CONTAMINATION OF CETRIMIDE AND OTHER FLUIDS WITH *PSEUDOMONAS PYOCYANEA*

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When wounds which can be adequately covered acquire bacterial infection in spite of a strict "no-touch" dressing technique (M.R.C. War Memo. No. 6, 1941; Clayton-Cooper and Williams, 1945), the sterility of materials applied during the dressing falls under suspicion. The usual methods of sterilizing gauze, cotton wool, bandages, and surgical instruments are effective if properly carried out, so it is more probable that a fluid or cream applied locally or used for holding sterile instruments might be conveying the infective agent. Soap solution, cetrimide, "dettol" and calamine lotion are commonly dispensed in large corked or stoppered bottles which are sometimes not replaced for many days, and during that time they are frequently opened. Contamination of such bottles is likely, and the survival or multiplication of some coliforms and especially of *Pseudomonas pyocyanea* is reported to be possible even in solutions of cetrimide (Williams, Clayton-Cooper, Faulkner, and Thomas, 1944).

It is common both in factory surgeries and in hospitals for injuries to be cleansed with soap solution or cetrimide and for Cheattle's forceps to be kept standing in dilute "dettol". The presence of added infection with *Ps. pyocyanea* in a number of adequately covered wounds prompted the sampling of various fluids and creams.

Isolation of *Ps. pyocyanea* from Hospital Fluids

Absorbent cotton wool swabs were dipped into bottles of 1% cetrimide solution, 2% soap solution, calamine lotion, 10% "dettol", penicillin cream, and a number of other fluids used in hospital dressing stations and operating theatres. Samples were taken from bottles which were actually in use, some of them containing only a remnant of fluid.

Swabs were inoculated on plates of a selective medium for *Ps. pyocyanea* (0.1% cetrimide agar; Lowbury, 1951) and in nutrient broth. After 24 hours' incubation at 37°C. the broth was subcultured to cetrimide agar plates. All plates were examined after 24 hours' and 48 hours' incubation at 37°C., and *Ps. pyocyanea* was

recognized by detecting green pigment and characteristic fluorescence under an ultra-violet lamp with a dark glass filter.

The results of sampling were as follows:

Fluid	No. of Bottles Sampled	<i>Ps. pyocyanea</i> Present
Soap solution . . .	18	18
1% cetrimide solution	23	6
Calamine lotion . .	14	10
10% "dettol" . . .	9	3
Penicillin cream . .	12	0
Penicillin jelly . .	5	0
Miscellaneous fluids (including sterile saline, water, eusol, sodium sulphate, flavine, collodion) . .	88	0

The bottles from which *Ps. pyocyanea* was isolated had corks. A separate sampling from the cork and inside of neck and from the cetrimide solution of 19 of the bottles sampled showed a higher proportion of positives from the cork (6/19) than from the cetrimide solution (3/23). *Ps. pyocyanea* was never isolated from the fluid when it was not also found on the cork. Moreover, in three of the bottles from which the organism was isolated, the load of contamination of the cork appeared to be heavier than that of the fluid.

Replacement of Corked by Sterile Screw-capped Bottles

It is probable that contamination of these fluids occurred through handling of the bottles rather than by deposition from the air. Moreover, once the organism has been introduced it is likely to remain when the bottle is refilled and might possibly contaminate other materials in the dispensary.

To prevent contamination and maintain sterility of these fluids, it seemed desirable and, perhaps, sufficient to dispense them (a) in small bottles, which would be replaced daily; (b) sterile, or at least in sterile bottles; and (c) with covers which

would overlap the necks of the bottles and protect them from handling. In practice it was found convenient to use screw-cap bottles of 100 ml. to 1,000 ml. capacity according to the amount of fluid used in a day. Cetrimide, soap, and "dettol" solutions were autoclaved in bottles at 10 lb. pressure for one hour, the caps being lightly screwed on. Calamine lotion was dispensed in sterile screw-cap bottles.

A second sampling of cetrimide, soap solution, and calamine lotion was carried out after all the stoppered bottles of these fluids in the hospital had been replaced by clean supplies in screw-cap bottles. The results were as follows:

Fluid	No. of Bottles Sampled	<i>Ps. pyocyanea</i> Present
1% cetrimide solution	17	0
Soap solution ..	17	0
Calamine lotion ..	12	0

Incidence of *Ps. pyocyanea* Infections before and after Issue of Screw-cap Bottles

The issue to all wards and to the operating theatres of cetrimide and soap solutions and of calamine lotion in screw-cap bottles, and the withdrawal of previous stocks of these fluids, was adopted between February and April, 1950. To find whether these changes had any immediate effect on the incidence of *Ps. pyocyanea* infection in the hospital, comparisons were made of the proportions of positive diagnostic swabs from pus, haematomata, wounds, and other surface lesions (except burns) submitted by surgeons to the clinical laboratory before and after the change in the method of dispensing fluids.

