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Research Papers

THE ASSESSMENT OF AIR-INLET FILTERS FOR INTRAVENOUS INFUSIONS IN GLASS BOTTLES

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SUMMARY

Glass infusion bottles require the influx of air into the containers to replace fluid during administration. A non-absorbent cotton wool plug, situated in the air-inlet set, acts as a filter to remove airborne micro-organisms. An enclosed air space artificially contaminated with viable bacterial spores was used to assess the contamination of intravenous infusions from an airborne source. Only small numbers of spores gained access to the infusion fluids when containers were used with commercially made air-inlet sets. Removal of the filter permitted the entry of spores, the numbers being related to the concentration present in the external air. The efficiency of cotton wool plugs was related to the weight of cotton wool in the air-inlet set. The compression applied to the cotton wool also significantly influenced filter efficiency. Although air-inlet sets were challenged with much higher levels of airborne contamination than would be expected in a hospital environment, results indicate that the air-inlet set should contain sufficient cotton wool. The compression of the filter material should also be considered.

INTRODUCTION

Intravenous infusions in glass bottles must be used in conjunction with an air-inlet set to permit the influx of air into the bottle during administration to replace infused fluid. The air is normally filtered through a cotton wool plug located near the proximal end of the air-inlet set. The efficiency of this plug as a filter to remove airborne micro-organisms has still to be evaluated but previous studies have reported that the introduction of unfiltered air into infusion containers can cause microbial contamination of the fluid contents (Arnold and Hepler, 1971; Hansen and Hepler, 1973; Percival, 1966). Misuse of an intravenous system can lead to the wetting of the cotton wool filter which is then often removed to restore the desired flow rate of fluid, thus allowing unfiltered air to enter the container and subsequently lead to contamination of the fluid.

A method for producing an environment artificially contaminated with bacterial

spores was developed to measure the microbial contamination of infusion containers from an airborne source. The efficiency of cotton wool plugs as bacterial air filters was assessed in this environment.

MATERIALS AND METHODS

Preparation of the bacterial spore suspension

Spores of *Bacillus megaterium* ATCC 8245 were prepared by the method of Hambleton (1964). The resulting suspension was stored at 4°C and heat-activated at 80°C for 10 min prior to use.

Design of the chamber

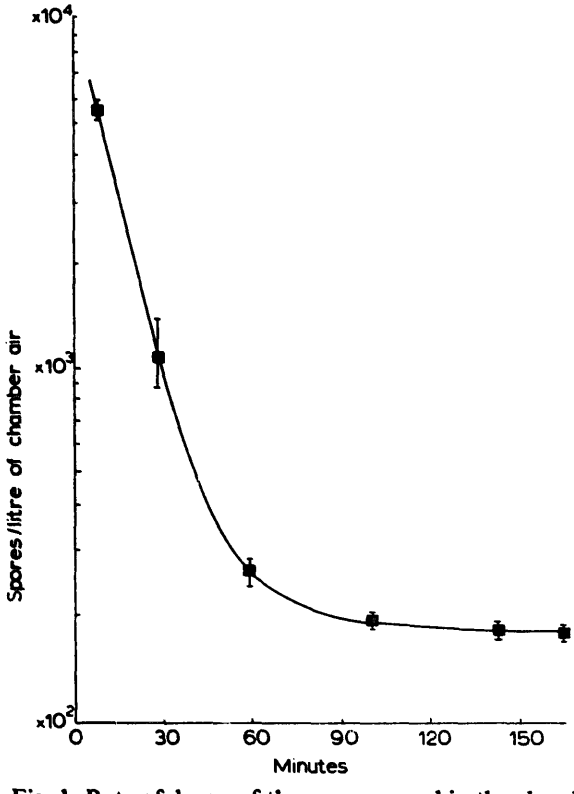
An enclosed area of about 1700 litres capacity was chosen as a suitable chamber in which to produce a bacterial aerosol. The front of the chamber was sealed over with a polyethylene sheet, through which two access ports were inserted. A fan was placed on the bottom of the chamber in a central position to maintain adequate circulation of air.

