

Stability of neomycin resistance in *Staphylococcus aureus*

G. A. J. AYLIFFE

From the Hospital Infection Research Laboratory, Summerfield Hospital, Birmingham

SYNOPSIS A strain of *Staphylococcus aureus* isolated from the skin of a subject with eczema showed a loss in resistance to neomycin and tetracycline after treatment with neomycin was stopped. Seven out of 22 strains of neomycin-resistant *Staph. aureus* showed a loss in resistance to neomycin and streptomycin after storage in nutrient broth for 14 days at room temperature, and it seems probable that resistance in these unstable strains was controlled by extra-chromosomal elements or plasmids. Strains of phage types 84/85 and 29/77 and related types were frequently isolated in general hospital wards and showed no loss in resistance on similar storage in nutrient broth. Five of the neomycin-unstable strains were isolated from patients with eczematous lesions. Multiple-resistant and antibiotic-sensitive strains of *Staph. aureus* of similar phage type were isolated from patients in a ward for patients with skin disease, and the possibility of transfer of resistance *in vivo* is discussed.

The selection of neomycin-resistant strains of *Staph. aureus* by neomycin or related compounds and the disappearance after discontinuing the use of these antibiotics has been shown in a burns unit (Lowbury, Babb, Brown, and Collins, 1964) and in a surgical ward (Alder and Gillespie, 1967). Neomycin resistance in the strains isolated from the burns unit appeared to be stable and the gradual disappearance of resistant strains over a period of six months was due to a reduction in the numbers of resistant organisms rather than to a specific loss of neomycin resistance in strains remaining in the unit. By contrast, a strain showing unstable resistance to neomycin was isolated from the skin of a profuse disperser with eczema who was being treated with a topical neomycin preparation (Ayliffe and Collins, 1967).

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In the present study, changes in resistance to antibiotics of *Staph. aureus* isolated from the eczematous subject after discontinuing treatment with neomycin were investigated. Studies were made on the loss of neomycin resistance on storage of neomycin-resistant strains isolated from a number of patients in different hospital environments. Variations in antibiotic sensitivity patterns of staphylococci of similar phage type isolated from patients in a skin hospital are also described.

Methods

SAMPLING OF PATIENTS

Nasal swabs and swabs from lesions were collected from patients in cross-sectional surveys of 14 hospitals in the Birmingham region. A

number of skin sites of patients in a hospital for skin diseases were sampled with Alne disposable contact plates (a modification of the method described by Hall and Hartnett, 1964). Samples were taken by pressing the agar surface of the contact plate onto the skin. The skin of the staphylococcal disperser (S) was sampled while he was treated with a topical neomycin preparation and two months after discontinuing its use. A varicose ulcer on the leg of a patient in a surgical ward was also sampled before and after a period of two months without antibiotic treatment.

SAMPLING OF THE ENVIRONMENT

In a cross-sectional survey of a hospital for diseases of the skin, floors were sampled with contact plates.

BACTERIOLOGY

Nasal swabs were cultured on nutrient agar containing phenolphthalein diphosphate (Barber and Kuper, 1951). Swabs from lesions were cultured on blood agar and McConkey's medium. Alne contact plates were filled with nutrient agar containing phenolphthalein diphosphate to provide a surface raised slightly above the rim of the plate. All plates were incubated for 18 hours at 37°C and colonies of presumptive *Staph. aureus* were confirmed by slide or tube coagulase tests. Strains of *Staph. aureus* were tested for sensitivity to antibiotics by the ditch-plate method. The ditches contained the following concentrations of antibiotics: benzylpenicillin, 10 units/ml; novobiocin, lincomycin, fusidic acid, erythromycin, and methicillin, 10 µg/ml; streptomycin, tetracycline, chloramphenicol, and neomycin, 50 µg/ml. Strains from the skin hospital were also tested for resistance to bacitracin (50 µg/ml in the ditch). Staphylococci were phage typed with the routine set of phages kindly supplied by the Central Public Health Laboratory, Colindale. Organisms were stored on agar slopes in bijou bottles at room temperature.

TESTS FOR STABILITY OF ANTIBIOTIC RESISTANCE OF *Staph. aureus* ISOLATED FROM TWO PATIENTS

One hundred and twenty-seven colonies of *Staph. aureus* (phage type 80/81 at 1,000 × routine test dilution) isolated from the skin of the staphylococcal disperser (S) were tested for sensitivity to antibiotics. Two months after discontinuing topical treatment with neomycin, the skin of the disperser was again sampled and 100 colonies of *Staph. aureus* were tested for sensitivity to antibiotics. One hundred colonies of neomycin-resistant *Staph. aureus* (phage type 85) from the varicose ulcer of another

patient were also tested for sensitivity to antibiotics before and after two months without antibiotic treatment. A number of colonies from both patients before and after treatment were phage typed.

