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Contamination of fluids from a hospital pharmacy

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SUMMARY

An investigation into the cause of bacterial contamination of bottles of non-injectable water has been reported. A method of monitoring such bottles has also been described. The roles played by autoclave spray-cooling water and inadequate bottle seals in the contamination of fluids have been examined. Possible methods of reducing the risk of contamination are discussed and the design of an improved method of closure of sterile bottled fluids is stressed. Bacteriological examination is shown to be a more accurate index of the true rate of contamination than measurement of dye concentrations of bottle contents.

INTRODUCTION

The dangers of contamination of intravenous fluids by micro-organisms are well known (Duma, Warner & Dalton, 1971; Phillips, Eykym & Laber, 1972; Felts, Schaffner, Melly & Koenig, 1972; Meers, Calder, Mazhar & Lawrie, 1973) but less well recognized are the hazards arising from contaminated non-injectable fluids which may also come into contact with, for example, peritoneal surfaces.

Microbiological laboratories are thus frequently faced with the problems of monitoring the reputed sterility of bottles containing 500 ml., or more, of fluids destined for topical or intravenous use. At the University Hospital of Wales, this work load is considerable since about 115,000 such bottles are produced annually. This communication reports an investigation into the problems associated with bottled fluids and inadequate seals and describes a routine method of monitoring which was found to be satisfactory.

MATERIALS AND METHODS

The bottles (M.R.C. Transfusion 500 ml. bottles with standard D.H.S.S. lacquered bungs and aluminium caps, and standard D.H.S.S. 1000 ml. bottles for non-injectable waters with rubber liners and aluminium caps) were filled in the Pharmacy and autoclaved in a British Sterilizer, 28 ft.^{3} spray cooled, air-ballasted autoclave at 121° C. (as registered with a thermocouple in a bottle simulator) and held for 30 min. The containers used were a mixture of new and old bottles, the latter surviving, on average, 8 cycles. The spray cooling water contained a dye,

Table 1. *Dye contamination of bottles containing* 500 *ml. or more after autoclaving cycle*

	Injectable solutions	Non-injectable water
No. autoclaved	76,450	39,250
No. contaminated	0	1,334
% Contaminated by dye	0·0 %	3·4 %

Table 2. *Bacterial contamination of bottles containing* 500 *ml. or more after autoclaving cycle*

	Injectable solutions	Non-injectable water
No. examined	476	553
No. contaminated	3	34
% Contaminated by bacteria	0·64 %	6·15 %

Amaranth B.P., to enable any visibly contaminated or obviously cracked bottles to be discarded (Table 1). The concentration of Amaranth in the spray cooling water was 8 mg./ml. and the minimum concentration of dye that could be detected visually was 0·32 μg./ml.

Random samples from each batch were taken from the remainder, in accordance with the *European Pharmacopoeia* (vol. II, 1973, p. 53) recommendations and sent for bacteriological examination.

The recovery medium used was casein-yeast-lactose-glucose (CYLG) medium (Marshall & Kelsey, 1960) which supports the growth of test organisms – *Staphylococcus aureus*, *Plectridium sphenoides* and *Candida albicans* – as stipulated in the *European Pharmacopoeia* (vol. II, 1973, p. 56). CYLG medium at 5 times normal strength was injected directly into the bottles under test. All procedures were carried out in a laminar flow cabinet and the bottle caps swabbed with 70 % (w/v) methylated spirits. Bottles of 1 l. were inoculated with 10 ml. of the CYLG medium and 500 ml. bottles with 5 ml. After incubation at 32° C. for 7 days contaminated bottles were plated onto blood agar plates and then incubated at 30–32° C.

RESULTS

Table 1 shows that 3·4 % of the bottles of non-injectable water produced were rejected at the Pharmacy because of contamination by dye (see Materials and Methods).

Random sampling (*European Pharmacopoeia*, vol. II, 1973, p. 53) of the remaining bottles not contaminated by dye revealed evidence of bacterial contamination in 34 bottles of 553 non-injectable fluids (6·15 %) when examined in the laboratory and in 3 of 476 bottles of intravenous fluids (0·64 %) (Table 2).

