

Coagulase-positive Staphylococcus aureus have poor survival times in coolant oils.

SURVIVAL OF COAGULASE-POSITIVE STAPHYLOCOCCI IN SOLUBLE CUTTING OILS

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INTRODUCTION

ALTHOUGH there is sufficient evidence of industrial dermatitis associated with excessive contact with soluble cutting oils, there is disagreement as to the role played by the pyogenic staphylococci in this process^{1, 2}. Unquestionably, healthy skin is not as susceptible to infection as skin continually exposed to the relatively high pH of soluble oils; not only are protective oils removed at this pH but also surface epithelial cells, leaving a much denuded epidermis. Since there is ample and continual inoculation from respiratory discharge as well as skin, one could expect a somewhat higher and clear-cut staphylococcal involvement in coolant dermatitis. Bennett and Wheeler³ did find large numbers of *Staphylococcus aureus* in cutting fluids handled by workers suffering from pyogenic infections, but they were unable to isolate this organism from samples one week after collection. The following study, using presently accepted profiles, was done to find the incidence of potentially pathogenic staphylococci and to evaluate their survival rate in soluble oil.

MATERIALS AND METHODS

One-gallon coolant samples were collected weekly for 12 consecutive weeks from 6 individual machining operations each of which contained approximately 300 gallons of a 4 percent heavy duty soluble oil in water. Staphylococcal medium 110 (BBL) plus egg yolk⁴ (called hereafter EYA) was found by Jay⁵ to

be superior to five other selective media for staphylococci. In a preliminary experiment in which coolant samples were seeded with coagulase-positive staphylococci and *Pseudomonas oleovorans*, we were able to differentiate and recover almost 100 percent of the seed using EYA. However, first attempts to isolate coagulase-positive staphylococci directly from used coolant were negative. Subsequently, isolations were made from pellets of 250-ml samples centrifuged at 3,000 rpm for 30 min. This rate of centrifugation had been found to sediment 95 percent of a known culture in a previous study. The pellets were resuspended in 5 ml 0.1M phosphate buffer, pH 7.4, three 1-ml aliquots being poured with EYA; plates were incubated at 37° C in CO₂ enriched atmosphere and examined after 18–24 hr. Colonies surrounded by a zone of precipitation were considered presumptively coagulase-positive and were subcultured onto Trypticase Soy Agar (TSA–BBL) for characterization and further study. The coolant isolates were examined in parallel with a coagulase-positive phage type 81 *S. aureus* recovered from a wound infection.

Characterization of *S. aureus* isolates. Following routine Gram staining, cultures were transferred 3 times in Brain Heart Infusion (Difco) and 0.5 ml of culture supernatant was added to 1 ml of Diagnostic Plasma (Warner-Chilcott) for coagulase determinations. A strain was labeled strongly positive if it coagulated plasma within 4 hr at 37° C and weakly positive if coagulation occurred within 14 hr. Mannitol fermentation

was detected in Phenol Red Mannitol Broth (Difco) after 24 and 48 hr incubation. Gelatinase production was determined by a modified Frazier method⁶. Hemolysis was detected in Tryptone Blood Agar (Difco) with 2 percent sheep, rabbit and human blood after incubation for 24 hr and examined again after 12 hr at 4° C. Pigmentation was assessed on SM 110 after incubation at room temperature for 48 hr and graded as golden, weakly golden or white. For the determination of antibiotic sensitivity 18–24-hr Trypticase Soy Broth (TSB–BBL) cultures were streaked on 2 percent blood agar. Anti-microbial discs (BBL) were added to the dried, inoculated plates which were then incubated 18 hr at 37° C. The method of Blair and Carr⁷ for phage typing of staphylococci was carried out on 12-hr

cultures from TSB. Results were read after 18–24 hr at 37° C.

Determination of survival. Five-ml aliquots of 24-hr TSB cultures of each isolate were added to 500 ml Erlenmeyer flasks containing 250 ml of one of the following: sterile Krebs–Ringer phosphate (pH 7.4), used coolant or unused coolant. The coolants were a 1:25 mixture of a heavy duty soluble oil with tap water. The flasks were maintained 2 wk at room temperature with agitation during which time plate counts were made on EYA at indicated intervals.

