J. Hyg., Camb. (1965), **63**, 285
With 1 *plate*
Printed in Great Britain

Staphylococcus aureus strains associated with the hedgehog, *Erinaceus europaeus*

BY JOHN M. B. SMITH*
Department of Microbiology, Medical School, University of Otago, Dunedin, New Zealand

(*Received* 6 *October* 1964)

Early in 1963, Smith & Marples (1964) showed the short eared hedgehog, *Erinaceus europaeus*, to be a natural selector and propagator of penicillin resistant forms of *Staphylococcus aureus*. Until this time, no satisfactory answer to the 'pre-penicillin era' occurrence of penicillin resistant strains had been found. This paper is the full report of the examination of free-living hedgehogs in New Zealand for coagulase positive staphylococci. Hedgehogs appear to constitute a hitherto unrecognized reservoir of penicillin resistant strains of *Staph. aureus*.

MATERIALS AND METHODS

Fifty-nine hedgehogs were collected from urban parks and gardens in various areas of New Zealand. The skin of fifty-six, paws of fifty-seven and anus of eleven were sampled by means of a moistened swab. Nasal samples were obtained from fifty-eight of the animals using a small wire loop which was gently rotated in each nostril.

All swabs and samples were incubated in 10 % salt broth for 12–18 hr. and, after that, each tube of salt broth was inoculated on blood agar. The only swabs which were plated direct, without enrichment in salt broth, were from the skin of six hedgehogs showing obvious skin crusting. All plates were examined after suitable incubation and samples of each colonial type of staphylococci were subcultured on fresh media. After incubation, these subcultures were examined for coagulase production using the tube method.

All coagulase positive strains were phage typed using the international set of twenty-two human phages. Strains typing with phage 81 were listed as phage group I strains. Sensitivity or resistance to disks of penicillin (3 units) was determined. In each case, the Oxford strain of *Staph. aureus* known to be sensitive to 0·03 units of penicillin was included. Fifty of the strains selected at random were examined for resistance to disks of the following antibiotics; chloramphenicol, 20 μg.; streptomycin, 66 units; erythromycin, 20 μg.; and celbenin, 10 μg. The Oxford staphylococcus was used as a control in each experiment. The ability of forty strains to produce β-lysin was determined by stab inoculation on to washed sheep erythrocyte agar plates.

* Present address: Department of Animal Health, Faculty of Veterinary Science, Massey University of Manawatu, Palmerston North, New Zealand.

Table 1. *Phage type and penicillin resistance of* Staphylococcus aureus *strains recovered from hedgehogs*

	Phage type		No.	Penicillin resistance
Phage group I	81	1*	5	5
	52	1*	4	4
	52/52A/80	—	3	3
	52A/81 +	1*	2	2
	79 +	1*	1	1
	52/52A	—	1	1
	29/52/52A/80	—	1	1
		4	17	17
Phage group II	3A	1*	1	—
	3C	—	1	1
	3A/3B/3C/55/77	—	1	1
		1	3	2
Phage group III	47	2*	8	5
	7/47	5*	5	5
	42E/75	—	3	3
	6/7/42E/47 +	—	2	1
	7/47/53/54/75	1*	2	2
	6/47/53/75	1*	1	1
	6/42E/47/54/75	—	1	1
	47/77 +	—	1	1
	42E	—	1	1
	6 +	—	1	—
		9	25	20
Untypable			12	7
Not typed			6	6

	Phage type		No.	Penicillin resistance
Unclassifiable mixed groups	42E/7/81	—	6	6
	7/79	4*	4	4
	42D/42E/81	—	4	4
	42D/42E/75	—	1	1
	42D/75/77 +	3*	3	3
	42D/42E/6/79/81	—	2	2
	42D/47	—	1	1
	42D/54/75/77	1*	1	1
	42D/42E/6/7/47/54/75/77/80/81	—	2	—
	52/42E/7/81	—	1	1
	54/80	—	1	1
	42E/7/77/81	—	2	1
	42E/6/7/47/75/77/79	—	2	1
	42E/6/7/47/75/81	—	1	1
	42E/6/7/47/55/77/81	—	4	3
	42E/6/7/47/77/81	—	1	1
	42E/6/7/47/79/77/81 +	—	2	2
	42E/6/47/53/77/79	—	1	1
	42E/6/47/53/75/77/79	—	1	1
	42E/47/77/79 +	—	2	2
	29/42D/42E/79/81	—	2	2
	29/42D/42E/7/47/81	—	2	2
	29/42D/42E/6/7/47/79/80/81	—	4	4
	29/42E/6/7/47/53/75/77/79/81	—	1	1
	29/52A/42E/6/7/47/79/80/81	—	2	2
	29/52/52A/47/80	—	3	3
	29/42E/47	—	1	1
	29/47	—	2	1
	29/42D/3A/3C/42E/6/7/47/54/79/81 +	—	1	1
	29/52/52A/42D/3A/3C/42E/6/7/47/54/79/80/81	—	1	1
		8	61	55

