Bacterial Contamination of Hospital Disinfectants

K C Keah, BSc. Hons*
M Jegathesan, FRCPath**
S C Tan, BSc. Hons***
S H Chan, B. Pharm Hons****
O M Chee, B. Pharm*****
Y M Cheong, MRCPath******
A B Suleiman, FRCPA*******

*Pathology Department,
Hospital Tengku Ampuan Rahimah, 41200 Klang, Selangor
**Institute for Medical Research,
Jalan Pahang, 50588 Kuala Lumpur
***Department of Laboratory Services,
General Hospital Kuala Lumpur, 50586 Kuala Lumpur
****Department of Pharmacy,
General Hospital, 70990 Seremban, Negeri Sembilan
*****Pharmacy Section, State Medical and Health Department,
70590 Seremban, Negeri Sembilan
******Pfizer (Malaysia) Sdn Bhd.
Lot 4, Jln 13/6, 46200 Petaling Jaya, Selangor
*******Ministry of Health, Jalan Cenderasari,
50590 Kuala Lumpur

Summary

A study to determine contamination of diluted disinfectants at different points in preparation and use in 6 Malaysian hospitals was done using the in-use test. A growth of ≥ 250 organisms/ml was taken as an indication of contamination. A total of 342 (7.9%) of the 4316 freshly diluted samples collected from disinfectant bottles in the pharmacy were found to be contaminated. The bacterial isolates obtained were *Pseudomonas* spp. (42.4%), *Moraxella* spp. (22.0%), *Flavobacterium* spp. (11.6%) and *Enterobacter* spp. (4.2%). Three hundred and sixty seven out of 2278 ward stock were contaminated. The isolates were *Pseudomonas* spp. (48.6%), *Moraxella* spp. (17.8%), *Acinetobacter* spp. (8.9%) and *Flavobacterium* spp. (7.0%). Of the 9265 disinfectants in-use, 1519 (16.4%) were contaminated. Among the organisms isolated were *Pseudomonas* spp. (44.3%), *Bacillus* spp. (13.0%), *Enterobacter* spp. (9.5%) and *Acinetobacter* spp. (7.3%). The results indicated a high level of contamination of diluted disinfectants prepared in the pharmacy, stored and used in the wards. This gave a high index of suspicion that recommendations for cleaning of disinfectant containers before refilling, handling of diluted stock solutions and using of disinfectants were not closely adhered to. Standard disinfection procedures outlined in the disinfection and sterilization policy by the Ministry of Health should therefore be followed.

Key Words: Disinfectant, In-use test, Contamination

Introduction

Disinfectants are chemical compounds used to destroy vegetative microorganisms present on objects and in the environment. In hospitals, disinfectants are used to destroy potentially pathogenic microorganisms present on medical instruments and make them safe for use on patients.

One cause of hospital infection which has been documented is the use of contaminated disinfectants. Seven cases of urinary tract infections in children after cystoscopy described by Mitchell and Hayward[1] were due to contaminated chlorhexidine solution used for disinfecting the bladder-irrigation reservoir. Dulake and Kidd[2] reported the isolation of *Alcaligenes faecalis* from urine of thirty gynaecological patients undergoing bladder drainage by indwelling catheter. The source was traced to the jar containing 0.1% chlorhexidine used for the storage of spigots.

The cause of contaminated disinfectants would include contaminated stock disinfectants, not changing disinfectants after prolonged usage, not washing disinfectant jars before refilling and refilling of contaminated containers.

The purpose of this study is to establish the extent of contamination of disinfectants in six Malaysian General Hospitals.

Materials and Methods

A random selection of 5% of the freshly diluted disinfectants were collected from each batch prepared in the pharmacy. The 10th, 30th, 50th, 70th and 90th bottle from a batch of every 100 were selected for testing. The date of preparation, batch number and serial numbers were tagged on these bottles and wards receiving these bottles were recorded. Two ml samples from the selected bottles were collected in sterile universal containers using a sterile pipette prior to distribution to the wards. The disinfectants sampled were alcohol, amphyl, carbolic acid, cetrimide, chlorhexidine, cidex, dettol, hypochlorite, lysol and povidone iodine at various dilutions. Disinfectants from the selected bottles were again sampled similarly prior to finishing at the wards (ward stock).