TESTS FOR STABILITY OF ANTIBIOTIC RESISTANCE OF *Staph. aureus* ON STORAGE
Twenty-five neomycin-resistant strains were tested again for sensitivity to antibiotics after storage on agar slopes for periods varying from three months to two years. Resistance to neomycin was lost in three strains, resistance to streptomycin was also lost in one of these strains (phage type 52A), and resistance to fusidic acid was also lost in another strain (phage type 29/52/52A at 1,000 × routine test dilution).

Single colonies from the other 22 neomycin-resistant strains were inoculated into nutrient broth and incubated for 18 hours at 37°C. Nutrient agar plates were inoculated with the broth cultures diluted to give approximately 200 colonies per plate. Replicates were made from these plates onto nutrient agar plates containing 20 µg/ml neomycin sulphate. The broth cultures were then stored for 14 days at room temperature after which they were again inoculated on agar plates, from which replica plates were taken on medium containing neomycin. In strains showing any neomycin-sensitive colonies, 100 colonies were selected at random from subcultures on agar plates made after 18 hours' incubation and after 14 days' storage. These colonies were tested for sensitivity to the full range of antibiotics.

Minimum inhibitory concentrations (MIC) of neomycin for resistant and sensitive variants of three strains, two strains showing stable resistance, and the Oxford staphylococcus were determined by a broth dilution method.

Extrachromosomal elements or plasmids controlling resistance to antibiotics may sometimes be eliminated by growing the organism in nutrient broth containing acriflavine (Mitsuhashi, Hashimoto, Kono, and Marimura, 1963) or in nutrient broth at 44°C (Asheshov, 1966). Tests for loss of resistance to neomycin using these methods were made with the 22 strains.

Results

STABILITY OF ANTIBIOTIC RESISTANCE OF *Staph. aureus* ISOLATED FROM TWO PATIENTS

Loss of resistance to neomycin and tetracycline of staphylococci isolated from the skin of the disperser (S) after discontinuing treatment for two months is shown in Table I. Resistance to neomycin or tetracycline was sometimes lost independently; resistance to novobiocin appeared

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to be stable. No loss in resistance to any antibiotic occurred in staphylococci isolated from the varicose ulcer after two months without antibiotic treatment. Phage types of strains isolated from both patients remained unchanged during the two-month interval without treatment.

Time of Isolation of <i>Staphylococci</i>	No. of Colonies Tested	Percentage of Colonies Sensitive to		
		Tetra-cycline	Novobiocin	Neomycin
During treatment	127	0	0	2
Two months after discontinuing treatment	100	40	0	69

Table I *Loss of antibiotic resistance in Staph. aureus on discontinuing treatment of disperser with neomycin cream*

STABILITY OF ANTIBIOTIC RESISTANCE ON STORAGE

The stability of neomycin-resistance in 22 strains is shown in Table II. Strains of phage types 84/85, 85, and 29/77 showed stable resistance in these tests, but most of the other strains showed varying degrees of reversion to sensitivity. The five strains isolated from the skin of eczematous patients were particularly unstable. However, three of these were of related phage patterns and isolated from patients in the skin hospital, although not all at the same time. One of the unstable strains (phage type 80/81 at 1,000 × routine test dilution) was isolated from the disperser (S). Five of the unstable strains were sensitive to penicillin, but were resistant to two or more other antibiotics.

Streptomycin resistance as well as neomycin resistance was lost in the unstable strains; not all neomycin-resistant strains, however, were

initially resistant to streptomycin. No other loss in resistance to other antibiotics was found.

The MIC of neomycin for sensitive variants was 2-4 µg/ml, which was slightly higher than that for the Oxford staphylococcus (0.5 µg/ml). The MIC for the resistant variants and for strains showing stable neomycin resistance was > 128 µg/ml.

No increased loss in resistance to neomycin occurred on growing the stable or unstable strains in nutrient broth containing acriflavine or at 44°C.

STRAINS ISOLATED FROM PATIENTS WITH SKIN DISEASES

Table III shows strains of similar phage pattern but different antibiotic sensitivity patterns isolated from patients in a hospital for diseases of the skin on one day. A strain resistant to five antibiotics and a strain sensitive to all the antibiotics tested was isolated from the skin of one patient. Resistance to neomycin and streptomycin, but not to other antibiotics, was lost on storing neomycin-resistant strains in broth for

No. of Isolates	Antibiotic Resistance Pattern	Phage Pattern (RTD)
4	P S T E Ne	6/47/53/54/75/83A
1	P S T E Ne	6/47/75/77
1	S T E Ne	6/47/53/54/75/77/42E/81
1	P T E	6/47/53/54/75/83A/85
2	P T E	6/47/53/75/77
2	P	6/47/53/54/75/83A
2	Sensitive	6/47/53/54/75/83A
2	Sensitive	6/47/53/83A/85

P = penicillin S = streptomycin T = tetracycline
E = erythromycin Ne = neomycin

Table III *Staph. aureus isolated from noses and skin lesions of patients in a ward for diseases of the skin*