RESULTS

Although over 70 separate samples were examined, only 3 colonies were isolated that initially produced precipitate zones on EYA. These were from 2 of

Table 1—Characteristics of *Staphylococcus aureus* Isolated from Hospital and Plant Machinists *

Determinations	Concentration	Source			
		Hospital Strain	Machine Strain #1	Machine Strain #2	Machine Strain #3
Gantrissin	0.25 mg	R	R	R	S
Penicillin	2 units	R	R	S	S
Neomycin	5 mcg	S	S	S	S
Chloramphenicol	5 mcg	S	S	R	S
Streptomycin	2 mcg	R	R	S	S
Demethylchlortetracycline	5 mg	S	R	S	S
Chlortetracycline	5 mcg	R	R	S	S
Erythromycin	2 mcg	S	S	S	S
Oleandomycin	2 mcg	S	S	S	S
Oxytetracycline	5 mcg	R	R	S	S
Triacetyloleandomycin	16 mcg	S	S	S	S
Novobiocin	30 mcg	S	S	S	S
Prostaphlin **	1 mcg	S	S	S	S
Staphcillin **	5 mcg	S	S	S	S
Phage type		81	non-typable	non-typable	
Coagulase		positive	positive	variable	negative
Gram stain		positive	positive	positive	positive
Mannitol fermentation		positive	positive	positive	positive
Egg yolk agar		white halo	white halo	white halo	no halo
Gelatinase		positive	positive	negative	negative
Hemolysin—rabbit		positive	positive	negative	negative
Hemolysin—sheep ***		negative	positive	negative	negative
Hemolysin—human		negative	negative	negative	negative
Pigmentation		golden	golden	weakly gold	golden

* Symbols used: R—resistant; S—sensitive, yellow halo.

** Bristol laboratories.

*** No change in hemolysis occurred with sheep cells after refrigeration.

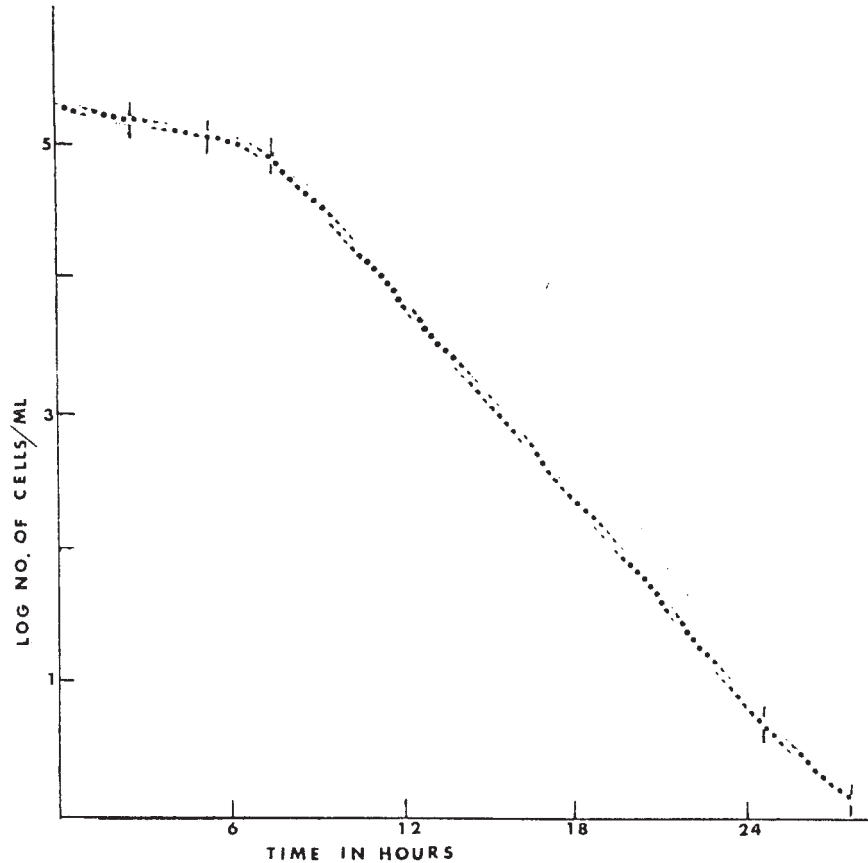


Figure 1—Survival rate of coagulase-positive *Staphylococcus aureus* type 81 in used coolant.