Disinfectants used for disinfecting medical instruments (disinfectants in-use) were also sampled similarly from disinfectant jars before being discarded.

All samples collected were sent to the laboratory within 1 hour of collection.

The in-use test was used to determine the presence of contamination in all the disinfectant samples. This study was conducted in 6 Malaysian General Hospitals over a period of 6 months (October 1990 – March 1991).

In-use Test

The in-use test was performed as recommended by Kelsey and Maurer[3]. This is also the method recommended by the Ministry of Health, Malaysia[4]. However, nutrient broth with 3% w/v Tween 80 was used as a diluent for all the disinfectants instead of nutrient broth alone for alcohols, aldehydes, hypochlorites and the phenolics.

One ml of disinfectant was diluted with 9ml of diluent. Ten drops of this diluted disinfectant were dropped onto 2 nutrient agar plates using a 50-dropper pipette. The plates were examined after incubation for 48 hours at room temperature and at 37°C. A growth of $\geq$ 250 organisms/ml indicated a failure of the disinfection process or contamination of disinfectants from pharmacy or ward stock. Identification of the isolates were then performed by convention methods[5], the API (Bio Merieux, France) or the Microbact (Disposable Products, Australia) in the 6 hospital laboratories.

Results

Table I shows the types of disinfectants and the sources from which they were taken for test.

Table II shows the contamination rate of disinfectants from the pharmacy. The contamination rates of disinfectants at the pharmacies of hospitals C and D were below 1%.

Table III shows the percentage of contamination from bottles of freshly diluted solutions of disinfectants from

Table I
Types of disinfectants tested from the pharmacy and wards

Types of disinfectant	Pharmacy	Ward stock	In-use
Alcohol 70%	170	103	47
Amphyl	384	153	1237
Carbolic acid	129	97	75
Cetrimide	560	357	19
Chlorhexidine	2705	1398	5803
Cidex	1	1	684
Dettol	22	13	1
Hypochlorite	45	28	895
Lysol	300	128	445
Povidone iodine (undiluted)	–	–	8
Others	–	–	51
Total	4316	2778	9265

the pharmacy, the ward stock and the disinfectants in-use. 4316 samples were tested from the pharmacy while only 2278 samples were tested from the ward stock. This was because a large number of bottled disinfectants used within the same day of preparation were missed by the Infection Control Nurses and not taken for test. 49.4% of the bottled disinfectants issued by the pharmacy for use in the wards were completely used on the same day.

Of the 367 contaminated ward stocks, 165(45%) were already contaminated when issued from the pharmacy. Thus the actual contamination which took place at the wards was 9.6%. Both the pharmacy and the ward stock dilutions of aqueous chlorhexidine 1:5000 had a contamination rate of >50%.

Table IV shows that the rate of contamination of disinfectant in-use was 16.4%. High rates of contamination was observed in chlorhexidine and amphyl in-use. The disinfectants in-use (where n tested >15) with the highest rate of contamination was aqueous chlorhexidine 1:2000 (51.3%) followed by chlorhexidine 1:1000 (50.7%) and chlorhexidine 1:100 (44%).

Table II
Contamination rate of freshly diluted disinfectants from pharmacy

Hospitals	No. of wards	Rate of contamination at pharmacy (no. of samples tested)
A	16	1.4% (1475)
B	14	6.0% (383)
C	11	0.5% (204)
D	11	0.6% (521)
E	14	19.5% (585)
F	11	15.8% (1148)
Total	77	7.9% (4316)

From the 342 contaminated diluted pharmacy stock solutions, 354 bacterial isolates were obtained. From the total of 202 disinfectants where contamination occurred in the wards alone, 214 isolates were obtained (Table V).

