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Intravenous infusion bottle plugs as a source of microbial contamination

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SUMMARY

A technique was described for the artificial contamination of the upper surface of intravenous infusion container plugs with viable micro-organisms. This technique was employed to assess the adequacy of decontamination of two designs of plug by the use of antiseptic swabbing. Results indicated that the MRC plug surface was more difficult to sterilize than that of the Travenol plug. The possibility of contamination of the infusion fluid during insertion of the giving-set needle was examined. Results indicated that there was a risk of contamination through the administration-set port of the MRC plug if swabbing was inadequate.

INTRODUCTION

It has been reported that micro-organisms can gain access to intravenous infusions during administration (Michaels & Ruebner, 1953; Deeb & Natsios, 1971; Duma, Warner & Dalton, 1971). It has been clearly established that microbial contamination can arise from the influx of unfiltered air (Arnold & Hepler, 1971), the addition of drugs (D'Arcy & Woodside, 1973) and by the migration of micro-organisms through the cannulae of the administration set (Maki, Goldman & Rhame, 1973). Recent studies indicate that infusions in collapsible plastic containers are less prone to contamination (Letcher, Thrupp, Shapiro & Boersma, 1972). An air inlet is not required and the administration-set port is relatively inaccessible to airborne or touch contamination. One further route of contamination is found with glass rather than plastic containers; this is associated with the insertion of the administration set through the rubber plug of the bottle. To reduce this risk, the plug surface is first swabbed with a germicidal solution. In this paper, we have examined the effect of the design of two different types of plug on the efficiency of decontamination by antiseptic swabbing, and the potential contamination of the infusion fluid if swabbing does not kill all of the contaminants on the plug surface.

MATERIALS AND METHODS

Preparation of bacterial suspensions

The organisms employed were *Staphylococcus epidermidis* NCTC 6513 and *Bacillus megaterium* ACTC 8245. After growth in nutrient broth (Oxoid) supplemented with 0·5% (w/v) yeast extract (Difco) at 37° C. for 18 hr., cells were

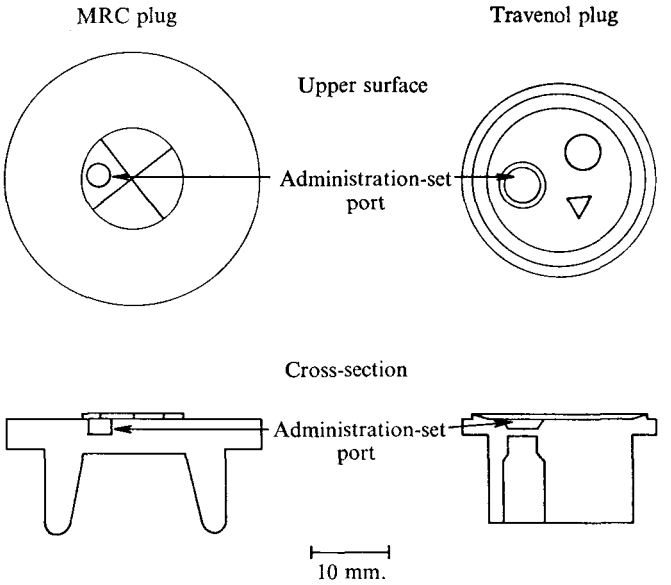

Fig. 1. Diagram to illustrate the upper surface and cross-section of MRC and Travenol infusion container plugs.

harvested by centrifugation at 6000 rev./min. for 10 min., washed twice and re-suspended in distilled water. Spores of *B. megaterium* were prepared by the method of Hambleton (1964).