Production and sampling of the spore aerosol

An aqueous suspension of *B. megaterium* spores (about 10^8/ml) was dispersed as an aerosol using a Laboratory Spray Gun (Shandon Scientific Co. Ltd., Willesden, Lond , England). Spores in the aerosol state show negligible loss of viability (Dimmick and Akers, 1969). The spray was directed through one of the ports to the centre of the chamber for 1–2 sec allowing the upward flow of air to circulate the aerosol. The concentration of airborne spores was measured using a Porton raised impinger (Kluyver and Vissell, 1950a; May and Harper, 1957). Spores were collected in distilled water, suitable volumes plated out in Nutrient agar (Oxoid) and incubated at 37°C for 24 hr. During the initial period after generation of the aerosol there was a rapid fall in the concentration of spores in the chamber air (Fig. 1). After 60 min the spore concentration decreased at a relatively slow and constant rate. It was found that homogenous mixing of the aerosol was achieved when the chamber was either empty or loaded with infusion containers.

Assessment of airborne contamination of infusion bottles using air-inlet sets

Ten 500 ml infusion bottles (Travenol Laboratories Ltd., Thetford, England) were assembled with blood administration sets (Travenol Laboratories Ltd.) and air-inlet sets (Travenol Laboratories Ltd.) using aseptic technique. The cotton wool filters were removed from 5 of the air-inlet sets prior to assembly. The containers were then suspended at random in an inverted position in the aerosol chamber. The far end of each administration set was allowed to extend outside the chamber and the needle inserted into an empty sterile MRC infusion bottle (B.S. 2463). The aerosol was then generated as previously described. After 60 min the vacuum in the infusion bottles was released by unclamping the air-inlet set and the infusion fluid collected in the MRC bottle over a period of 60 min (about 8 ml/min). During this 60 min period air in the chamber was sampled every 20 min. The number of spores/litre of air was determined for each period, and the mean of the three samples calculated to give the average number of spores/litre of air during the experiment. The content of each MRC bottle was filtered through a Millipore membrane



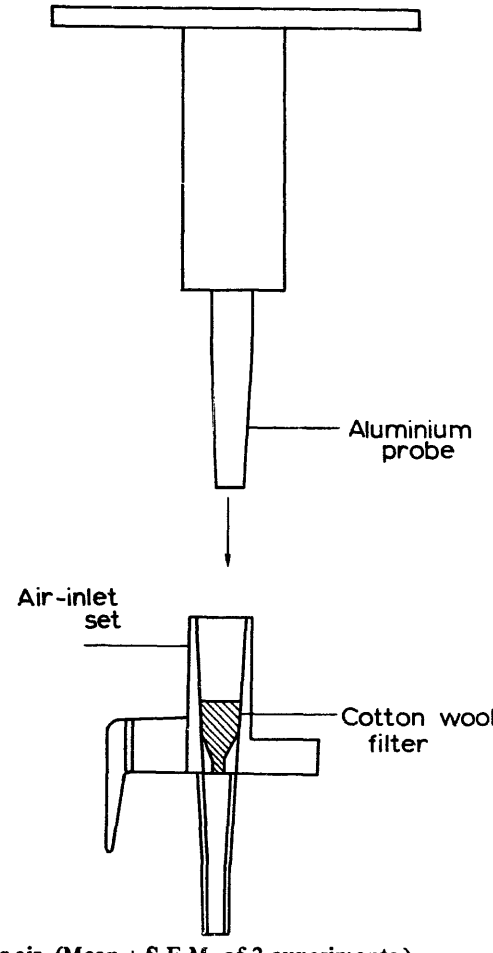


Fig. 1. Rate of decay of the spore aerosol in the chamber air. (Mean ± S.E.M. of 3 experiments.)

Fig. 2. Diagram to illustrate the aluminium probe used to compress the cotton wool into air-inlet sets.

(45 mm; 0.45 μm pore size), the bottles rinsed with 20 ml of sterile distilled water and the rinsings added to the same filter. Finally each filter membrane was transferred to Nutrient agar plates and incubated at 37°C for 24 hr. The experiment was performed ten times. Similar experiments were conducted to compare air-inlet sets from two sources: Travenol Laboratories Ltd. and Avon Medicals Ltd. (Birmingham, England).