Table III
Percentage of contaminated freshly diluted disinfectants from pharmacy and ward stock

Disinfectant	No. contaminated/total tested	
	Pharmacy	Ward
Alcohol 70%	25/170 (14.7%)	24/103 (23.3%)
Amphyl 1:50	0/3 (0.0%)	0/3 (0.0%)
1:100	1/1 (100%)	1/1 (100%)
1:200	1/380 (0.3%)	1/149 (0.7%)
Carbolic acid 1:20	0/129 (0.0%)	1/97 (1.0%)
Cetrimide undiluted	ND	0/2 (0.0%)
1:50	7/59 (11.9%)	15/31 (48.8%)
1:100	37/501 (7.3%)	32/324 (9.9%)
Chlorhexidine (in 70% alcohol)		
1:200	9/979 (0.9%)	18/543 (3.3%)
1:2000	0/35 (0.0%)	0/24 (0.0%)
Chlorhexidine (aqueous)		
1:20	0/7 (0.0%)	ND
1:200	8/250 (3.2%)	16/151 (10.6%)
1:1000	2/25 (8.0%)	3/12 (25.0%)
1:2000	211/1336 (15.8%)	234/631 (37.1%)
1:5000	18/31 (58.1%)	8/15 (53.3%)
Chlorhexidine (with 1% NaNO2)		
1:2000	ND	0/10 (0.0%)
1:5000	8/42 (19.1%)	3/12 (25.0%)
Cidex 2%	0/1 (0.0%)	0/1 (0.0%)
Dettol 1:20	7/7 (100%)	5/5 (100%)
1:40	3/15 (0.2%)	5/8 (62.5%)
Hypochlorite 1:10	3/45 (6.7%)	0/28 (0.0%)
Lysol undiluted	ND	0/1 (0.0%)
1:20	1/231 (0.4%)	1/87 (1.1%)
1:40	1/69 (1.5%)	0/40 (0.0%)
	342/4316 (7.9%)	367/2278 (16.1%)

From the 1519 contaminated disinfectants in-use, 1592 bacterial isolates and 28 fungi were obtained (Table VI).

Discussion

This survey revealed a high rate of contamination of disinfectants obtained from the pharmacy as well as those stored and used in the wards. The contamination rates of freshly diluted disinfectants from the pharmacy, ward stock and disinfectants in-use were 7.9%, 9.6% and 16.4% respectively.

Most of the bacteria isolated from the disinfectants

Table IV
Percentage of contaminated disinfectants in-use

Disinfectant		No. contaminated/total tested	
Alcohol 70%		18/47	(38.3%)
Amphyl	1:20	0/2	(0.0%)
	1:40	0/2	(0.0%)
	1:50	18/41	(43.9%)
	1:100	35/91	(38.5%)
	1:200	481/1099	(43.8%)
	1:2000	1/2	(50.0%)
Carbolic acid	1:20	3/68	(4.4%)
	1:40	1/7	(14.3%)
Cetrimide	1:100	1/19	(5.3%)
Chlorhexidine	(in 70% alcohol)		
	1:200	224/4643	(4.8%)
	1:2000	3/24	(12.5%)
Chlorhexidine	(aqueous)		
	1:100	11/25	(44.0%)
	1:200	48/167	(28.7%)
	1:1000	68/134	(50.7%)
	1:2000	363/707	(51.3%)
	1:5000	9/11	(81.8%)
Chlorhexidine	(with 1% NaNO2)		
	1:2000	3/14	(21.4%)
	1:5000	17/78	(21.8%)
Cidex 2%		43/684	(6.3%)
Dettol	1:20	1/1	(100%)
Hypochlorite	1:10	49/672	(7.3%)
	1:20	8/110	(7.3%)
	1:40	1/109	(0.9%)
	1:100	1/4	(25.0%)
Lysol undiluted		0/15	(0.0%)
	1:10	0/35	(0.0%)
	1:20	67/365	(18.4%)
	1:40	7/30	(23.3%)
Povidone iodine undiluted		0/8	(0.0%)
Others		38/51	(74.5%)
		1519/9265	(16.4%)

obtained from the pharmacy and ward stock were gram-negative non-fermentative organisms. The most common were *Pseudomonas* spp. and *Moraxella* spp. From chlorhexidine, the organisms isolated were *Pseudomonas* spp., *Moraxella* spp., *Flavobacterium* spp. and *Acinetobacter* spp. This group of organisms was also the most common isolates from the disinfectants in-use. Our results were similar to an earlier study by Khor and Jegathesan[6] in 1977 where the gram-negative non-fermentative organisms were also the most common isolates. *Pseudomonas* spp. was the most common contaminant in chlorhexidine (60.1%) and the phenolics in-use (33%). *Pseudomonas* spp. comprises 44.3% of all the organisms isolated from the contaminated disinfectants in-use. Gram-negative non-fermentative organisms are ubiquitous organisms that can be found in aquatic habitat in nature, soil as well as the hospital environment. They are usually of low virulence but are capable of causing infections in debilitated patients and patients with predisposing illness. Hence, contamination of disinfectants by such organisms should not be taken lightly.