Inoculation of plug surfaces

Two types of plug were employed: the MRC infusion bottle plug B.S. 2463 (1962) and the plug from infusion bottles supplied by Travenol Laboratories Ltd, Thetford, Norfolk, U.K. (Fig. 1). Plugs were cleaned by boiling in sterile distilled water for 2 hr. After drying, 20 μl. of a cell suspension, delivered from a Gilson pipette (Anachem Ltd, Luton, Beds., U.K.), was spread over the total upper surface of each plug using a sterile wire loop and dried by placing the plugs in an incubator with fan at 37° C. Travenol plugs dried in *ca.* 20 min. MRC plugs dried in a similar period provided that the relatively deep recessed administration-set port was opened to extend its surface area. The plug was lightly clamped across a diameter so that the surface was slightly convex. It was necessary to achieve approximately equal drying times of the bacterial film because prolonged drying led to an excessive reduction in the number of viable cells surviving on the plug surface. Experiments designed to investigate the potential introduction of bacteria via the administration-set port during insertion of the administration-set used the same procedure except that the inoculum was placed entirely in the administration-set port. Travenol plugs were inoculated and dried without removal from the Travenol infusion container in *ca.* 20 min. However, in order to conduct efficient drying within the same period, the MRC plugs were sterilized, inoculated with test inoculum and dried. The plugs were then placed on the MRC infusion bottle using aseptic technique.

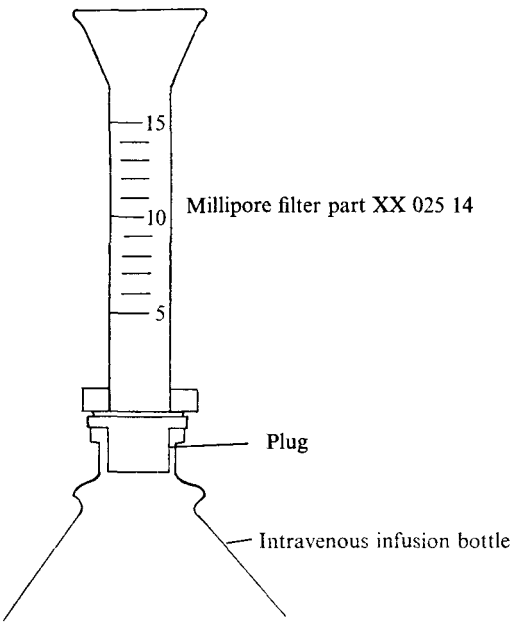

Fig. 2. Diagram to show how the Millipore filter holder was secured over a plug.

Swabbing of surfaces

Swabs containing 70% iso-propyl alcohol and 0·5% chlorhexidine acetate (Steret H swab, Prebbles Ltd., Liverpool, U.K.) were used. Swabbing consisted of a 'once over' wipe by hand taking *ca.* 2–3 sec., using moderate pressure.

Recovery of bacteria from test surfaces

In order to assess the number of viable bacteria present following inoculation as described both before and after swabbing, a Millipore filter holder (25 mm. XX 025 14) was secured over the plug as shown in Fig. 2 using the corresponding clamp (XX 025 03). This produced a water-tight seal. 2·5 ml. of sterile 0·5% Tween 80 solution was placed in the holder. Removal of organisms from the surface of the plug was facilitated by agitation with a sterile test-tube brush. Tests showed there to be negligible loss of viable organisms on to the brush. The suspension of micro-organisms was removed with a pipette, the washing procedure repeated, the two suspensions combined and plated out in nutrient agar (Oxoid) supplemented by 0·5% yeast extract (Difco) (concentrated medium was prepared to allow for the addition of the 5 ml. of cell suspension). Plates were incubated at 37° C. for 48 hr.

Recovery of Staph. epidermidis *from the infusion fluid after insertion of the administration-set*

After inoculation onto plug surfaces as described, sets were inserted through the appropriate port in the plug (Fig. 1) employing a straight pushing action, sterile gloves being worn to avoid touch contamination. The infusion container was then shaken for 15 sec. to facilitate the removal of bacteria from the set needle and

inner surfaces of the plug. The plug was removed and the fluid (0·9 %, w/v, saline) filtered through a Millipore filter (45 mm.; 0·45 μm. pore size). The bottle was rinsed with 20 ml. of sterile water and the rinsings transferred to the same filter. Finally each filter membrane was transferred to nutrient plates (nutrient agar +0·5 % yeast extract) and incubated at 37° C. for 48 hr.

