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Neomycin-resistant *Staphylococcus aureus* in a burns unit

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Hospital strains of *Staphylococcus aureus* resistant to neomycin have been reported in the United States (Quie, Collin & Cardle, 1960; Griffith, Ostrander, Smith & Beswick, 1961; Cohen, Fekety & Clough, 1962) and more recently in Great Britain (Robertson, 1963; Jacobs & Willis, 1963). Many of the strains isolated in this country have proved difficult to type and are associated with patterns of inhibition by various phages of group III at 1000 R.T.D. (routine test dilution); strains showing this pattern have been designated 'type A' (Temple & Blackburn, 1963; Jacobs, Willis, Ludlam & Goodburn, 1963). They differ from the American strains, which were typable by phage 54 at 1000 R.T.D.

From 1954 to 1957 and for a period in 1961 all strains of *Staph. aureus* isolated from burns in this unit were tested for sensitivity to neomycin; although many patients were treated with local application of the antibiotic in three controlled trials, resistant strains were not found (Lowbury, 1955; Cason & Lowbury, 1960; Lowbury, Miller, Cason & Jackson, 1962). In 1963 routine neomycin sensitivity tests on staphylococci from burns were started again, and a large proportion of the strains were found to be resistant. We describe here some studies on the neomycin-resistant staphylococci and discuss the emergence and spread of these organisms.

MATERIALS AND METHODS

Strains of Staphylococcus aureus

Staph. aureus was isolated by methods described elsewhere (Cason & Lowbury, 1960) from swabs taken from the burns of patients in the Burns Unit. After the detection of neomycin-resistant staphylococci in burns, nasal swabs were taken also from the nurses in the burns wards and in all the other wards of the Accident Hospital, and from a series of 309 patients attending the Casualty Department; 320 strains of *Staph. aureus* isolated in the hospital from miscellaneous infective lesions other than burns were kindly supplied by Dr S. Sevitt. From each site a single colony of each colonial form was picked and subcultured on blood agar; recognition of *Staph. aureus* was determined by a tube coagulase test with 10% human plasma broth examined after overnight incubation.

Antibiotic sensitivity tests

Strains of *Staph. aureus* were tested for sensitivity to a range of antibiotics by a ditch plate test, with 10 μg. neomycin sulphate per ml. nutrient agar in the

ditch for tests of that antibiotic. Some of the strains were also tested for sensitivity by a tube dilution method, with an inoculum of 0·02 ml. of a 1/1000 dilution of overnight broth cultures of the organisms added to nutrient broth in tubes containing doubling dilutions of neomycin.

Phage typing

Staphylococci were typed with phages kindly supplied by the Central Public Health Laboratory, Colindale (Blair & Williams, 1960).

Method of study

From 5 June 1963, when neomycin-resistant staphylococci were first observed in the unit, all strains of *Staph. aureus* were tested for sensitivity to neomycin, and phage type was determined on one strain from each patient at each sampling (swabs being taken daily from exposed burns and at all changes of dressings from those treated by the covered method). For many months before this time staphylococci from burns and from the nares of patients in the burns ward had been preserved for further study; on detection of the first neomycin-resistant staphylococci in June, these preserved strains were tested for sensitivity to neomycin and phage typed.

EMERGENCE OF NEOMYCIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

A series of 829 strains isolated between 4 December 1962 and 4 June 1963 was tested for sensitivity to neomycin. The first resistant strain was isolated on 23 February (week 8) 1963, and from that time a rapidly increasing proportion of the swabs from burns was found to be carrying neomycin-resistant *Staph. aureus*, reaching a peak of 74% in the week of 9–15 April (week 15) (see Fig. 1), after which the proportion of resistant strains fluctuated and then fell gradually. Neomycin-resistant strains were resistant also to kanamycin. The proportion of patients carrying neomycin-resistant strains increased more gradually, reaching a peak of 42% in the week 2–8 July (week 27), after which it fluctuated and fell. Since 15 November (week 47) until the present time (7 December), no neomycin-resistant staphylococci have been isolated in the Burns Unit.

