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EVALUATION OF SOME HAND WASHING AND DISINFECTION METHODS IN THE REMOVAL OF TRANSIENT BACTERIAL FLORA

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Summary

Hand washing is generally considered the most important procedure in preventing nosocomial infections. The effectiveness of 5 disinfectants and unmedicated bar soap were studied for the removal of transient bacteria. Pevidine, chlorhexidine and cetrimide were found to be the most effective in removing both gram positive and gram negative transient bacteria. High bacterial reductions by soap and water reinforced the point that in the vast majority of situations in the hospital this procedure alone, if properly carried out, is adequate.

INTRODUCTION

The value of chemical disinfectants in hand washing was clearly established in 1847 by Semmelweiss who used chloride of lime to prevent the transfer of organisms of puerperal infection from the postmortem room to the labour wards. Later, in 1867, Lister introduced the use of 2.5 and 5 percent phenol for the disinfection of skin and instruments respectively.[1]

The human skin has a unique ecosystem composed of non-living components and viable bacteria, all living more or less in equilibrium. The bacterial flora of the skin may be divided into two major groups – the "transient" bacteria and the "resident" bacteria, the nature and number of which will vary with individuals and in the same individuals in different situations.' The transient bacteria, acquired mainly by contact, are abundant on exposed skin, beneath nails, etc. and are comprised of many varieties, pathogenic and non-pathogenic, which can be removed with relative ease. The residents form a comparatively stable flora, are abundant on more protected skin and contain relatively few pathogenic bacteria. Frequent or prolonged exposure of skin to pathogenic contaminants may result in a resident flora of virulent organisms. Hospitalized patients and hospital personnel, in contact with the more pathogenic bacteria of a hospital environment may become chronic carriers of these organisms for they become part of the resident flora.'

The practice of washing hands with disinfectants is to prevent cross infection from hospital personnel to patients as well as to prevent cross infection from patient to patient or from fomites to patients. The sites of nosocomial infections are diverse, but they commonly include urinary tract infections, lower respiratory tract infections, intravenous catheter associated bacteriaemias, neonatal skin infections and surgical wound infections. Organisms transmitted on the hands of physicians, nurses and technicians may cause infections at these sites and many outbreaks have occurred, particularly in nurseries, where this was thought to be the means of cross infection.[3] Because many different types of nosocomial infections might be prevented by appropriate personnel hand washing, this is generally considered the most important single procedure in their prevention.

Various antiseptic soaps, detergent emulsions, creams and jellies for the disinfection of the hands have become available during the last few years.[4] Skin antiseptics, ideally, should be

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not merely bacteriostatic but bactericidal to a wide variety of organisms, and should be harmless to skin and underlying tissues, either directly or through sensitization.[2] Skin disinfectants may be compared by their killing effect on normal skin flora or bacteria applied to the skin. Pathogenic bacteria are nearly always transient on the skin surface where they can be killed by suitable chemicals.[5] Skin cleansing and disinfection can be tested for effectiveness against transient bacteria by spreading bacterial suspensions on the skin which are allowed to dry, and the numbers of bacteria before and after cleansing or disinfection of the skin are compared.[6]

In the investigation reported here this technique was used to test the efficacy of five disinfectants against four known organisms deposited on the skin (palms) of volunteers. These five skin disinfectants are commercially available in Malaysia and are used widely in hospitals in this country. The efficiency of these antiseptics were tested against three common organisms – ***Escherichia coli, Pseudomonas aeruginosa*** and ***Staphylococcus pyogenes.*** The fourth organism was *Flavobacterium meningosepticum,* a gram negative bacillus, which has recently come into prominence as a cause of neonatal meningitis in this country.[7]

MATERIALS AND METHODS

Pure cultures of the following organisms, isolated from clinical specimens from patients in the University Hospital were grown on human blood agar – ***Staph. pyogenes, Ps. aeruginosa, E. coli*** and ***F. meningosepticum*** Type C. Five colonies of each organism were inoculated into nutrient broth (Oxoid) and incubated overnight at 37°C.