Design of experiments

For each experiment, 20 plugs were inoculated and dried. Ten of these plugs were used as controls to assess the number of colony-forming units present. The remainder were employed to assess the efficiency of antiseptic swabbing.

All manipulations were performed in a laminar flow hood employing strict aseptic technique.

RESULTS

The proportion of *B. megaterium* spores that could be removed after inoculation onto plug surfaces was 68·5 % (S.D. 10·4) from Travenol plugs and 66·4 % (S.D. 9·8) from MRC plugs (means of ten different colony counts). This finding suggests that the method described is capable of recovering nearly 70 % of any inoculum. However, employing various species of vegetative bacteria much lower recovery rates were obtained. *Staph. epidermidis* was the organism most able to withstand the inoculation procedure although considerable variation occurred between experiments. For example, recovery rates from Travenol plugs in different experiments were 22·0 % (S.D. 9·8), 37·7 % (S.D. 15·4) and 24·5 % (S.D. 13·3). The recovery of vegetative micro-organisms from inoculated plug surfaces is therefore less satisfactory than that of spores.

The effect of antiseptic swabbing on the survival of *Staph. epidermidis* on infusion bottle plugs is shown in Table 1. Swabbing of Travenol plugs reduced the viable bacteria to a very small number (less than 1 % of the control) even at the relatively high inoculum size of 320 viable cells per plug (Expt. C). In comparison, it appears to be more difficult to inactivate viable cells from the surface of the MRC plug, even with the lower inoculum size (Expt. D), since there was a greater recovery of viable cells after swabbing. This may be due to the design of the administration-set port which is deeper than the Travenol port and straight-sided, as can be seen in Fig. 1. Consequently, the likelihood of contact between the swab and inner port surface is reduced and the penetration of antiseptic into the administration-set port depends on the degree of pressure applied to the swab during decontamination. Statistical analysis of results, comparing experiments B and E, in which the control colony counts (without swabbing) were approximately equal on both Travenol and MRC plugs, showed that the number of viable cells recovered after swabbing was not, however, significant ($F^1_{18} = 1{\cdot}98$; tabulated value = 4·41; $P = 0{\cdot}05$). This was due to the considerable variation between individual colony counts. This observation was even more apparent in experiments designed to assess the value of swabbing plugs with antiseptic in order to prevent the introduction of micro-organisms contaminating the administration-set port into the infusion fluid during insertion of the set. Tests showed that this could lead to the

Table 1. *The effect of swabbing with Steret H on the recovery of* Staphylococcus epidermidis *from plug surfaces*

		Colony count (mean of 10 counts)			
		Without swabbing		After swabbing	
Plug	Experiment	No.	S.D.	No.	S.D.
Travenol	A	52·6	21·3	0·7	1·2
	B	119·5	45·0	0·5	0·6
	C	319·6	153·3	1·65	3·3
MRC	D	41·0	11·6	8·3	14·1
	E	129·0	64·0	13·4	15·2

Table 2. *The effect of swabbing with Steret H on the contamination of intravenous infusions by* Staphylococcus epidermidis *during the insertion of the administration-set*