the 6 machines studied. In fact, 2 isolates were from a machine treated with a mixed quaternary ammonium germicide. On subsequent transfer to EYA, one of these isolates no longer produced a precipitate. Table 1 summarizes the characteristics of the 4 (3 coolant and 1 hospital) isolates. It is worth noticing that the frankly coagulase-positive industrial strain has almost the identical profile of the type 81 hospital organism and that the coagulase-negative organism seemed susceptible to all the agents employed.

The survival patterns of all 4 strains in used and unused coolant seemed related to coagulase-positivity; the more positive, the shorter the survival time (Figures 1-4). The hospital strain (Figure 1) lasted only 30 hr in contrast to the industrial coagulase-positive strain

which lingered 8 da. There were less striking differences among the industrial strains. In addition, survival in used coolant was slightly less than for unused coolant. Table 2 summarizes the pH's at the beginning and end of each 2-week experiment as well as the total populations of the used coolants. It can be seen that the pH of fresh coolant is slightly higher and that this factor does not appear to be responsible for the difference noted in staphylococcal survival.

DISCUSSION

In isolating coagulase-positive *S. aureus* from cutting oils it is necessary to contend with as many as 10^9 competing organisms/ml. DiGiacinto and

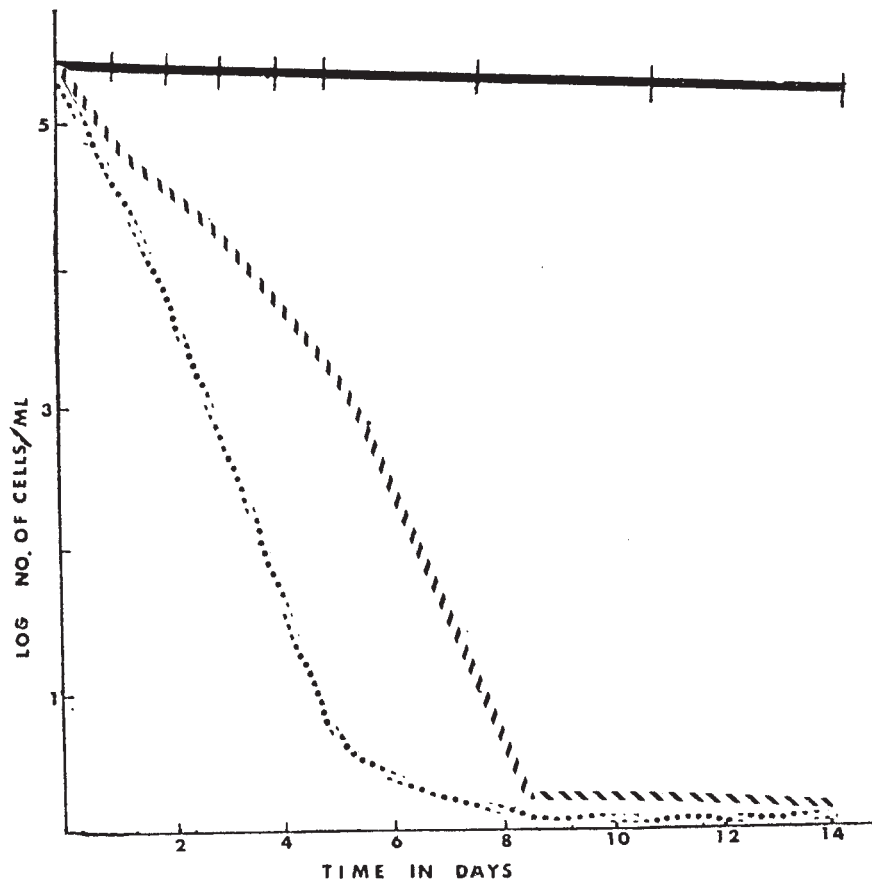


Figure 2—Survival rate of coagulase-positive *Staphylococcus aureus* (untypable) in used coolant (dots), unused coolant (stripes) and buffered (pH 7.4) salt solution (solid line).

Frazier⁸ recently reported on the effect of competing Gram-negative species on the growth of *S. aureus* and found that with ratios of Gram-negatives to *S. aureus* of 100:1 in the initial inoculum, the latter never reached a previously selected end-

point of 5×10^6 /ml. For the survival study in used coolant the same initial ratio was used. It is possible that growth products from these competing organisms were a factor in decreasing survival times of *S. aureus*. However, this fact

Table 2—Total Plate Count and pH at the Beginning and End of Survival Experiments

Figure	Coolant	pH		Total Plate Count	
		Start	Finish	Start	Finish
Fig. 1	Used	8.75	7.75	3.6×10^7	3.8×10^7
Fig. 2	Used	8.35	7.85	3.0×10^7	1.0×10^8
	Unused	9.0	8.3	0	0
Fig. 3	Used	8.35	7.85	2.8×10^7	3.0×10^7
	Unused	9.0	8.7	0	0
Fig. 4	Used	8.35	7.80	2.8×10^7	7.4×10^7
	Unused	9.0	8.3	0	4.5×10^4

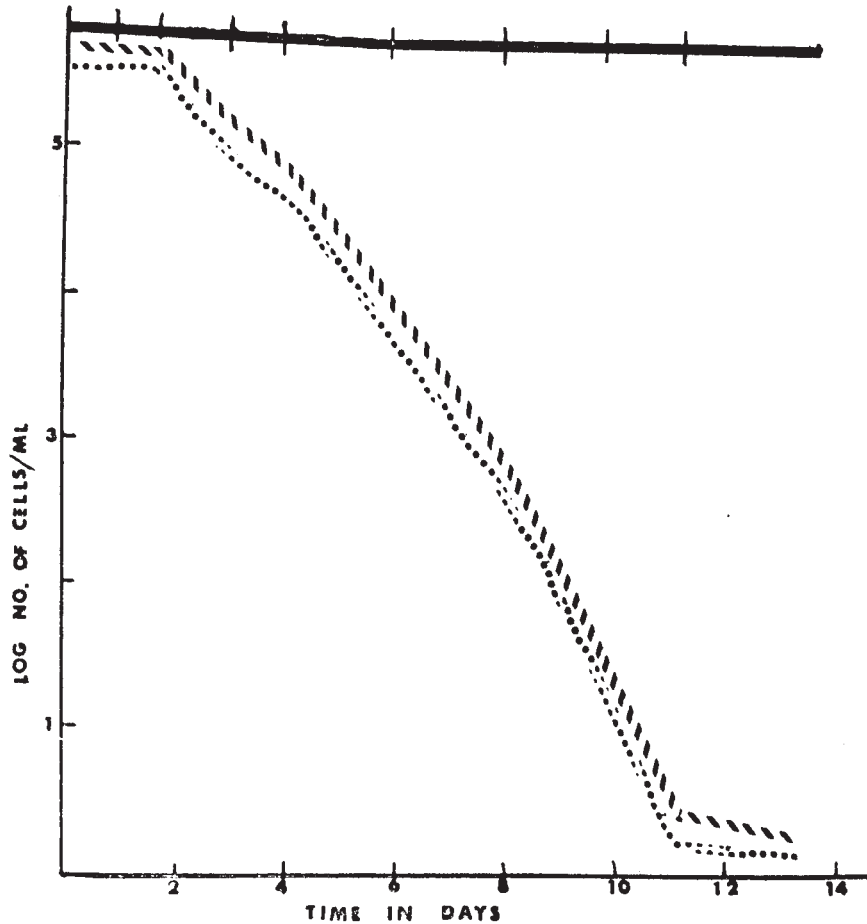


Figure 3—Survival rate of coagulase-variable *Staphylococcus aureus* (untypable) in used coolant (dots), unused coolant (stripes) and buffered salt solution (solid line).

