In-use testing of disinfectants in Malaysian government hospitals

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Introduction

THE CONCENTRATIONS of disinfectants recommended for use in hospitals are calculated from the results of tests under controlled laboratory conditions. However, a laboratory test cannot reproduce the wide range of conditions which exist when the disinfectant is in use. It is therefore advisable to carry out in-use tests for bacterial contamination (Kelsey & Maurer, 1966; Prince & Ayliffe, 1972) when a new disinfectant is introduced into a hospital and at intervals afterwards.

In this study, samples of disinfectants from various discard jars in four Malaysian Government Hospitals were assessed for bacterial contamination by an "in-use" test.

Method

"In-use" testing was carried out by the method of Kelsey and Maurer (1966). One ml of the disinfectant in the discard jars or bowls was pipetted into 9 ml of diluent. The diluent for alcohols, aldehydes, hypochlorites & phenolics was Nutrient Broth, while Nutrient Broth + Tween 80 (3% W/V) was used for diguanides, hypochlorites + detergents, phenolics + detergents, quartenary ammonia compounds (QAC's) and iodophors (Maurer, 1974). The diluted disinfectant was plated out within one hour by dropping 10 drops with a 40-dropper (40 drops per ml) Pasteur pipette onto a Nutrient Agar plate and incubated at 37°C for 2 days. Samples showing more than 6 colonies from the 10 drops, that is, more than 240 live bacteria per ml, were considered to be contaminated. In the original method of Kelsey and Maurer (1966), 5 colonies from 10 drops of a 50-dropper pipette or 250 live bacteria per ml, was used as a criterion for contamination. The contaminating bacteria were then picked and subcultured for identification. Identification was done using the manuals for the identification of Enterobacteriaceae (Cowan & Steel, 1965; Edwards & Ewing, 1962) and several schemes for the identification of Gram-negative non-fermentating bacteria (Pickett & Pedersen, 1970; Gilardi, 1971; Sandlin, 1974; Kantor *et al.*, 1975).

The resistance of the bacterial isolates to disinfectants was assessed by subculturing the isolates in solutions of the original disinfectant as well as other disinfectants. Dilutions of the disinfectants were made in tap water and one drop (of a 40-dropper) of an overnight broth culture of the isolate was added to the disinfectant solution. After 3 days and 7 days, the solution was tested for survivors by plating drops from a 40-dropper onto Nutrient Agar. A very rough estimate of the number of survivors was given by recording the results thus: CF = confluent growth, SC = semi-confluent growth, or as the number of organisms/drop.

Results

Bacterial contamination of discard jars:

The results of "in-use" testing of the discard jars are shown in Table I. Table I shows that in Hospital A, two out of eight samples taken were contaminated (more than 240 organisms/ml) and both were from the same ward; one from a thermometer jar filled with Dettol and another from a forceps jar, also filled with Dettol. Both jars were contaminated with *Moraxella* sp.

Table I Results of "in-use" testing

Hospital	No. contaminated / No. sampled	Article in contaminated jars	Disinfectant & Conc.	Degree of contamination (per ml)	Bacteria isolated	Isolate No.
A	2/8	Thermometer	Dettol	$>10^5$	*Moraxella* sp.	1
	,,	Forceps	Dettol	16×10^3	*Moraxella* sp.	2
B	3/10	Thermometer	Dettol 1/20	$>10^5$	*Moraxella* sp.	3
	,,	Forceps	Dettol 1/10	14×10^3	*Moraxella* sp.	4
	,,	Trolley mop	Dettol 1/20	4×10^4	*Moraxella* sp.	5
C	6/32	Forceps	Dettol	$>10^5$	*Alcaligenes* sp.	6
	,,	Thermometer	Dettol	$>10^5$	*Moraxella* sp.	7
	,,	Trolley mop	Dettol	$>10^5$	*Moraxella* sp.	8
	,,	Trolley mop	Hibitane 1/2000	$>10^5$	*Flavobacterium* sp.	9
	,,	Thermometer	Hibitane 1/200	$>10^5$	*Moraxella* sp.	10
	,,	Cheatle forcep	Hibitane 1/200	$>10^5$	*Moraxella* sp.	11
D	5/17	Thermometer	Water	$>10^5$	*Pseudomonas aeruginosa*	12
	,,	Thermometer	Water	$>10^5$	*Klebsiella aerogenes*	13
	,,	Thermometer	Water	$>10^5$	*Acinetobacter* sp.	14
	,,	Cheatle forcep	Dettol	$>10^5$	*Moraxella* sp.	15
	,,	Scrubbing brush	Dettol 1/10	8×10^3	*Moraxella* sp.	16
	,,	Thermometer	Dettol 1/10	$>10^5$	*Pseudomonas* sp.	17
	,,	Cheatle forcep	Hibitane 1/200	$>10^5$	*Pseudomonas* sp.	18

In Hospital B, three out of ten samples were contaminated. Two of them were from a trolley mop and forcep jars from one ward and the other from a thermometer jar in the labour room. All three samples were found to be contaminated with *Moraxella* sp. and all contained Dettol.

