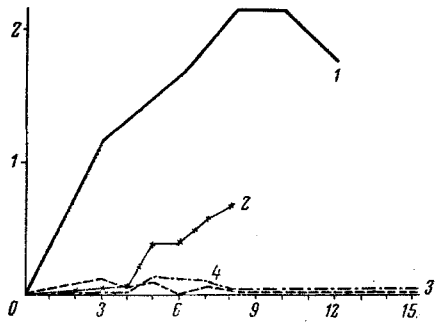

Fig. 1

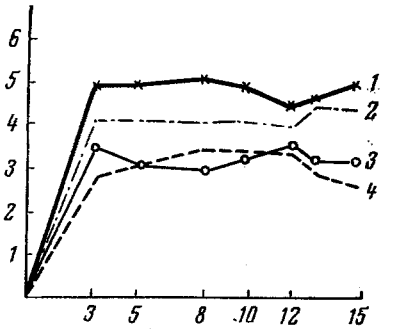

Fig. 2

Fig. 1. Effect of preliminary neomycin treatment of donor or recipient cells on transmission of resistance factors in staphylococci in a suppurative focus: 1) animals injected with untreated microorganisms; 2) animals injected with treated donor and untreated recipient cells; 3) animals injected with untreated donor and treated recipient cells; 4) animals injected with treated donor and treated recipient cells. Here and in Fig. 2: ordinate – log of number of microorganisms; abscissa – day of observation.

Fig. 2. Effect of preliminary neomycin treatment of donor and recipient cells on their numbers in suppurative focus: 1) untreated donor cells; 2) donor cells treated with neomycin; 3) untreated recipient cells; 4) recipient cells treated with neomycin.

TABLE 2. Disturbance of Transmission of Extrachromosomal Determinant in Staphylococci in Vivo after Neomycin Treatment of Donor Microorganisms

Day of observation after introduction of culture of microorganism into focus					
3rd	4th	5th	6th	7th	8th
0	7	257	212	71	0
0	0	0	0	0	0
0	0	1	0	0	0
1	3	4	5	39	22
0	0	3	2	21	392
0	0	0	0	1	19
0	3	0	0	1	0
0	0	0	0	0	0
1	0	7	0	0	0
6	1	0	15	128	130
5	0	0	0	0	0

In the investigation described below, the effect of preliminary treatment of donor and recipient microorganisms with neomycin on the transmission of extrachromosomal resistance factors was studied in staphylococci in vivo.

EXPERIMENTAL

Strains of Staphylococcus aureus 8325-1 (recipient with chromosomal resistance to streptomycin) and 8325-R 11 (de) (donor with extrachromosomal resistance to erythromycin) were used in the experiments [5, 6]. An experimental model of a suppurative inflammatory focus was produced in rats [2]. The extrachromosomal determinant was transmitted by spontaneous transduction with temperate phage RP when both donor and recipient microorganisms were present together in the focus [4, 6]. The cultures of microorganisms were grown by the method described previously [4, 6].

The bacterial cells were treated with neomycin as follows: a suspension of donor or recipient microorganisms was transferred in a volume of 1 ml to two different flasks containing 9 ml Hottinger's broth with 10 μg/ml neomycin [1] and grown at 37°C on a shaker for 18 h. After incubation the cultures were sedimented by centrifugation and the residue washed twice with an excess of physiological saline. The animals were infected with suspensions of microorganisms concentrated five times. At various times after infection, 1-2 ml exudate was taken from the suppurative focus by means of a syringe and seeded on selective agarized media [4]. Four groups of animals were used in the experiments. The animals of group 1 were injected with a suspension of untreated donor and recipient cells, those of group 2 with a suspension of treated donor and untreated recipient cells, group 3 with a suspension of untreated donor and treated recipient cells, and those of group 4 with a suspension of treated donor and recipient cells.

EXPERIMENTAL RESULTS

The results given in Fig. 1 show that treatment of the donor or recipient bacteria with neomycin sharply inhibited the formation of transductants in the exudate. The differences between the number of transductants in the exudate of the control animals and in the exudate of animals of each experimental group were statistically significant throughout the period of observation ($P = 0.001$). The number of donor and recipient cells remained practically identical to all groups for 15 days (Fig. 2).

TABLE 3. Disturbance of Transmission of Extrachromosomal Determinant in Staphylococci in Vivo after Neomycin Treatment of Recipient Microorganisms

Day of observation after introduction of culture of microorganisms into focus									
3rd	4th	5th	6th	7th	8th	10th	12th	13th	
0	0	0	0	0	0				
0	12	64	48	22	5				
0	0	0	0	0	0				
3	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	0	0	0	0	0				
0	—	0	—	—	0	0	0	0	0
0	—	0	—	—	0	0	0	0	0
0	—	0	—	—	0	0			
0	—	0	—	—	0	0	0	0	0
0	—	0	—	—	0	0			
0	—	0	—	—	0	0	0		
0	—	0	—	—	0	0	0	0	0
0	—	0	—	—	0	0	0	0	0
1	—	0	—	—	0	0			
0	—	0	—	—	0	0	0		
0	—	0	—	—	0	0	0		
0	—	0	—	—	0	0	0	0	

The results of a study of the dynamics of transductant formation in the various groups of animals are given in Tables 1-3. During the first few days after infection staphylococci resistant to streptomycin and erythromycin were found in the exudate from all the control animals (Table 1).

Treatment of donor microorganisms with neomycin slightly reduced the number of animals in whose exudate the factors of drug resistance were transmitted (Table 2). Microorganisms with simultaneous resistance to two antibiotics failed to appear in only 18% of the animals, while in the rest their number varied from 1 to 392.

Neomycin treatment of the recipient population was much more effective (Table 3). Under these conditions no transductants were isolated in 88% of the animals before the 15th day after infection. This period of observation could not be increased, because as a rule the abscesses burst on the 13th-15th day after injection of the culture. Injection of treated donor and treated recipient microorganisms into the focus (group 4) did not increase the effectiveness of disturbance of the transmission of the extrachromosomal determinant.

The most effective method of disturbing the transmission of extrachromosomal determinants of drug resistance in staphylococci in vivo is therefore by neomycin treatment of the recipient population.

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