Influence of the weight and compression of cotton wool on filter efficiency in air-inlet sets

Cotton wool filters were removed from 4 groups of 8 air-inlet sets (Travenol Laboratories Ltd.) and into each group was placed either 10, 20, 30 or 40 mg of non-absorbent cotton wool. The cotton wool was compressed in the air-inlet sets using a close-fitting aluminium probe (Fig. 2) on which different weights could be placed. Two of each group of 8 filters were compressed under a 0.1, 0.5, 1 or 5 kg weight, respectively. Spores passing through the filter were collected in a Swinnex-25 filter unit (0.45 μm pore size, Millipore Corp. Ltd., London, England) inserted at the proximal end of the air-inlet tube

50 mm from the cotton wool filter. Each group of 8 air-inlet sets was inserted through the rubber closure of a single MRC infusion bottle placed in the aerosol chamber. A group of Swinnex filter units was also placed in the chamber without air-inlet sets being attached. The spore aerosol was then generated. After equilibration, 3 litres of air was drawn simultaneously through each group of 8 air-inlet sets by suction over a period of 30 min. The chamber air was sampled at 0, 15 and 30 min. On completion of the experiment, the membrane filters were removed from the Swinnex units, placed on Nutrient agar plates and incubated at 37°C for 24 hr. The experiment was performed 5 times.

RESULTS

Airborne contamination of infusion fluid containers

When bottles were fitted with commercially made air-inlet sets, spores were subsequently recovered from the contents of 80% of the infusion bottles. A small number of spores (<1% to 5% of the number in the chamber air) were recovered from all except 5 of the contaminated bottles (Table 1). These 5 bottles became contaminated with larger numbers of spores, indicating that the efficiency of the cotton wool filter may be subject to some variation. The removal of the cotton wool air filter permitted the fluid contents of all bottles to be contaminated with spores, the numbers ranging from about 20 to 200 spores per bottle. Results from experiments involving bottles both with and without air

TABLE 1

NUMBER OF SPORES RECOVERED FROM BOTTLES AFTER INFUSION WHEN USED WITH AND WITHOUT COTTON WOOL AIR FILTERS

Experiment	Average no. of spores/litre of chamber air	Mean [a] number of viable spores/container	
		Air-inlet sets with filters	Air-inlet sets without filters
A	212.9	1.2	34.0
B	284.7	2.4	56.8
C	208.3	2.2	49.4
D	175.6	20.0 [b]	78.2
E	191.2	3.4	84.0
F	289.2	2.0	112.8
G	376.8	23.0 [c]	135.2
H	291.5	1.6	109.0
I	328.5	3.6	154.8
J	156.2	2.8 [d]	44.0
Mean of 10 experiments	251.5		85.8

[a] Mean of 5 containers.
[b] Two bottles contained 19 and 66 viable spores, respectively.
[c] Two bottles contained 52 and 53 viable spores, respectively.
[d] One bottle contained 12 viable spores.

filters were subjected to a 2-factor analysis of variance. The difference between the number of spores gaining access to bottles in the presence or absence of an air filter was significant ($P < 0.001$). No significant correlation was found between the numbers present in the surrounding air during administration and those gaining access to the fluid if an air filter was present (correlation coefficient $r = 0.24$; tabulated value = 0.63 for 8 degrees of freedom, $P = 0.05$). In contrast, a significant correlation did exist when the air filter was removed from the air-inlet set ($r = 0.77$; tabulated value = 0.63 for 8 degrees of freedom, $P = 0.05$). These results confirm that, if no air filter is present, micro-organisms from the surrounding air readily gain access to the infusion fluid via the air-inlet. No significant difference was observed between the number of spores recovered from the contents of infusion containers when utilizing air-inlet sets from two different manufacturers ($P = < 0.5$). The mean weight of cotton wool per air-inlet set was 19.5 ± 2.9 mg although greater variation was noted in individual sets. Considerable variation in the packing of filters was also observed. Therefore, the influence of the weight and compression of cotton wool on filter efficiency was assessed.

Assessment of non-absorbent cotton wool as air-inlet filters

When the cotton wool filters were absent from the air-inlet sets, 31.3% (S.D. 8.0) of the spores present in the chamber were recovered as indicated by colony counts on the filter membranes derived from the Swinnex-25 units. The entry of spores into the