Resistant strains from other sources

Table 1 shows the neomycin sensitivity of strains of *Staph. aureus* isolated between 11 June and 2 September from the nursing staff of all wards in the hospital, from 134 patients attending the Casualty Department, and from 320 miscellaneous lesions other than burns examined between 25 May and 15 October. One nurse in the burns ward and one specimen (urine) examined in the pathology department were found to be colonized by neomycin-resistant staphylococci.

Relation of neomycin resistance to clinical use of the antibiotic

Three trials of chemotherapy were in progress at the time when neomycin-resistant strains were detected: (1) a comparison of neomycin–chlorhexidine tulle gras (Lowbury *et al.* 1962) with chlorhexidine tulle gras for local prophylaxis of

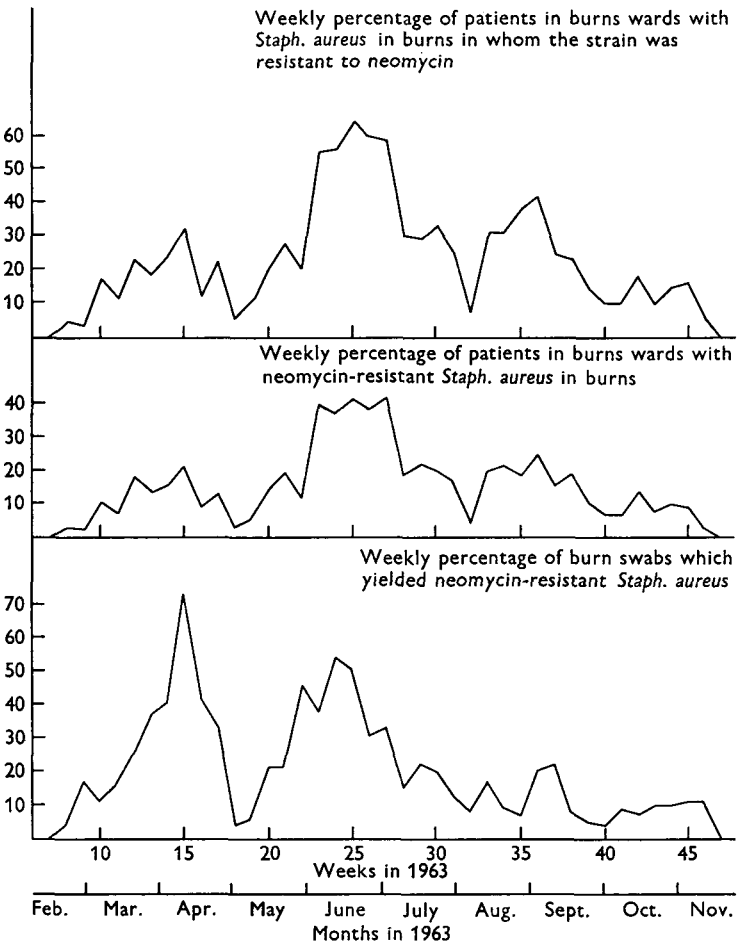

Fig. 1. Weekly percentage of strains of *Staph. aureus* from burns of in-patients which were resistant to neomycin between 18 February and 7 December.

Table 1. *Neomycin resistance of* Staphylococcus aureus *from various sources other than burns*

Sources	Samples	Number of samples yielding *Staph. aureus*	Number of samples yielding Neomycin-resistant strains
Nares of nurses in			
Burns Unit	13	10	1
Other wards	76	24	0
Nares of patients attending Casualty Department	309	134	0
Miscellaneous infections (not burns)	320	320	1

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covered burns involving less than 20% of the body surface. Neomycin was first used in this trial on 25 September 1962; (2) a comparison of 'polybactrin' spray (polymyxin, neomycin and bacitracin in a volatile suspending fluid) with chlorhexidine powder for local prophylaxis of exposed burns; the trial began on 21 January 1963; and (3) a therapeutic trial in patients with severe burns (more than 35% of the body surface) of systemic kanamycin, colistine methane sulphonate and cloxacillin; this trial began on 6 September 1962.

The first resistant strain was isolated on 23 February 1963 in a patient who was being treated with polybactrin. The proportion of resistant strains fluctuated greatly during the trials, but the rise in the proportion of resistant strains occurred during a period when neomycin and kanamycin were in use; after the withdrawal of these antibiotics from the ward when resistant staphylococci were detected (in June 1963), there was a fluctuating fall and eventually an elimination of resistant strains from the ward. Patients continued to carry resistant strains for long periods in hospital and also after discharge from hospital.

