J. clin. Path., 1972, **25**, 713-715

Effect of antibiotic resistance on the survival of *Staphylococcus aureus*

R. W. LACEY

From the Department of Bacteriology, University of Bristol

SYNOPSIS The survival of strains of *Staphylococcus aureus* on glass at 30°C, 37°C, and room temperature was compared with derivatives of them that had either lost or gained naturally occurring antibiotic resistance. In other properties the sets of strains were identical. Neither loss nor gain of antibiotic resistance (methicillin, penicillinase, streptomycin, tetracycline, erythromycin, neomycin) altered survival.

Multiresistant strains of *Staphylococcus aureus* recently isolated from hospital sources give bacteriophage typing patterns predominantly in group III (eg, Jevons, John, and Parker, 1966; Willis, Smith, and O'Connor, 1966; Jessen, Rosendal, Bulow, Faber, and Eriksen, 1969). Group III strains survive less well than group I and II strains on both skin and glass (Lacey, Alder, and Gillespie, 1970) and this could be a factor why the multiresistant group III strains have not become more widespread outside hospitals (Goldie, Alder, and Gillespie, 1971). It has not been established whether it is the antibiotic resistance or the phage type that is essentially associated with poor survival. Antibiotic resistance could alter the survival of an organism—other than in withstanding the effect of the antibiotic—in several ways. For example, the biochemical resistance mechanism itself or genes linked to those conferring resistance could affect survival.

In order to determine whether antibiotic resistance had such an effect, the survival on glass of wild strains was compared with derivatives from them that had either lost or gained resistance to one of several antibiotics. Only naturally occurring resistances were investigated.

Materials and Methods

STRAINS

Strains nos. 13136, 13137, 2273, 9463, and 11164 are naturally occurring, methicillin-resistant strains obtained from the Cross-Infection Reference Laboratory, Colindale. Strains nos. 609, 6936, 7024, and 7081 were recently isolated from Bristol hospitals.

Received for publication 25 May 1972.

LOSS OF ANTIBIOTIC RESISTANCE

The antibiotic-sensitive derivatives were isolated on storage and resembled the wild strains in phage typing pattern, sensitivity to other antibiotics, haemolysin production, Tween 80 reaction, and pigmentation; the only associated loss was resistance to mercury and cadmium with penicillinase production (for methods see Lacey, 1971).

CONSTRUCTION OF MULTIRESISTANT STRAINS

Antibiotic resistances were transduced from various donor organisms after mitomycin C induction (Lacey, 1971). None of the resistant recipients was lysogenic for the transducing phage, and each resembled the corresponding wild strains in all the above properties.

Survival on glass

This was determined at 30°C and 37°C over six hr and at room temperature over 24 hr by the method described previously (Lacey *et al*, 1970). In some experiments, instead of using inocula containing mixtures of three strains, strains were inoculated singly (about 10^4 cocci spread over areas of glass microscope slides 40 × 24 mm), including a standard strain. The glass slides were sampled by irrigating each for 30 sec with 5 ml nutrient broth in a petri dish and performing counts on this broth using milk agar as recovery medium. Counts were expressed as the percentage survival of the test strain relative to the standard. The survivals were similar whether single or mixed inocula were used and this excluded the remote possibility that there were strain interactions on the glass. Mixed inocula were therefore used subsequently. No attempt was made to control the relative humidity. Survival for

times longer than six hr at 30°C and 37°C was not tested because few organisms survived beyond this time.

Results

REPRODUCIBILITY OF RESULTS

Preliminary experiments showed that when two strains (no. 11164 and the standard) were tested daily for survival over six hr at 37°C there was considerable variation. The relative survival of strain no. 11164 varied from 1·08 to 19·7%. However the survival figures of replicate experiments performed concurrently showed good agreement (2·5, 1·9, 2·7, 3·3, 2·8, and 2·4%). In all subsequent experiments the survival of wild strains was always investigated simultaneously with that of their sensitive or resistant derivatives.

EFFECT OF LOSS OF ANTIBIOTIC RESISTANCE ON THE SURVIVAL OF *STAPH. AUREUS*

Many of the wild strains had markedly different survivals to that of our standard. For example, the relative survivals of strain no. 11164 were 2·6,

Strain No.	*Antibiotic Resistance Lost*	*Percentage Survival Relative to a Standard Strain at*		
		37°C (6 hr)	*30°C (6 hr)*	*Room Temperature (24 hr)*
	None	86	142	124
13136	Methicillin	92	151	135
	Penicillinase	103	115	150
	Tetracycline	72	127	137
	None	94	147	127
13137	Methicillin	71	131	127
	Penicillinase	65	110	160
	Tetracycline	87	118	144
	None	2·6	6	11
11164	Methicillin	3·1	5	9
	Erythromycin	2·0	10	8
	None	43	94	72
	Methicillin	58	53	79
2273	Pennicillinase	65	72	102
	Erythromycin	69	80	85
	Tetracycline	38	61	95
	None	147	140	183
9463	Methicillin	150	196	204
	Penicillinase	195	192	152
	Tetracycline	167	188	195
	None	84	51	96
609	Penicillinase	66	63	59
	Neomycin	98	72	65
7024	None	41	31	64
	Neomycin	25	24	79
7081	None	53	62	49
	Neomycin	56	39	60