Twenty-eight isolates of fungi were obtained from the phenolics in-use. These isolates were not identified further. They could be fast growing saprophytic fungi of yeasts. The short incubation period of 48 hours together with the use of nutrient agar and not Sabouraud agar could probably account for the lack of fungi isolated.

Organisms resistant to commonly used disinfectants had been isolated[7]. It is therefore unwise to depend on the self-disinfecting properties of the disinfectants to remove contaminants from the bottles as resistant organisms would not be killed by the recommended concentration of disinfectant. Rinsing bottles without taking into consideration the temperature of water used could not be depended upon to kill all the contaminants. This was reported by Burdon and Whitby[8] who isolated *Pseudomonas* spp. surviving a temperature of up to 70°C from chlorhexidine.

45% of the contaminated bottles from the pharmacy ended up in the wards for use. Mitchell and Hayward[1] reported that chlorhexidine 1:5000 contaminated by pseudomonads was the cause of urinary tract infection in children following cystoscopy.

Table V
Frequency of organisms isolated from contaminated diluted stock disinfectants from pharmacy and wards

Disinfectant	Organism	Pharmacy	Ward
Alcohol 70%	*Enterobacter* spp.	6 (24.0%)	3 (37.5%)
	**Pseudomonas* spp.	11 (44.0%)	1 (12.5%)
	Others	8 (32.0%)	4 (50.0%)
Chlorhexidine	*Acinetobacter* spp.	14 (5.2%)	19 (11.1%)
	Alcaligenes spp.	8 (3.0%)	6 (3.5%)
	Enterobacter spp.	9 (3.4%)	1 (0.6%)
	Flavobacterium spp.	41 (15.3%)	15 (8.8%)
	Moraxella spp.	59 (22.0%)	33 (19.3%)
	**Pseudomonas* spp.	117 (43.7%)	82 (48.0%)
	Unknown	10 (3.7%)	11 (6.4%)
	Others	10 (3.7%)	4 (2.3%)
Hypochlorite	Unknown	3 (100%)	0
Phenolic	*Moraxella* spp.	10 (71.4%)	3 (60.0%)
	Others	4 (28.6%)	2 (40.0%)
Cetrimide	*Moraxella* spp.	9 (20.5%)	2 (6.7%)
	**Pseudomonas* spp.	22 (50.0%)	21 (70.0%)
	Others	13 (29.5%)	7 (23.3%)

** includes Ps. aeruginosa*

This report therefore suggests the importance of ensuring that diluted disinfectants do not get contaminated before distribution to the wards.

A total of 50.6% of the diluted disinfectants were kept and used for more than 1 day in the wards. Prolonged storage and repeated use of the same container of disinfectant over an extended period of time could result in contamination of its contents. Anderson and Keynes[9] showed that bacteria from the caps and area around the opening of containers could contaminate the disinfectant it holds. We were however not able to demonstrate the proportion of contamination due to this phenomenon and to deterioration because of prolonged storage.

Higher rates of contamination were observed in higher dilutions of aqueous chlorhexidine as was found in the pharmacy and the ward. (Tables III and IV). Besides being easily contaminated, the high rate of contamination of chlorhexidine in-use could be attributed to 2 factors: higher dilutions resulting in less effective concentrations being used and not changing some of the disinfectants in-use regularly. The concentration of chlorhexidine recommended for disinfection of medical instruments is 0.5%. The chances of failure in using lower concentrations would therefore be much higher[10]. In many instances, the only satisfactory way to process instruments is to sterilize them by heat[4]. Should disinfectants be required, correct optimal dilutions of freshly prepared ones must be used.