Six out of thirty two samples from Hospital C involving three wards were contaminated. From the 1st ward, a forceps jar with Dettol was contaminated with *Alcaligenes* sp., and *Moraxella* sp. were isolated from the thermometer and trolley mop jars filled with Dettol. In the 2nd ward, *Flavobacterium* sp. was isolated from a trolley mop jar filled with Hibitane 1/2000. *Moraxella* sp. was isolated from thermometer and Cheatle forcep jars filled with Hibitane 1/200 in the 3rd Ward.

In Hospital D, contamination of five out of seventeen samples was shown involving four wards. In the 1st ward *Pseudomonas aeruginosa, Klebsiella aerogenes* and *Acinetobacter* species were isolated from a thermometer jar filled with water alone. From the 2nd ward a Cheatle's forceps jar filled with Dettol was contaminated with *Moraxella* sp. A scrubbing brush jar and thermometer jar filled with Dettol 1/10 from the 3rd ward were found to be contaminated with *Moraxella* sp. and *Pseudomonas* sp. respectively. Finally, a Cheatle's forceps jar filled with Hibitane in the 4th ward was contaminated with *Pseudomonas* sp.

Resistance of the Bacterial Isolates:

The results of the experiments to determine the resistance of the isolates to disinfectants are given in Table II. Isolates 1, 2, 4, 5, 6, 8, 15 & 16 were resistant to Dettol 1/10. All were originally isolated from Dettol Solutions in the hospitals. Isolates 3 & 7 were killed by Dettol 1/10 but grew in Dettol 1/20. The isolates from the Hibitane containing jars, 9, 10, 11 & 18 were sensitive to Hibitane 1/2000 but 10, 11 & 18 flourished in

Hibitane 1/4000 and Zephiran 1/1000. Isolates 12, 13 & 14 (from the thermometer jar filled with water) were sensitive to all disinfectants with the exception of Isolate 12 (Pseudomonas aeruginosa) which grew in Dettol 1/80.

Discussion

The choice of the "in-use" test diluent is very important. The use of an unsuitable diluent gives misleadingly good results and a false sense of security. Simple dilution is usually satisfactory for bactericidal disinfectants; but bacteriostatic disinfectants must be diluted in fluids that inactivate the bacteriostatic effect without inhibiting bacterial growth. The diluents used for in-use testing must be checked before the test results can be considered valid. A method for checking the diluent (Maurer, 1974) was followed in our study. Many inactivators have been recommended (Mackinnon, 1974; Bergan & Lystad, 1972), but in our study, an attempt wa smade to simplify matters by using the diluents recommended by the Disinfection Reference Laboratory of the Central Public Health Laboratory, London (Maurer, 1974).

Out of a total of 67 disinfectant solutions (26 of Dettol, 36 of Hibitane, 2 of Cetavlon, 2 of Milton, 1 of water) tested, 16 (24%) were found to be contaminated; 12 (18%) with greater than 10^5 organisms/ml and 4 (6%) with greater than 10^3 organisms/ml. 11 of the contaminated jars were filled with Dettol, that is, 11/26 (42%) of the jars filled with Dettol were contaminated. Out of 36 jars of Hibitane, 4 or 11% were contaminated.

Dettol is well documented as a disinfectant that is easily inactivated by organic matter and allows growth of *Pseudomonas* sp. and other Gram-

Table II Results of the resistance of the isolates to disinfectants

Isolate Number	Isolated from:	No. of survivors per drop after 3 days*							
		Dettol 1/10	Dettol 1/20	Dettol 1/80	Hibitane 1/2000	Hibitane 1/4000	Zephiran 1/1000	Lysol 1/100	Cetavlon 1/200
1	Dettol	100 (CF)	CF	NT	NT	0 (0)	0 (0)	0 (0)	NT
2	Dettol	SC (SC)	SC	NT	NT	0 (0)	0 (0)	0 (0)	0 (0)
3	Dettol 1/20	0	100 (CF)	NT	NT	0 (0)	0 (0)	0 (0)	NT
4	Dettol 1/20	30 (CF)	CF	NT	NT	0 (0)	0 (0)	0 (0)	NT
5	Dettol 1/10	37 (CF)	NT	NT	NT	0 (0)	0 (0)	0 (0)	NT
6	Dettol	35 (CF)	NT	NT	NT	0 (0)	0 (0)	0 (0)	NT
7	Dettol	CF	SC (CF)	NT	NT	0 (0)	0 (0)	0 (0)	0 (0)
8	Dettol	25 (CF)	NT	NT	NT	0 (0)	0 (0)	0 (0)	NT
9	Hibitane 1/2000	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NT
10	Hibitane 1/200	NT	0 (0)	0 (0)	0 (0)	5 (0)	CF (CF)	0 (0)	NT
11	Hibitane 1/200	NT	0 (0)	0 (0)	0 (0)	CF (0)	CF (CF)	0 (0)	NT
12	Water	NT	0 (0)	CF (CF)	0 (0)	0 (0)	0 (0)	0 (0)	NT
13	Water	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NT
14	Water	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NT
15	Dettol	CF (SC)	CF	SC	NT	0 (0)	0 (0)	0 (0)	NT
16	Dettol 1/10	CF (CF)	CF	NT	NT	0 (0)	0 (0)	0 (0)	0 (0)
17	Dettol 1/10	0 (0)	0 (0)	CF (CF)	0 (0)	0 (0)	0 (0)	0 (0)	NT
18	Hibitane 1/200	0 (0)	0 (0)	0 (0)	0 (0)	SC (CF)	CF (CF)	0 (0)	NT