		Colony counts (mean of 10 counts)					
		Inoculum in administration-set port		In the infusion after administration-set insertion			
				Without swabbing		After swabbing	
Plug	Experiment	No.	S.D.	No.	S.D.	No.	S.D.
Travenol	F	263·0	77·1	51·2	34·1	1·2	1·3
	G	458·0	114·4	—	—	1·2	1·6
MRC	H	91·0	75·8	24·8	19·3	10·6	11·6
	I	250·0	154·9	58·0	34·6	19·4	17·8

ingress of micro-organisms from contaminated plug surfaces (Table 2). Swabbing of Travenol plugs before insertion of the administration-set greatly reduced the likelihood of the introduction of viable micro-organisms into the infusion from the administration-set port. The number of viable organisms recovered from the infusion after germicidal swabbing of the plug followed by insertion of the administration-set was assessed. This was found to be negligible, even at the higher level of contamination employed in Expt. G. In contrast, a greater number of viable cells could be recovered, after swabbing and insertion of the administration-set, from the contents of the MRC infusion container. For instance, statistical comparison of the results from Expts. F and I proved that the recovery rate from MRC containers (the mean of which was 19·4 viable cells) was significantly greater than from the contents of Travenol bottles, despite the large variances between colony counts ($F'_{18} = 13{\cdot}72$; tabulated value 4·41; $P = 0{\cdot}05$). Since the same swabbing procedure was used for each plug this area of the MRC upper plug surface is, therefore, more difficult to sterilize by antiseptic swabbing. Subsequent tests in which the swab was squeezed on the plug surface to release liquid to flood the administration-set port resulted in no viable bacteria being recovered from the infusion after administration-set insertion.

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DISCUSSION

The results affirm the validity of swabbing of bottle plugs with germicide before the setting up of intravenous therapy systems. Care must be taken to ensure that the antibacterial solution comes into contact with all parts of the upper surface of the plug, especially the less accessible inside surface of the administration-set port. This is particularly important with the MRC plug design where this port, being relatively deep, narrow and straight-sided, is difficult to decontaminate, as shown by the results. The Travenol plug appeared to be less difficult to effectively swab with germicide, presumably because the port is shallower and the sides of the port are slanted, thus presenting a more accessible surface for swabbing. The probability and degree of contamination of plug surfaces in practice has not been studied; these are difficult to predict, being to a large extent governed by the previous history of the bottle and closure. Travenol bottle plugs are provided with a metal disk cover applied before sterilization and are therefore likely to have a very low incidence of microbial contamination. MRC bottle plugs are more likely to be exposed to contamination after sterilization. There is no protective cover applied before sterilization and, in consequence, the plug surface may be exposed to aerial and touch contamination before the application of a non-sterile aluminium foil or plastic over-seal.

The results reported in this study indicate that adequate careful swabbing of the upper plug surface before insertion of the giving-set needle is sufficient to kill even relatively large numbers of micro-organisms that could be present. However, any residual viable organisms may be introduced into the fluid from the administration-set port and thus pose a potential hazard to the patient.

REFERENCES

Arnold, T. R. & Hepler, C. D. (1971). Bacterial contamination of intravenous fluids opened in unsterile air. *American Journal of Hospital Pharmacy* **28**, 614–19.

D'Arcy, P. F. & Woodside, W. (1973). Drug additives – a potential source of bacterial contamination of infusion fluids? *Lancet* ii, 96.

Deeb, E. N. & Natsios, G. A. (1971). Contamination of intravenous fluids by bacteria and fungi during preparation and administration. *American Journal of Hospital Pharmacy* **28**, 764–7.

Duma, R. J., Warner, J. F. & Dalton, H. P. (1971). Septicemia from intravenous infusions. *New England Journal of Medicine* **284**, 257–60.

Hambleton, R. (1964). The germination of spores of *Bacillus megaterium* in the presence of cetrimide. M.Sc. thesis, University of Manchester.

Letcher, K. I., Thrupp, L. D., Shapiro, D. J. & Boersma, J. E. (1972). In-use contamination of intravenous solutions in flexible plastic containers. *American Journal of Hospital Pharmacy* **29**, 673.

Maki, D. G., Goldman, D. A. & Rhame, F. S. (1973). Infection control in intravenous therapy. *Annals of Internal Medicine* **79**, 867–87.

Michaels, L. & Ruebner, B. (1953). Growth of bacteria in intravenous infusions. *Lancet* i, 773.