Table 2. *Neomycin-resistant* Staphylococcus aureus *in burns during controlled trials of neomycin and kanamycin*

Trials	Total swabs	Swabs with *Staph. aureus*	Swabs with neomycin-resistant *Staph. aureus*
Kanamycin	811	318	148
Control	320	191	7
Polybactrin	299	196	69
Control	170	76	4
Neomycin–chlorhexidine tulle gras	328	166	55
Control	41	24	0

Table 2 shows the numbers of neomycin-resistant staphylococci isolated from the burns of patients treated with neomycin or kanamycin and from the control patients who were not treated with these antibiotics. The admission of patients to control or treatment groups in the prophylactic trials on the basis of odd or even hospital numbers led (by chance) to a disproportionately small number of swabs in the control series, but there was consistently a much larger proportion of swabs yielding resistant staphylococci from the burns of patients who were in the series treated with neomycin or kanamycin.

Phage type of resistant strains

All the strains of neomycin-resistant *Staph. aureus* showed an almost identical pattern of inhibition by certain phages of group III (6, 47, 54, 77, and often also 7, 53, 75 and 75B) at 1000 R.T.D. None of the neomycin-sensitive staphylococci isolated before or during the early weeks after resistant strains appeared showed the same pattern of inhibition by phages (see Table 3). At a later stage (on 17 and 19 March) 2 patients were found to be carrying neomycin-sensitive strains with this

phage inhibition pattern, one of them being the patient in whose burns resistant strains were first isolated here, and from whom they were still being isolated; strains of this description were not found in later specimens from the ward.

Table 3. *Phage group and inhibition pattern of neomycin-sensitive and neomycin-resistant* Staphylococcus aureus *in Burns Unit*

Time of isolation	Phage group or inhibition pattern 6/47/54/77, etc.	Strains of *Staph. aureus*		
		Neomycin sensitive	Neomycin resistant	Total
Week before first neomycin-resistant strain appeared (week 7 in 1963)	Group I	6	0	6
	Group II	1	0	1
	Group III	37	0	37
	Miscellaneous	0	0	0
	Not typable	7	0	7
	Inhibition pattern 6/47/54/77, etc.	0	0	0
Week when first neomycin resistant strain appeared (week 8 in 1963)	Group I	3	0	3
	Group II	0	0	0
	Group III	28	0	28
	Miscellaneous	1	0	1
	Not typable	11	0	11
	Inhibition pattern 6/47/54/77, etc.	0	2	2
Twelfth week after first neomycin resistant strain appeared (week 20 in 1963)	Group I	11	0	11
	Group II	1	0	1
	Group III	24	0	24
	Miscellaneous	9	0	9
	Not typable	2	0	2
	Inhibition pattern 6/47/54/77, etc.	0	30	30

Degree of resistance of neomycin-resistant staphylococci

The minimal inhibitory concentration (M.I.C.) of neomycin for two strains tested by a tube dilution method was 64 μg./ml.

Stability of resistance

Cultures of neomycin-resistant staphylococci were subcultured daily for a period of 2 months. The strains were still fully resistant after this procedure.

A resistant culture was spread on a blood agar plate and 100 single colonies obtained after overnight incubation were tested for sensitivity to neomycin. All the colonies yielded fully resistant subcultures.

In vitro *habituation of sensitive strains to neomycin*

A preliminary experiment was made with two neomycin-sensitive strains showing the same phage inhibition pattern as the resistant staphylococci (63/2882B and 63/2535A), and with two other neomycin-sensitive staphylococci (63/8747, type 80 and 63/9025, type 80/81). The M.I.C. was determined by a tube dilution test. The tubes containing the highest concentration of neomycin in which the

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staphylococci had grown were subcultured to broth tubes containing a range of doubling dilutions of neomycin. After ten transfers, each of the strains showed the appearance of small-colony forms with approximately the same increase in resistance to neomycin (the M.I.C. rising from 0·25 or 0·5 μg./ml. to 32 or 64 μg./ml.). One of the strains which showed the same phage inhibition pattern as the naturally occurring neomycin-resistant staphylococci (63/2882B) showed a small proportion of normal full-sized staphylococcal colonies together with the small colonies. In a replicate experiment strain 63/2882B was again the only one from which colonies of increased resistance but normal size and appearance were obtained after a series of 7 transfers in medium containing neomycin; two other control strains were used in this experiment (63/2892, type 75/75B/77 and 63/2884, type 52/52A/80).