* Indicates typed at RTD.

The sex, weight, and presence of skin parasites *Caparinia tripilis* and *Trichophyton mentagrophytes* var. *erinacei*, was recorded for each animal.

RESULTS

Incidence of Staphylococcus aureus

Fifty (85 %) of the fifty-nine hedgehogs yielded strains of *Staph. aureus*, from either the paws, skin, nasal cavity or anus. The phage types of the strains at RTD and 1000 × RTD are shown in Table 1. Altogether 124 strains were obtained. A summary of the isolations is shown in Table 2. Skin carriage was more frequent than carriage on other sites.

Table 2. *Isolation of* Staphylococcus aureus *from hedgehogs*

Area of animal	No. examined	No. positive	% positive	No. strains
Nasal	58	23	40	27
Skin	56	38	68	44
Paw	57	36	63	45
Anal	11	6	55	8

Table 3. *Phage groups of* Staphylococcus aureus *recovered from hedgehogs in various areas of New Zealand*

Area	Phage group I	II	III	Unclassified	Untyped	Not typed
Dunedin (Nov. 1962/Mar. 1963)	11	1	1	12	2	3
Dunedin (Nov. 1963/Jan. 1964)	4	1	5	11	3	3
Upper Hutt (Jan. 1963)	—	1	14	18	2	—
Hamilton (Feb. 1964)	1	—	3	18	4	—
Other areas (1962–64)	1	—	2	2	1	—

Phage groups obtained

Phage typing was attempted on 118 of the 124 strains. Seventeen were of phage group I, 3 of phage group II, 25 of phage group III, 61 were typable but unclassifiable into any of the phage groups, and 12 were untypable. At RTD, 96 of the 118 strains were untypable and of these ninety-six, 84 typed at 1000 × RTD.

No predominance of phage groups in any body area was apparent.

Geographical distribution

Shown in Table 3 are the phage groups of staphylococci recovered from hedgehogs collected in various areas of New Zealand. Although ungroupable phage types predominated in each area, phage group I types were relatively common in the early Dunedin groupable strains, while phage group III staphylococci were the main classifiable types recovered from Upper Hutt.

Sex distribution

Male hedgehogs more frequently harboured pathogenic staphylococci than did females. If the number of strains recovered from each sex is compared with the number of body areas sampled, pathogenic staphylococci were recovered from 69·2 % male areas and only 40·0 % female areas. Phage groups were equally spread amongst the two sexes.

Age distribution

All ages of hedgehogs were found infected with staphylococci. Animals were divided into two groups depending on weight: 'young' hedgehogs which weighed less than 500 g. and 'old' hedgehogs which weighed more than 500 g. These represented ages of up to 6 months in the case of 'young 'animals, and between 9 and 18 months for 'old' animals. Few hedgehogs in New Zealand live beyond 18 months (Brockie, 1958). Table 4 reveals that both 'young' and 'old' hedgehogs were colonized by coagulase positive staphylococci to the same extent.

Table 4. *Isolation of* Staphylococcus aureus *from young and old hedgehogs*

	Nasal		Skin		Paw		Anal	
Age	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive
Young	23	8	23	17	24	15	6	4
Old	26	11	24	17	24	15	5	2

Table 5. *Distribution of mites and* Staphylococcus aureus *on the skin of forty-three hedgehogs*

Normal animals			Slightly scabby animals			Very scabby animals		
No. examined	Staph. aureus	Mites	No. examined	Staph. aureus	Mites	No. examined	Staph. aureus	Mites
17	10	1	14	11	5	12	12	9

Effect of parasitic mites

Hedgehogs in New Zealand are commonly infected with the acarine mite *Caparinia tripilis* (Brockie, 1958). How these parasites influence the skin microflora is difficult to assess. Coagulase positive staphylococci were found on both normal and scabby looking animals. Mites on the other hand appeared to be correlated directly with skin scabbiness and were usually absent from normal animals. These points are shown in Table 5.