The hands of volunteers were first rinsed for two minutes in 70% ethyl alcohol (surgical spirit) to reduce the numbers of resident bacteria, and dried using a hair dryer. One drop (0.02 ml) of the bacterial suspension (nutrient broth culture containing approximately 3×10^8 organisms/ml) was inoculated on the centre of the right palm of each volunteer. Then, using a sterile wire loop, the drop of inoculum was spread over a standard area 3 cm. in diameter, and allowed to dry in air.

The hands were then washed or treated with one antiseptic in the manner described below, or left untreated for use as controls. Bacteriological samples were taken from the inoculated areas by rubbing in a standard way, under a measured volume (5 ml) of sterile Ringer's solution containing appropriate neutralisers. The washing solution was retained in a sterile empty petri dish. Ten fold dilutions of these were made and 0.2 ml amounts of each of the dilutions were spread on the surface of nutrient agar plates with a bent glass rod. Specific neutralisers for the disinfectants were included in both the sampling fluid as well as the culture media. These were sodium thiosulphate (0.5%) and ox serum (5%) for the pevidine and a 2% egg yolk agar for aqueous chlorhexidine, aqueous cetrimide and resiguard. The plates were incubated aerobically for 24 and 48 hours at 37°C before counting of bacterial colonies.

Six preparations for cleansing or disinfecting the hands were studied and compared with untreated controls; in each case the period of treatment was 30 seconds and a standard number of strokes. – palm to palm, palm over dorsum and with fingers interlaced were used, either for rinsing in a bowl containing 100 ml of antiseptic solution or for washing under a running tap. The following preparations were studied:

1. Unmedicated bar soap (Lux) and water
2. Aqueous solution of chlorhexidine gluconate 0.05% (Hibitane)
3. Aqueous solution of chloroxylenol 2.5% (Dettol)
4. Aqueous cetrimide 1%
5. Povidone-iodine (Pevidine), an antiseptic solution containing 1% available iodine.
6. Resiguard antiseptic solution (0.63%) which contains picloxydine digluconate 1% and benzalkonium chloride 12%
7. No treatment (control)

Each organism was tested against each of the above seven methods of cleansing or disinfection, eight times using 56 volunteers and the results of these comprise 8 sets per organism. Thus a total of 224 volunteers took part in the experiment resulting in 8 sets of results for each organism. There were sufficient volunteers of doctors, nurses, medical students and laboratory technicians so that each took part in the experiment only once.

RESULTS

Tables la-ld show the actual numbers of the viable organisms of the deposited bacteria present in 0.02 ml of the sampling fluid after cleansing, disinfection or no treatment (control). Most of the counts for each organism are around the mean value except for some instances where the counts are much higher than that of the mean, for example; in the experiment using ***Staph. pyogenes,*** set 2 when using resiguard, sets 3 and 5 after soap and water and sets 1 and 8 after treatment with dettol. Similar high counts can be seen for E. ***coli*** in sets *1* and 5 after soap and water, for *Ps.* ***aeruginosa*** in set *1* after treatment with pevidine and chlorhexidine and sets **4** and 5 after dettol. For F. ***meningosepticum*** again some high counts compared to the mean were observed. The reasons for these variance from the mean would include factors such as individual variance as well as sampling errors.

Table 2 shows the mean bacterial counts per 0.02 ml of the washing fluid after treatment with the various disinfectants, cleansing with soap and water and the controls. Recovery of the organisms in the controls was highest with *Ps.* ***aeruginosa,*** next being ***Staph. pyogenes*** followed by ***E. coli*** and F. ***meningosepticum.*** This reduction in the number of viable bacteria without any form of treatment could be due to factors such as drying and dessication but the most important factor could well be the "self-sterilizing" or "autodisinfecting" powers that the normal skin has due to the presence of fatty acids derived from skin lipids.[8]

Table 3 shows the mean percentage reduction of colony counts after contamination of the hands and subsequent cleansing or disinfection. The range of efficacy of the various disinfectants against each of the four organisms can be considered using the mean percentage reduction as the criteria. Pevidine, chlorhexidine and cetrimide were the most efficient skin disinfectants for all the four organisms, each causing a mean percentage reduction in the mean viable bacterial counts of 99.99%.