DISCUSSION

The neomycin-resistant staphylococci which appeared in the Burns Unit all showed the same pattern of phage inhibition. In tests kindly made by Dr M. T. Parker of the Central Public Health Laboratory, Colindale, these strains were lysed by certain experimental phages and could be described as an atypical type 83A; in this respect they are similar to those isolated in other parts of Britain in the past year. Since none of the many neomycin-sensitive staphylococci isolated in the period when the resistant strain emerged have shown this inhibition pattern it seems likely that the strain was brought into the hospital by a patient or a visitor; alternatively, it is possible that a loss or a change in phage susceptibility may have appeared on the emergence of a neomycin-resistant mutation (e.g. Harrison, Beavon & Griffin, 1959).

A striking feature was the emergence of a single strain as the dominant staphylococcus in the Burns Unit (cf. Lowbury & Collins, 1964). The emergence of this staphylococcus was clearly determined by the use of neomycin and kanamycin; it appeared during trials of these antibiotics, and it dwindled and disappeared after they were withdrawn from the ward. Patients who were not treated with neomycin or kanamycin were much less often colonized by the resistant strain, and the staff in other wards were never found to carry it in their nares; indeed, only one of the nurses in the Burns Unit carried this strain (probably a strain acquired from the contaminated environment rather than the source of that contamination).

Although a few neomycin-sensitive staphylococci showing the phage inhibition pattern of the resistant strain have appeared, including one in a patient who also carried resistant strains, there was evidence that resistance to neomycin was stable. Patients tended to continue carrying resistant strains for prolonged periods (including periods while they were at home), and resistant strains preserved and subcultured in the laboratory kept their full resistance without exposure to neomycin.

Eleven years elapsed between the introduction of neomycin (Waksman & Lechevalier, 1949) and the description of resistant pathogenic staphylococci by Quie *et al.* (1960). In our unit there was a lag of 9 years between our first use of the

antibiotic in 1954 and the first resistant strains which we detected in 1963—a surprising lag in a burns unit, where the emergence of resistant strains is a notorious hazard. It would appear that a neomycin-resistant mutant capable of growing in human wounds or nares is a great rarity, but one which can rapidly spread in environments where the antibiotic is much used and where cross-infection occurs readily. In view of these hazards it is recommended that neomycin and the related antibiotics kanamycin and framycetin should be applied in combination with another unrelated agent in the nares or on wounds when used for prophylaxis, and that sensitivity tests should be made whenever neomycin-containing applications are used.

SUMMARY

Early in 1963 neomycin-resistant *Staph. aureus* appeared in the burns of patients in a burns unit; after a period of 7 weeks three-quarters of the strains of *Staph. aureus* isolated from patients in the unit were resistant to neomycin, and after 22 weeks almost half of the patients in the burns wards were carrying the organism on their burns. When treatment with neomycin and kanamycin was stopped in the Burns Unit, neomycin-resistant strains gradually diminished in numbers and were no longer found in the ward after 6 months.

The neomycin-resistant staphylococci appeared during controlled trials of local neomycin and systemic kanamycin, and were much more frequently isolated from the burns of patients treated with these antibiotics than from patients in the control series.

During the previous 9 years local neomycin application had been used on many patients; though all staphylococci were tested for sensitivity to neomycin for a considerable part of this time, no resistant staphylococci were found.

All the neomycin-resistant staphylococci showed a pattern of inhibition by phages 6, 47, 54 and 77, and many also by phages 7, 53, 75 and 75B at 1000 R.T.D. No neomycin-sensitive staphylococci with this phage pattern were found at the time when resistant strains first appeared (though two such strains were found later); it seemed likely, therefore, that the resistant strain was introduced from outside the hospital. Preliminary tests of habituation to neomycin of sensitive strains with the phage inhibition pattern are described.

Back mutation to sensitivity was not found in tests on neomycin-resistant staphylococci.

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