It is important to follow proper procedures for cleaning disinfectant containers, handling diluted stock solutions and disinfectants in-use. To reduce contamination disinfectant containers and caps need to be sterilized. However, if sterilization is not possible and disinfection has to be done, the temperature of water must be as near 100°C as possible. Disinfectants should be freshly diluted for daily use and storage of dilutions in the

Table VI
Frequency of organisms isolated from disinfectants in-use

Disinfectant	Organism	No. of isolates	(%)
Alcohol 70%	*Enterobacter* spp.	6	(33.3%)
	Hafnis alvei	1	(5.6%)
	Staphylococcus spp.	6	(33.3%)
	**Pseudomonas* spp.	5	(27.8%)
	Others		
Chlorhexidine	*Acinetobacter* spp.	72	(10.4%)
	Alcaligenes spp.	26	(3.8%)
	Bacillus spp.	19	(2.7%)
	Enterobacter spp.	53	(7.7%)
	Flavobacterium spp.	24	(3.5%)
	Hafnia alvei	17	(2.5%)
	Moraxella spp.	38	(5.5%)
	**Pseudomonas* spp.	415	(60.1%)
	Unknown	13	(1.9%)
	Others	14	(2.0%)
Cidex	*Bacillus* spp.	7	(15.6%)
	Enterobacter spp.	13	(28.9%)
	Staphylococcus spp.	6	(13.3%)
	**Pseudomonas* spp.	11	(24.4%)
	Others	8	(17.8%)
Hypochlorite	*Acinetobacter* spp.	5	(7.0%)
	Bacillus spp.	9	(12.7%)
	Enterobacter spp.	5	(7.0%)
	**Pseudomonas* spp.	18	(25.4%)
	Unknown	13	(18.3%)
	Others	21	(29.6%)
Phenolic	*Acinetobacter* spp.	41	(5.4%)
	Alcaligenes spp.	9	(1.2%)
	Bacillus spp.	176	(23.2%)
	E. coli	8	(1.1%)
	Enterobacter spp.	77	(10.1%)
	Flavobacterium spp.	10	(1.3%)
	Fungi	28	(3.7%)
	Klebsiella spp.	19	(2.5%)
	Moraxella spp.	56	(7.4%)
	**Pseudomonas* spp.	251	(33.0%)
	Unknown	59	(7.8%)
	Others	26	(3.4%)
Cetrimide	**Pseudomonas* spp.	1	(100%)
Others	**Pseudomonas* spp.	16	(47.1%)
	Unknown	8	(23.5%)
	Others	10	(29.4%)

* *includes Ps. aeruginosa*

wards discouraged. Working solutions of disinfectants in-use should be changed regularly.

Acknowledgement

This project was funded by a Research and Development grant (No. 3-08-01-041) from the Ministry of Science Technology and the Environment. We thank the medical superintendents and matrons of the 6 hospitals for their support throughout the project, the microbiologists and the infection control nurses for technical assistance.

References

1. Mitchell RG, Hayward AC. Postoperative urinary tract infection caused by contaminated irrigating fluid. Lancet 1966;1 : 793-5.

2. Dulake C, Kidd E. Contaminated irrigating fluid. Lancet 1966;1 : 980.

3. Kelsey JC, Maurer IM. An in-use test for hospital disinfectants. Mon Bull Ministry Health Lab Services. 1966;25 : 180.

4. Ministry of Health, Malaysia. Disinfection and Sterilization Policy and Practice, 1990 (2nd ed).

5. Lennette EH, Balows A, Hausler WJ, Shadomy HJ. Manual of Clinical Microbiology (4th ed). Washington D.C. : American Society for Microbiology, 1991.

6. Khor SY, Jegathesan M. In-use testing of disinfectants in Malaysian Government Hospitals. Med J Malaysia 1977;32 : 85-9.

7. Shiraoshi T, Nakagawa Y. Review of disinfectant susceptibility of bacteria isolated in hospital to commonly used disinfectants. Postgrad Med J 1993;69 : 570-7.

8. Burdon DW, Whitby JL. Contamination of hospital disinfectants with *Pseudomonas* spp.. Br Med J 1967;2 : 153-5.

9. Anderson K, Keynes R. Infected cork closures and the apparent survival of organisms in antiseptic solutions. Br Med J 1958;2 : 274.

10. Lowbury EJ, Ayliffe GAJ, Geedes AM, Williams JD (comps). Disinfection(1) Types of chemical disinfectant and formulation of policy for disinfection. In: Control of Hospital infection. London: Chapman & Hall, 1982 : 63-71.