Table I *Effect of loss of resistance to five antibiotics on the survival of* Staph. aureus *on glass*

Strain No. of Wild Strain (Overall Resistance[1]*)*	*Strain No. of Donor from which Resistance Gene Transduced*	*Genetic Locus of Resistance Acquired*	*Percentage Survival Relative to Standard Strain at*		
			37°C	*30°C*	*Room Temperature*
609 (PN)	—	—	81	63	71
609 (PNE)	2273 (E)	Plasmid	67	64	79
609(PTNE)	13136(T)	Plasmid	92	85	96
609(PSTNE)	13136(S)	Chromosomal	96	55	61
6936	—	—	88	120	136
6936(P)	13136(P)	Plasmid	104	138	125
6936(PS)	2273(S)	Chromosomal	112	144	161
6936(PSN)	609(N)	Plasmid	74	112	126
6936(PTSN)	11164(T)	Plasmid	65	137	119
6936(PTSNE)	11164(E)	Plasmid	93	105	148

Table II *Effect of acquisition of antibiotic resistance on the survival of* Staph. aureus *on glass*

[1]P = penicillinase producer, N = resistance to neomycin, E = resistance to erythromycin, T = resistance to tetracycline, S = resistance to streptomycin.

6, and 11% at 37°C, 30°C, and room temperature respectively. In contrast the survivals of strain no. 9463 were 147, 140, and 183%. But there was no change in survival associated with antibiotic resistance loss (Table I).

EFFECT OF ACQUISITION OF ANTIBIOTIC RESISTANCE ON THE SURVIVAL OF *STAPH. AUREUS*

Results in Table II show that survival was not altered with acquisition of antibiotic resistance. The multiresistant derivative of strain no. 6936 (6936 PTSNE) possesses genetic material considerably different from strain no. 6936 wild, having acquired four plasmids and one chromosomal gene. Yet the survivals of 6936 PTSNE are unchanged—93, 105, and 148% compared with 88, 120, and 136% of the wild strain.

Discussion

Comparisons have been made between strains similar except for gain or loss of naturally occurring antibiotic resistance. The results indicate that antibiotic resistance in itself has no effect on the survival of the staphylococcus at 37°C, 30°C, or room temperature. Since the survival of a staphylococcus is similar on glass and on exposed skin (Lacey *et al*, 1970) it is probable that these findings also relate to the survival on skin.

Phage group III staphylococci therefore possess two independent properties, an ability to acquire antibiotic resistance and poor survival. The latter may be a factor confining multiresistant group III strains to hospitals where antibiotics are used intensively, whilst strains of variable phage-typing patterns which are resistant to few antibiotics predominate outside hospitals. Other factors that may also favour this division are the more rapid disappearance of group III strains from nasal carriers (Thompson and Gillespie, 1958) and the spontaneous loss of plasmid-borne genes from group III strains.

References

Goldie, D. J., Alder, V. G., and Gillespie, W. A. (1971). Changes in the drug resistance of *Staphylococcus aureus* in a non-hospital population during a 20-year period. *J. clin. Path.*, **24**, 44-46.

Jessen, O., Rosendal, K., Bülow, P., Faber, V., and Eriksen, K. R. (1969). Changing staphylococci and staphylococcal infections: a ten-year study of bacteria and cases of bacteremia. *New Engl. J. Med.*, **281**, 627-635.

Jevons, M. P., John, M., and Parker, M. T. (1966). Cultural characters of a newly recognized group of hospital staphylococci. *J. clin. Path.*, **19**, 305-312.

Lacey, R. W. (1971). Transfer of tetracycline-resistance between strains of *Staphylococcus aureus* in mixed cultures. *J. gen. Microbiol.*, **69**, 229-237.

Lacey, R. W., Alder, V. G., and Gillespie, W. A. (1970). The survival of *Staphylococcus aureus* on human skin: an investigation using mixed cultures. *Brit. J. exp. Path.*, **51**, 305-313.

Thompson, M. E. M., and Gillespie, W. A. (1958). Nasal carriage of *Staphylococcus aureus* by nurses. *J. Path. Bact.*, **75**, 351-355.

Willis, A. T., Smith, J. A., and O'Connor, J. J. (1966). Properties. of some epidemic strains of *Staphylococcus aureus*. *J. Path. Bact.*, **92**, 345-358.