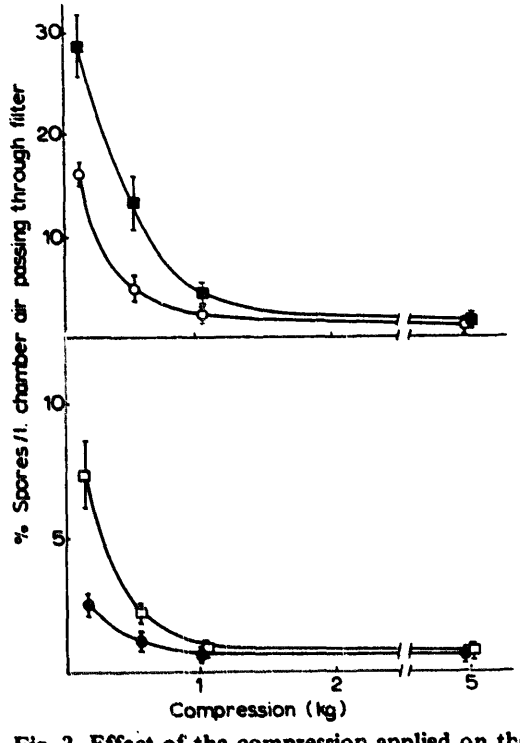


Fig. 3. Effect of the compression applied on the % (± S.E.M.) of spores in the chamber air recovered after passage through the filter consisting of 40 mg (•), 30 mg (□), 20 mg (○) and 10 mg (▪) of non-absorbent cotton wool.

Swinnex-25 units was probably hindered by the rapid and uneven flow of circulating chamber air, thus preventing a proportion of the spores from being diverted into the small opening of each filter unit. Similar results were obtained if no air-inlet set was attached to the Swinnex-25 unit (31.9%; S.D. 8.6) indicating that spores were not entrained in the air-inlet tubing. A significant correlation existed between the number of spores in the chamber air and the number recovered from the Swinnex-25 units when no filter was present in the air-inlet set ($r = 0.89$; tabulated value = 0.88 for 3 degrees of freedom, $P = 0.05$). Fig. 3 shows the effect of the degree of compression applied to the cotton wool on filter efficiency. For each weight, the number of spores penetrating the filter decreased with increasing weights of compression. However, this effect was less pronounced with the higher weights of cotton wool employed in the air-inlet set. Increasing the weight of cotton wool also enhanced filter efficiency. A 3-factor analysis of variance of the number of spores recovered from membrane filters revealed that the variance in the results due to different weights (w) and compressions (c) of cotton wool was significant ($wP < 0.001$; $cP < 0.001$). A significant interaction between the weight and compression of cotton wool was also shown to exist ($cwP < 0.001$). A 2-factor analysis of variance of the number of spores recovered from membrane filters for each individual compression employed showed that, in every case, the weight of cotton wool significantly affected the results ($P < 0.001$), except when 5 kg compression was applied ($P < 0.05$). A 2-factor analysis of variance for individual weights of cotton wool showed that, in each case, the compression of the filter material significantly affected the results ($P < 0.001$).

DISCUSSION

The use of an artificially contaminated environment enabled infusion systems to be challenged with high concentrations of airborne viable spores. Infusion fluids contained in bottles allowing the influx of unfiltered air were contaminated with relatively high numbers of spores. A significant correlation existed between the numbers of spores recovered from the infusion fluid and the numbers present in the surrounding air. The incorporation of a cotton wool filter, as in commercially prepared air-inlet sets, greatly reduced the number of spores gaining access to the infusion fluid. It was observed, however, that a relatively large number of spores was recovered from five of these containers. The presence of a loosely packed air filter may have been responsible for the occasional ingress of larger numbers of micro-organisms into the infusion bottle contents. Studies by Kluyver and Vissel (1950b) of air sterilization using cotton wool filters have indicated that channelling of the cotton wool caused by loose packing and air leakage between the wall of the filter holder and the cotton wool could lead to the passage of micro-organisms through the filter. Channelling through the filter, which probably accounted for the high contamination rate of isolated bottles, would presumably be reduced by increasing the weight of cotton wool used. It would also be prevented if sufficient compression were applied to the cotton wool mass in the air-inlet tubing. Results indicated that both increasing the amount of cotton wool or the force of compression applied to the filter significantly increased filter efficiency. There was a clear relationship between weight of cotton wool and compression applied. The influence of the degree of compression on filter efficiency was most evident with lower weights of cotton wool. It is evident that

the degree of compression must be taken into account when packing air-inlet sets with cotton wool to provide an adequate filter to remove airborne contamination from air entering the infusion container.

In these experiments, large numbers of spores were used to challenge the filters. The level of airborne contamination in the hospital ward environment is only a fraction of that used for this study. Previous studies have indicated that the hospital environment contains 1–5 micro-organisms/litre air (Davies and Noble, 1962; Green et al., 1962). Therefore, the likelihood of any micro-organisms gaining access to the contents of intravenous infusion containers is considerably less than was found in the present study. However, any inconsistency in the quantity and packing of filters resulting in poor filter efficiency, or complete removal of the filter, could lead to the introduction of micro-organisms into the infusion container from the surrounding air during administration.

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