Resiguard was most active against *Ps. aeruginosa* and least active against ***Staph. pyogenes,*** the mean percentage reduction being 99.95% and 99.72% respectively.

The range of efficacy of dettol varied widely between a mean percentage reduction of 99.98% against E. ***coli*** and only 62.30% against ***Staph. pyogenes.***

Soap and water showed a fairly narrow range in the reduction of viable bacteria from a mean of 99.97% against F. ***meningosepticum*** to 99.59% against ***Staph. pyogenes.***

The range of efficacy of the disinfectants for each of the organisms can also be considered by looking at Table 3. For all the four organisms the most efficient disinfectants were pevidine, chlorhexidine and cetrimide. For ***Staph. pyogenes*** the next in line is resiguard with a mean percentage reduction of 99.72% and then comes soap and water (99.59%) and last is dettol (62.30%).

For *E.* ***coli,*** after pevidine, chlorhexidine and cetrimide, the next best disinfectant was dettol, then resiguard and last was soap and water. For *Ps.* ***aeruginosa*** the results were similar to E. ***coli*** except that soap and water seemed marginally better than resiguard.

Cleansing with soap and water was definitely more efficient than dettol or resiguard against F. ***meningosepticum;*** the mean percentage reduction being 99.97% for soap and water and 99.92% and 99.81% for dettol and resiguard respectively.

DISCUSSION

The bacterial counts were greatly reduced by all the four disinfectants as well as by soap and running water. But in considering the overall efficacy of the tested disinfectants against the four organisms, pevidine, chlorhexidine and cetrimide seemed to be the most effective for the removal of transient organisms deposited on the skin. Their range of effectiveness covered both the gram positive cocci ***(Staph. pyogenes)*** as well as the three gram negative bacilli that were used. The mean percentage reduction for the above three disinfectants was only 99.99% which shows that none of the three were efficient enough always to remove all the ***Staph. aureus*** nor the gram negative bacilli. Nevertheless, the aim being practical rather than theoretical, the effect looked for in hand washing techniques is the virtually complete killing of the bacteria, or "virtual" disinfection as described by Gardner.[9] Both cetrimide and chlorhexidine are cheap enough and physically acceptable for the disinfection of hands when it is necessary. But pevidine is not cheap and the

TABLE la

VIABLE COUNTS FROM 0.02ML OF SAMPLING FLUID USING STAPH. PYOGENES

Set No.	1	2	3	4	5	6	7	8
Control	208,000	23,900	370,000	106,000	95,000	158,000	111,000	210,000
Resiguard	720	2,460	85	3	15	137	75	31
Dettol	196,000	502	501	518	630	166	164	89,000
Soap & water	151	700	2,030	224	1,160	390	444	75
Pevidine	0	0	0	1	2	0	1	0
Chlorhexidine	0	1	6	6	5	1	0	0
Cetrimide	0	7	0	0	5	1	1	0

TABLE lb

VIABLE COUNTS FROM 0.02ML OF SAMPLING FLUID USING E. *COLI*

Set No.	1	2	3	4	5	6	7	8
Control	40,000	17,600	188,000	32,500	27,400	119,000	12,200	59,000
Resiguard	139	1	69	8	0	204	1	250
Dettol	5	0	2	12	2	30	0	1
Soar, & Water	580	29	43	34	357	4	7	2
Pevidine	0	3	1	0	0	1	1	0
Chlorhexidine	0	0	0	0	5	38	0	0
Cetrimide	0	0	0	25	1	0	2	0

TABLE lc
VIABLE COUNTS FROM 0.02 ML OF SAMPLING FLUID USING *PS. AERUGINOSA*

Set No.	1	2	3	4	5	6	7	8
Control	1,230,000	42,000	59,000	1,020,000	550,000	290,000	143,000	45,900
Resiguard	91	760	2	68	410	122	178	22
Dettol	1	1	5	282	605	42	2	0
Soap &water	49	318	24	238	355	8	80	12
Pevidine	59	0	0	0	1	1	0	0
Chlorhexidine	220	0	0	0	0	2	0	0
Cetrimide	0	1	13	38	16	0	0	0