* Figures in parenthesis give the number of survivors after 7 days.
NT = not tested
CF = confluent growth
SC = semiconfluent growth

negative organisms (Public Health Laboratory Service Report, 1965; Maurer, 1974). In view of all the documented evidence plus the results of the "in-use" tests, it must be strongly emphasized that the widespread practise of using Dettol in discard jars be discontinued. Results of Capacity Tests (Khor & Jegathesan, unpublished data) showed that while 10% Dettol passes the test for both "clean" & "dirty" conditions, 8% Dettol fails the test for "dirty" situations. Thus it can be seen that if Dettol is to be used, the concentrations must be at least 10%, which is expensively high.

Contamination of the disinfectant solutions could possibly be due to the following:-

(i) inaccurate measurement of the disinfectant concentrations

(ii) infrequent changing of the solutions

(iii) refilling or topping up discard jars without cleaning the jars

(iv) the presence of inactivating material or soiling

(v) an inappropriate choice of disinfectant

Most of the contaminating bacteria isolated were Gram-negative non-fermenters with the exception of one *Klebsiella* sp. They include *Moraxella* sp. (11), *Pseudomonas* sp. (3), *Acinetobacter* sp. (1), *Alcaligenes* sp. (1) and *Flavobacterium* sp. (1). Although these bacteria are ubiquitous in nature and to some extent are indigenous to man, they may be opportunistic pathogens under appropriate conditions. Many non-fermentating bacteria have generally been accepted as secondary invaders but a growing number of literature have implicated these bacteria as a primary cause of infection especially in infants and old people (Snell, 1973; Pederson et al, 1970).

There have been reports of bacteria isolated from disinfectant solutions but these bacteria were subsequently killed on subculture in the same disinfectant solutions (Prince & Ayliffe, 1972; Basset et al, 1970). However, some workers have isolated bacteria from disinfectant solutions which subsequently grow on subculture in the same disinfectant solution (Palmer & McCracken, 1970; Burdon & Whitby, 1967).

Most of the isolates were shown to have some degree of resistance to the disinfectant from which they were isolated. Nine of the isolates, originally from Dettol, were resistant to Dettol at a concentration 1/10. These tests were carried out with no addition of nutrient broth or organic matter. Clearly these organisms have adapted to survival at concentrations which are usually bactericidal. Prince and Ayliffe (1972) have reported adaptation of a *Pseudomonas* sp. to increasing concentrations of a phenolic disinfectant by continual sunculture in increasing phenolic concentrations.

Five other isolates grew in reduced concentrations of the disinfectants, and three isolates were killed by all disinfectants. Possibly these organisms had adapted to the higher concentrations but this property was rapidly lost on subculture. Another possibility was that these organisms could have been protected by a layer of organic material in the original solutions which was lost on subculture. Support for the theory that the organisms have adapted to resistance to the disinfectants by exposure to the disinfectants, is shown by the finding that the organisms isolated from the thermometer standing in water were sensitive to most of the disinfectants.

In all 4 hospitals surveyed, "in-use" testing was not carried out routinely or even when the disinfectant was first introduced. The results obtained in this study show the usefulness of "in-use" testing in the surveillance of disinfectant usage in hospitals.

Summary

Sixty seven samples of disinfectants were obtained from various discard jars in the wards of four Malaysian Hospitals. Bacterial contamination was assessed by an "in-use" test. 16/67 (24%) samples were found to be contaminated with Gram-negative bacilli. Further experiments showed that most of these bacteria had some degree of resistance to the disinfectants from which they were originally isolated.

Acknowledgement

We would like to thank Mr. Wong Fook Sang and Miss M. Kanaganayagy for technical assistance rendered, Mr. Thean Yit Sang for typing the manuscript and the Director, IMR for his kind permission to publish this paper.

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