No. of Strains Tested	Sites of Origin	Antibiotic Resistance Pattern	Phage Pattern		Percentage of Colonies Sensitive to Neomycin	
			At Routine Test Dilution	At 1,000 × Routine Test Dilution	Unstored (18 hours in broth at 37°C)	After Two Weeks' Storage in Broth (room temperature)
10	Noses and lesions	P S T E Ne	85 or 84/85	—	0	0
3	Noses and wound	P S T E M Ne	29/77	—	0	0
1	Floor	Ne	53/83A	—	0	0
1	Burn	P S T E Ne	N.T.	N.T.	0	0
1	Nose	T Ne	N.T.	29	0	3
1	Nose	S T Ne	52A/79	—	0	5
1	Skin	T No Ne	N.T.	80/81	1	88
1	Skin	T Ne	N.T.	54/75/77	4	95
1	Skin	S T E No Ne	N.T.	54/75/77	1	60
1	Skin	P S T E Ne	N.T.	N.T.	11	86
1	Skin	P S T E Ne	6/47/53 54/75/83A	—	15	88

P = penicillin S = streptomycin T = tetracycline E = erythromycin No = novobiocin Ne = neomycin M = methicillin
N.T. = not typable — = not tested.

Table II *Stability of neomycin resistance in Staph. aureus on storage*

14 days. The neomycin-resistant strains were not resistant to bacitracin.

Discussion

Loss of resistance to an antibiotic at a higher rate than might be expected if the change were due to a chromosomal deletion is one of the characteristics of resistance controlled by extra-chromosomal elements or plasmids. Plasmid-controlled antibiotic resistance in *Staph. aureus* has been reported for penicillinase production, for resistance to tetracyclines, macrolides, chloramphenicol, and kanamycin or neomycin (eg, Novick, 1963; Harmon and Baldwin, 1964; May, Houghton, and Perret, 1964; Chabbert, Baudens, and Gerbaud, 1964; Hashimoto, Kono, and Mitsuhashi, 1964; Mitsuhashi *et al*, 1965; Novick and Richmond, 1965; Asheshov, 1966). Evans and Waterworth (1966) reported a loss in resistance to fusidic acid and penicillin in one strain of *Staph. aureus* and in another strain a simultaneous loss of resistance to fusidic acid, tetracycline, and kanamycin.

In the present study, resistance to neomycin (or kanamycin) and streptomycin was simultaneously lost in some strains on storage for 14 days in broth and a loss of neomycin and tetracycline resistance was found in staphylococci isolated from a patient with eczema after discontinuing neomycin treatment. It seems likely that resistance in these strains was controlled by plasmids, although no increased loss was observed on growing in acriflavine or at 44°C, and attempts at transduction of neomycin or streptomycin resistance have so far been unsuccessful.

Neomycin-resistant strains of *Staph. aureus* were isolated in most of the hospitals visited; the overall incidence in noses of patients and staff was 102/5,084 (2%) and in lesions 53/820 (6%). Phage types 84/85 and 29/77 and related strains were the commonest types of neomycin-resistant *Staph. aureus* isolated in general hospitals and were usually resistant to five or six antibiotics, which often included methicillin. The neomycin-resistant strains originally isolated in the USA, the United Kingdom, and other parts of the world were predominantly phage type 84/85 or related types, but recently phage types 29/77 or 77 have been isolated with increasing frequency in hospitals in the Birmingham region. Neomycin resistance in the strains tested was stable on storage in nutrient broth for 14 days. The neomycin-unstable strains were mainly isolated from patients with skin lesions and were different in phage type from most of the resistant strains isolated in general wards. A high incidence of neomycin-resistant strains in skin lesions (6/17, 35%) was probably related to the widespread use of neomycin in

dermatology. Lacey (1968) has shown that neomycin forms insoluble complexes with long-chain fatty acids *in vivo* and suggests that the persistence of neomycin in the skin after treatment might encourage the appearance of resistant strains.

A wide range of antibiotic sensitivity patterns of *Staph. aureus* with similar phage type was found in a survey of a ward for patients with skin diseases. A strain resistant to five antibiotics and one of a similar phage type, which was sensitive to all the antibiotics, were also isolated from different sites on one patient. It seems possible that in some circumstances, a strain of *Staph. aureus* growing on a patient may gain or lose resistance to a number of antibiotics together, although only neomycin and streptomycin resistance was shown to be lost in the resistant strains isolated in this ward. Transfer *in vivo* of antibiotic resistance in *Staph. aureus* by transduction has been reported in mouse experiments (Jarolmen, Bondi, and Crowell, 1965; Novick and Morse, 1967), but no evidence is available of transfer of resistance in *Staph. aureus* in man. However, the large populations of *Staph. aureus* on the skin and in the environment of wards in skin hospitals and in burns units provide an unusual opportunity for transfer of genetic material between staphylococci.

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