TABLE Id
VIABLE COUNTS FROM 0.02 ML OF SAMPLING FLUID USING F. *MENINGOSEPTICUM*

Set No.	1	2	3	4	5	6	7	8
Control	16,400	35,000	46.000	12,400	65,000	73,000	80,000	99,000
Resiguard	211	57	59	7	20	8	148	290
Dettol	5	1	11	2	240	42	3	3
Soap &water	1	7	10	0	6	60	2	17
Pevidine	1	4	1	1	3	1	2	0
Chlorhexidine	1	0	0	0	1	11	0	3
Cetrimide	0	14	0	2	0	0	0	26

TABLE 2
MEAN VIABLE BACTERIAL COUNTS

	Mean counts per 0.02 ml of washings			
	Staph. pyogenes	*E. coli*	*Ps. aeruginosa*	*F. meningosepticum*
Control	160,237.5	61,962.5	422,487.5	53,350.0
Resiguard	440.8	84.0	206.6	100.0
Dettol	60,435.0	6.5	117.3	38.4
Soap & water	646.8	132.0	135.5	12.9
Pevidine	0.5	0.8	7.6	1.6
Chlorhexidine	2.4	5.4	27.5	2.0
Cetrimide	1.8	3.5	8.5	5.3

TABLE 3
MEAN PERCENTAGE REDUCTION OF COLONY COUNTS AFTER DEPOSITION OF BACTERIA ON SKIN AND SUBSEQUENT CLEANSING OR DISINFECTION

Cleansing1 disinfection method	Bacteria deposited on skin:			
	Staph. pyogenes	*E. coli*	*Ps. aeruginosa*	F. *meningosepticum*
Pevidine) Chlorhexidine) Cetrimide)	99.99	99.99	99.99	99.99
Resiguard	99.72	99.86	99.95	99.81
Dettol	62.30	99.98	99.97	99.92
Soap & water	99.59	99.78	99.96	99.97

physical appearance is not conducive to hand washing. It is described by the manufacturers (Berk, England) as an agent that kills gram positive and gram negative bacteria, protozoa, yeasts, fungi, some viruses as well as many bacterial and fungal spores. Therefore pevidine is not indicated where the aim is only disinfection of hands outside the operation theatre.

Both resiguard and dettol were more effective against the gram negative bacilli than the gram positive *Staph. pyogenes* and both preparations are not usually used for the disinfection of hands. Soap and running water was fairly effective in the reduction of both gram positive and gram negative bacteria and this supports the practice of using only soap and water for normal hand hygiene in many hospital situations.[10]

The time of 30 seconds for washing was chosen on the basis of published reports[6,11] but Ojajarvi[12] reported that 15 seconds was closer to the average hand washing time in the wards. Whether this would have made any difference to our results is difficult to predict.

The volunteers selected for this laboratory study did not have any skin problems. Hospital staff, on the other hand, are at least to some extent unselected. The skin of their hands is

often dry and subject to repeated washing. Laboratory tests may therefore yield unrealistic information.[12] Drying or cracking of the skin and frequent hand washing were shown to be associated with disinfection failures.[11] This brings to light the necessity of abstaining from certain nursing procedures by personnel with skin problems.

The main goal of hand washing in hospital is to cut the route of transmission of pathogenic micro-organisms to patients and outside the operation theatre, transient bacteria play a major role in the transmission of cross-infections.[12] Therefore the situation where true disinfection is necessary must be determined and then the appropriate disinfectant and hand washing method selected. Frequency of hand washing and disinfection has to be taken into account as there is indirect evidence that within five hours of disinfection, areas of skin regained original bacterial counts when protected from external contamination.' But it is not unusual for nursing staff to complain of irritation of the skin of the hands caused by soaps, detergents or hand disinfectants. All these considerations must be taken into account when choosing hand disinfectants and frequency of usage. The ultimate goal is to promote the safety of the patient, but the working ability of the nursing staff must also be protected.[10]

The hands of nurses, doctors, physiotherapists and others who handle patients are probably the most important vehicles of cross-infection, and it is essential that effective methods should be used to minimize this hazard.[13] For most ward procedures a thorough washing of hands with soap and water is sufficient. But when a higher degree of disinfection is desirable as in "high infection risk" situations, washing of hands with a suitable disinfectant is necessary.

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