does not explain the somewhat similar results in unused coolant. Bennett and Wheeler³ have found that Gram-positive organisms generally survive poorly in cutting fluids. One possible reason for their rather rapid demise could be the relatively high ambient pH. From the low incidence of coagulase-positive isolations and their subsequent poor survival, it can be assumed that isolation must follow closely the inoculation of the coolant system itself. Although weekly collections were made from each machine, it was never possible to recover isolates the week following the original finding. There was no way to associate the few coagulase-positive isolations with any clinical condition since plant

dispensary records did not show any worker infection related to the machines being studied, and it was not permissible to interrogate the machinists at any time. At any rate, the public health hazard from coolant containing coagulase-positive *S. aureus* is probably minimal since these organisms seem to have the poorest survival time among those studied.

ACKNOWLEDGMENT

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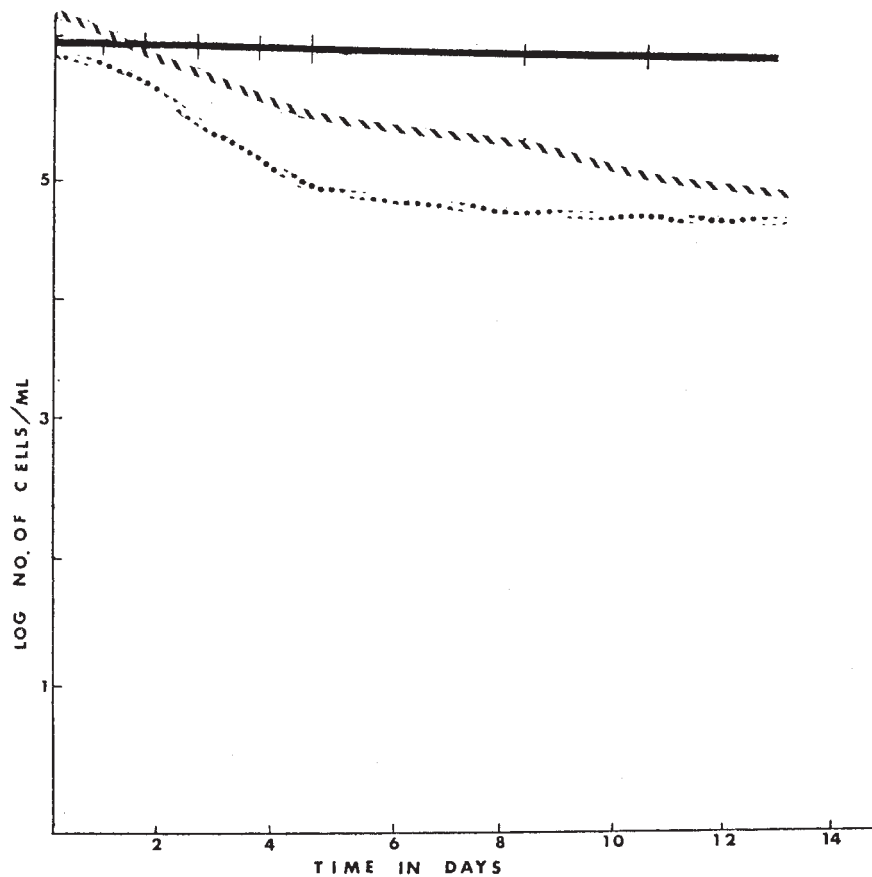


Figure 4—Survival rate of coagulase-negative *Staphylococcus aureus* in used coolant (dots), unused coolant (stripes) and buffered salt solution (solid line).

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