Staph. aureus strains could easily be recovered from scabby animals. Direct plating of swabs from the six hedgehogs with obvious skin scaling resulted in almost pure growths of *Staph. aureus*. Enrichment in salt broth was required to recover staphylococci from 'normal' animals. Thus the presence of mites did appear to favour the increased multiplication of staphylococci. Plate 1 is a picture of the snout and ears of a wild hedgehog naturally infected with mites, *T. mentagrophytes* var. *erinacei*, and pathogenic staphylococci.

Effect of fungi

Hedgehogs in New Zealand are also heavily infected with the dermatophyte *T. mentagrophytes* var. *erinacei* (Smith & Marples, 1963). The presence of this fungus in the skin of the animals did not appear to influence the distribution of coagulase positive staphylococci. Of thirty-three hedgehogs suffering from ringworm twenty-seven (82 %) also harboured staphylococci, while twenty-one (81 %) of twenty-six fungus-free animals were colonized by these bacteria.

Antibiotic sensitivities

Of the 124 coagulase positive strains isolated, 107 (86·3 %) were resistant to penicillin G (3 units). All fifty strains examined were sensitive to streptomycin, chloramphenicol, tetracycline, erythromycin and celbenin.

β-lysin production

Of the forty strains examined for β-lysin production, 33 (83 %) were positive.

DISCUSSION

It appears that hedgehogs are very suitable hosts for coagulase positive staphylococci. Unfortunately it is difficult to compare the distribution of staphylococci in hedgehogs with that occurring in other animals, for, apart from man, surveys on the normal animal incidence of pathogenic staphylococci have been largely neglected. Hedgehogs carry coagulase positive staphylococci on the skin much more frequently than is found in man, for these organisms were recovered from the skin of 68 % and paws of 63 % hedgehogs. The corresponding normal human figures are 16 % for skin and 40 % hands (Williams, 1963). Nasal carriage in hedgehogs and man is at approximately the same level (40 %). It would seem that in the hedgehog the skin is the primary site of staphylococcal multiplication.

The growth of dermatophytes in animal skin provides an ideal environment for the colonization and rapid multiplication of *Staph. aureus* (Smith, unpublished). Presumably the high incidence of *T. mentagrophytes* var. *erinacei* in New Zealand hedgehogs is partly responsible for the high incidence of staphylococci on the skin. The acarine mite, *Caparinia tripilis*, may also assist in the establishment of coagulase positive staphylococci by damaging the skin surface. Sections of intact mites revealed the presence of numerous micrococci. These parasites could be responsible for the spread of *Staph. aureus* between individual hedgehogs.

Hedgehog strains of *Staph. aureus* appeared to be relatively susceptible to human staphylophages, only twelve of 118 strains being untypable with a set of twenty-two human phages. However, only 19 % of the strains were typable at RTD, the majority typing satisfactorily only at 1000 × RTD. Cattle and other animal strains do not type satisfactorily with human phages (Marshall, 1964). This worker found only about 60 % of his strains to be typable with human phages. As forty-five of the 106 typable hedgehog strains were groupable into recognized human groups, and as animal strains of staphylococci have been shown to be capable of infecting humans (Moeller, Smith, Shoemaker & Tjalma, 1963), it is reasonable to assume that hedgehogs may provide a reservoir for human infections. Phage types 52, 52/52 A, 80 and 81 or combinations of these types represented 16 % of the typable strains. Phage group III made up 24 % of the typable strains with types 47 and 7/47 most prominent. While many of the strains were groupable into human phage groups the majority of these were of animal origin, for they produced β-lysin. In general the finding of β-lysin producing strains in routine material is about ten times more likely if the strain originated from animal sources (Elek & Levy, 1950).

The presence of one phage type in hedgehogs did not appear to inhibit the carriage of a second type. This is contrary to the human results reported by Williams (1963), who found that prior infection with one strain excluded the acquisition of other strains. However, the carriage of one phage group in hedgehogs did influence the presence of other groups (see Table 3).