Out of 545 swabs examined between May 1, 1949, and April 30, 1950, 101 (18.5%) contained *Ps. pyocyanea*; an average of 8.3 swabs yielded the organism each month (ranging from 3 to 16). In the three months from May 1, 1950, to July 31, 1950, three out of 104 (2.8%) of the swabs examined contained *Ps. pyocyanea*, two of the positives being from an old case with long-standing *Ps. pyocyanea* infection. A comparison was also made of the proportion of patients from whose lesions *Ps. pyocyanea* was isolated during the periods May 1, to July 31, in 1948, 1949, and 1950. Monthly counts of the cases swabbed and the number of these which yielded *Ps. pyocyanea* were added together and gave the following results:*

Period	Cases Swabbed	Cases Yielding <i>Ps. pyocyanea</i>	
		No.	Per-centage
May 1 to July 31, 1948	106	11	10.4
" " 1949	89	14	15.7
" " 1950	76	2	2.6

Interpretation of the findings from such an arbitrary group of specimens is, of course, very uncertain. The data quoted are preliminary, and even if further records show a continued reduction in the incidence of *Ps. pyocyanea* it is still impossible to exclude the influence of other factors (e.g., improvements in ward hygiene and the use of specific chemotherapy in other wards). It does, however, seem likely that contaminated soap solution and cetrimide were responsible for a proportion of the added infections, since these fluids were used as a routine for the cleansing of wounds and of operation sites.

Reasons for the Survival of *Ps. pyocyanea* in Disinfectant Solutions

Soap is known to be weak in its disinfectant action against Gram-negative bacilli (McCulloch, 1945). In our studies soap solution inoculated with small amounts of a *Ps. pyocyanea* broth culture was found still to be contaminated after standing 24 hours at room temperature. Cetrimide, though it has been shown to be peculiarly inactive against *Ps. pyocyanea* (Williams and others, 1944; Harper and Cawston, 1945; Hood, 1948), is reported to kill the organism in concentrations considerably lower than 1% (Davies, 1949). In experimental contamination of aqueous cetrimide solutions with peptone water cultures we found that several strains of *Ps. pyocyanea* were killed at concentrations of 0.015% and above in one hour.

The survival of an organism in an environment which is normally lethal to the species might be due to (a) the presence or development of resistant strains; (b) protection of the organisms or neutralization of the disinfectant by proteins or other organic compounds; and (c) other factors, for example, intermittent contact with the disinfectant.

A few experiments were made with the purpose of finding if one or more of these mechanisms might account for the presence of *Ps. pyocyanea* in bottles of cetrimide and 10% "dettol".

Experiments to Find Resistant Strains

Experiment 1.—The resistance to cetrimide of two strains of *Ps. pyocyanea* was estimated, one of the

*The differences between the proportion of positives in 1949 and 1950 ($\chi^2 = 8.0$; $P < .01$) and 1948 and 1950 ($\chi^2 = 4.0$; $P < .05$) were both significant.

strains having been isolated from a stock bottle of 1% cetrimide and the other having no history of contact with cetrimide. The method used was as follows.

Amounts, each of 0.04 ml. 24-hour peptone water cultures of *Ps. pyocyanea*, were added from a dropping pipette to 0.5 ml. amounts of a range of doubling dilutions of cetrimide in distilled water. After standing at room temperature for periods of one hour, six hours, and 24 hours, the tubes were shaken and loopful subcultures to peptone water were made. Peptone water tubes were examined for turbidity after overnight incubation at 37°C. and subcultured to agar plates for confirmation. After the 24 hours' subculture, the 0.5 ml. amounts of cetrimide were diluted with 10 ml. "lemco" broth and incubated overnight to detect small numbers of surviving organisms. The results showed no difference in sensitivity to cetrimide of the two strains, the highest concentrations (g.%) from which *Ps. pyocyanea* was isolated being as follows:

	Standing Time (hours)		
	1	6	24
Strain A (no history of contact with cetrimide)	0.015	0.007	0.007
Strain E.1051 (from cetrimide bottle)	0.015	0.007	0.007

Experiment 2.—Using the same method, the sensitivity to "dettol" of two strains of *Ps. pyocyanea* isolated from bottles of 10% "dettol" was compared with the sensitivity of two other strains. The highest concentrations (%) from which *Ps. pyocyanea* was isolated were as follows:

Strain	Standing Time (hours)		
	1	6	24
E.1024 (from "dettol") ..	12.5	6.25	<6.25
E.1025 (from "dettol") ..	12.5	6.25	<6.25
A	12.5	6.25	<6.25
B	6.25	6.25	<6.25

Resistant strains or mutants were apparently not responsible for the contamination of the fluids from which the organisms under test were isolated. Moreover, several attempts to induce *Ps. pyocyanea* to acquire resistance to cetrimide were unsuccessful.

Experiments to Determine Protection by Organic Compounds

Experiment 3.—Twenty-four hour peptone water cultures of four strains of *Ps. pyocyanea* were subcultured to a range of doubling dilutions of cetrimide from 10% to 0.15% in broth. The strains of *Ps. pyocyanea* had not been in contact with cetrimide since their isolation. After incubation for 24 hours at 37°C. all four strains were found to be growing in all dilutions of cetrimide broth. The experiment was repeated with

peptone water solutions of cetrimide, and the results were the same as with broth.