The outstanding feature of hedgehog *Staph. aureus* strains was the high percentage (86·3 %) resistant to penicillin and only penicillin. This resistance was due to penicillinase production as all strains were sensitive to celbenin. Penicillin resistance in human pathogenic staphylococci is often coupled with resistance to other antibiotics (Wentworth, 1963). The normal incidence of penicillin-resistant types in the *Staph. aureus* population of man is only about 25 % (Rountree & Rheuben, 1956).

In staphylococcal strains from man, penicillin resistance appears to favour certain phage types (Parker, 1958). This was not the case with hedgehog strains where antibiotic resistance was spread equally throughout phage groups I, II and III and unclassifiable and untypable strains.

The hedgehog in New Zealand provides a hitherto unrecognized reservoir of penicillin-resistant strains of *Staph. aureus*. Further work is required to determine to what extent these organisms may be transmitted to the human population.

SUMMARY

1. Strains of *Staphylococcus aureus* were obtained from the nostrils of twenty-three of fifty-eight hedgehogs; the skin of thirty-eight of fifty-six hedgehogs; the paws of thirty-six of fifty-seven hedgehogs and the anus of six of eleven hedgehogs.

2. Of 118 strains, 106 (90 %) were typable with human staphylophages. Seventeen were phage group I, three phage group II, twenty-five phage group III, sixty-one were typable but unclassifiable into groups, and twelve were untypable.

3. Male hedgehogs were more heavily infected than females, while all ages of hedgehogs appeared equally susceptible to infection.

4. Of the 124 coagulase positive strains obtained, 107 (86·3 %) were resistant to penicillin. Resistance to other antibiotics—chloramphenicol, streptomycin, tetracycline, erythromycin, celbenin—was not encountered.

5. Thirty-three (83 %) of forty strains produced β-lysin.

6. Mites (*Caparinia tripilis*) and fungi (*Trichophyton mentagrophytes* var. *erinacei*) did not appear to directly influence the carriage of *Staphylococcus aureus* on the hedgehog skin.

These investigations were carried out in the Microbiology Department, Medical School, University of Otago, Dunedin, New Zealand. I am indebted to Associate Prof. Mary J. Marples, Microbiology Department, for her continued help and encouragement during these investigations, and to Prof. J. A. R. Miles, Microbiology Department, for providing research facilities. Financial assistance given by the Medical Research Council of New Zealand is gratefully acknowledged. I would also like to thank Mrs Jean Markham for phage typing the staphylococcal strains.

REFERENCES

BROCKIE, R. E. (1958). *The Ecology of* Erinaceus europaeus, *Linn. in Wellington, New Zealand.* M.Sc. Thesis, Victoria University of Wellington, New Zealand.

ELEK, S. D. & LEVY, E. (1950). Distribution of haemolysins in pathogenic and non-pathogenic staphylococci. *J. Path. Bact.* **62**, 541.

MARSHALL, R. B. (1964). Staphylococci from dairy cattle. *N.Z. vet. J.* **12**, 57.

MOELLER, R. W., SMITH, I. M., SHOEMAKER, A. C. & TJALMA, R. A. (1963). Transfer of hospital staphylococci from man to farm animals. *J. Amer. vet. med. Ass.* **142**, 613.

PARKER, M. T. (1958). Some cultural characteristics of *Staphylococcus aureus* strains from superficial skin lesions. *J. Hyg., Camb.*, **56**, 238.

ROUNTREE, P. M. & RHEUBEN, J. (1956). Penicillin-resistant staphylococci in the general population. *Med. J. Aust.* i, 399.

SMITH, J. M. B. & MARPLES, M. J. (1963). *Trichophyton mentagrophytes* var. *erinacei. Sabouraudia*, **3**, 1.

SMITH, J. M. B. & MARPLES, M. J. (1964). A natural reservoir of penicillin-resistant strains of *Staphylococcus aureus. Nature, Lond.*, **201**, 844.

WENTWORTH, B. B. (1963). Bacteriophage typing of Staphylococci. *Bact. Rev.* **27**, 253.

WILLIAMS, R. E. O. (1963). Healthy carriage of *Staphylococcus aureus*: its prevelance and importance. *Bact. Rev.* **27**, 56.

EXPLANATION OF PLATE

Ears and snout of a hedgehog naturally infected with *T. mentagrophytes* var. *erinacei, Caparinia tripilis*, and *Staphylococcus aureus.*