Experiment 4.—Twenty-four hour peptone water cultures of two of the strains used in Experiment 3 were centrifuged and re-suspended in horse serum. Amounts, each of 0.04 ml., of these suspensions and of the original peptone water cultures were added by dropping pipette to 0.5 ml. amounts of a range of doubling dilutions of cetrimide from 10% to 0.001%. Subcultures of these suspensions were made as in Experiment 1, and the highest concentrations of cetrimide from which *Ps. pyocyanea* could be isolated were as follows:

Strain	Suspended in	15 minutes	1 hour	6 hours	24 hours
A	serum	0.03	0.015	0.015	0.015
	peptone water	0.007	0.007	0.004	0.004
B	serum	0.125	0.06	0.015	0.015
	peptone water	0.007	0.007	0.004	0.004

Experiment 5.—Experiment 1 was repeated, using 1% cetrimide solution taken from a bottle in which *Ps. pyocyanea* had been found and at the same time a freshly-made cetrimide solution.

The highest concentrations of both solutions from which *Ps. pyocyanea* could be cultured were 0.015 after one hour and 0.007 after six hours. Since there was no difference in the disinfectant power of the two solutions, survival of *Ps. pyocyanea* in the cetrimide bottle had obviously not been due to substances in the solution itself.

From these experiments it may be inferred that organic substances in solution with cetrimide can neutralize its disinfectant action against strains of *Ps. pyocyanea* and that there is a degree of protection of these strains by serum added with the organism to aqueous cetrimide solution. The cetrimide solution in a contaminated stock bottle, however, does not contain enough of such solutes to protect added *Ps. pyocyanea*.

Artificial Contamination of a Cetrimide Bottle

Growth of *Ps. pyocyanea* from a culture plate was spread on the inside of a cork covering a bottle of 1% cetrimide. During the first six hours the bottle was inverted about once every hour. Swabs taken from the cork one hour, three hours, six hours, and 24 hours after inoculation were heavily contaminated with *Ps. pyocyanea* and swabs from the fluid were positive one hour, three hours, and six hours after the cork had been inoculated.

Discussion

The isolation of *Ps. pyocyanea* from fluids applied to wounds has revealed a preventable reservoir of wound infection which may be common and is of particular importance in the infection of adequately covered wounds. Measures to ensure and maintain the sterility of certain fluids, including such disinfectants as cetrimide and 10% "dettol"

are necessary and have been achieved by dispensing them in autoclaved screw-cap bottles. Since the introduction of such sterile fluids the incidence of wound infection with *Ps. pyocyanea* has fallen.

The question arises why *Ps. pyocyanea* and other organisms survive in disinfectant fluids at concentrations which are lethal to these organisms in distilled water in a test tube. Strains of *Ps. pyocyanea* isolated from cetrimide and "dettol" were no more resistant to these disinfectants than other strains. The fact that *Ps. pyocyanea* will readily grow in culture media containing 1% and more of cetrimide suggests a mechanism by which organisms deposited on the corks of bottles may survive intermittent contact with this fluid. This explanation gains support from the observation of Nelson (1942), who isolated coliform bacilli from bottles of sterile distilled water, and showed that growth-promoting substances could be derived from cork. Deposited on moistened corks by contaminated fingers, *Ps. pyocyanea* might be expected to survive in bottles of cetrimide and "dettol" at a concentration which kills the organism when it is unprotected. An envelope of dried exudate from the wound in which the contaminating organism had previously grown would contribute further protection. The organisms probably persist on the cork, from which they are periodically washed into the fluid when the bottle is tipped up or inverted. Special stress is therefore laid on the disadvantages of corks which not only fail to protect the rim of the bottle neck from contamination but also provide a nidus on which bacteria persist and may even multiply. The replacement of large corked bottles by small screw-cap bottles should be attainable in all industrial medical departments.

Other precautions are desirable wherever possible. The bottles should be sterilized before, or preferably after, filling with the fluid. In industrial medical departments where facilities for autoclaving are

not available, it would be reasonable to boil screw-cap bottles of cetrimide, soap solution, and 10% "dettol" in the sterilizer. Such a simple daily routine using water starting up from cold should be neither time-consuming nor cause too great a loss from cracked bottles.

Summary

Ps. pyocyanea was isolated from bottles of 1% cetrimide solution, 2% soap solution, 10% "dettol", and calamine lotion used in hospital dressing stations and operating theatres. The contaminated fluids were in bottles with corks.

Fluids dispensed in small sterile screw-cap bottles were found to be free from contamination by *Ps. pyocyanea* and there was evidence of a reduction in the rate of infection of wounds by the organism after the new method of dispensing was introduced.

The survival of bacteria in solutions of disinfectant is briefly discussed.

Special care is recommended in the dispensing of such fluids for hospitals and industrial medical departments. Small screw-cap bottles should be used, when possible filling the bottles before they are autoclaved or boiled.

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