The contaminating micro-organism

From every contaminated bottle, bacteria with similar characters were isolated.

Colonial morphology	Smooth pinkish-orange colonies, 2 mm. in diameter with an entire edge.
Growth	On plain agar after 16 days incubation at 32° C. Good growth at 4° C.–32° C. Poor growth at 37° C.
Media	Good growth on plain agar, blood agar and heated blood agar.
Gram stain	Gram positive rods 3–6 μm. in length with many asteroid forms. Non-sporing. Non-motile.
Biochemistry	Catalase positive. Oxidase negative. Hugh and Leifson O/F Test – no reaction. Attacked glucose on ammonium salt medium but other carbohydrate and standard tests negative.

Inactivation curves of the coryneform were determined and the organism was found to have a decimal reduction time (D 10 value) of < 1 min. at 80° C.

At one stage it seemed possible that the organism was *Corynebacterium bovis* but the pigmented colonies made this unlikely. Pink colonies are produced by *Corynebacterium equi* but this organism is nitrate positive and urease positive, unlike the bacteria described. The micro-organism was finally labelled as a non-specific 'coryneform'. (Cultures of this coryneform are maintained in the laboratory.)

Examination of possible sources

This coryneform could only be recovered from filled bottles after autoclaving. The autoclave was therefore examined together with auxiliary pipes and the surfaces of the autoclave-plant room. Sampling was performed using a sterile throat swab moistened with CYLG medium in which it was then incubated.

The coryneform was isolated from all the above sites and also repeatedly from the cooling water within the autoclave itself.

It was concluded that contamination of the bottles had occurred via the spray cooling water and that an aerosol from the exhaust pipe had contaminated the immediate environment of the autoclave.

DISCUSSION

More than 6% of the bottles examined were bacteriologically contaminated. Such figures are unacceptable since the non-injectable water concerned is used for a variety of purposes in the operating theatre and could, therefore, be a vector of infection.

Removal of the micro-organism from the autoclave by filtration and chemical disinfection were considered but found to be totally inadequate. Maintenance of the temperature of the cooling water in the storage tank at 80° C. or conversion of the spray-cooling water system to a closed pipe system held at 5 lb./in.^{2} and 109° C. for the duration of the autoclaving cycle may well have a beneficial effect in reducing the bacterial population but no guarantee of permanent sterility of the

cooling water can be given. The only way to ensure that the bottle fluids remain uncontaminated is *not* to use spray cooling water. If an increase in through-put by use of a spray-cooling water system is required then the risk of contamination of some bottles has to be accepted.

Here the difference in contamination rate between injectable and non-injectable fluids has some relevance (Tables 1 and 2). When compared with injectable fluids, the bottles for non-injectable water were found to be contaminated to a much greater extent with both dye and bacteria. These differences were statistically significant. (Dye contamination $\chi^2 = 2628$, $P < 0{\cdot}001$. Bacterial contamination $\chi^2 = 24{\cdot}09$, $P < 0{\cdot}001$.)

With the injectable fluids a very good seal is made by means of a flanged rubber plug held in place by an aluminium screw cap or spun-on retaining ring (Medicines Commission Report, 1972). The bottle holding non-injectable water is capped by means of a rubber disk with an aluminium screw cap (see Plate). The seal thus depends on the pressure of the rubber liner on the narrow rim of the neck of the bottle.

The failure of this seal has been shown to be due to variations in pressure, temperature and distortion of the aluminium screw caps during spray cooling (Beverly, Hambleton & Allwood, 1974) thus permitting the entry of any contaminated water into the bottle.

However, even with a good seal, contamination can still occur and the design of an improved method of closure of sterile bottle fluids for both topical and intravenous use must be considered as a matter of urgency. Our results, moreover, demonstrate that bacteriological examination provides a more accurate index of the true rate of contamination and should be used in conjunction with an initial visual dye test in the Pharmacy.

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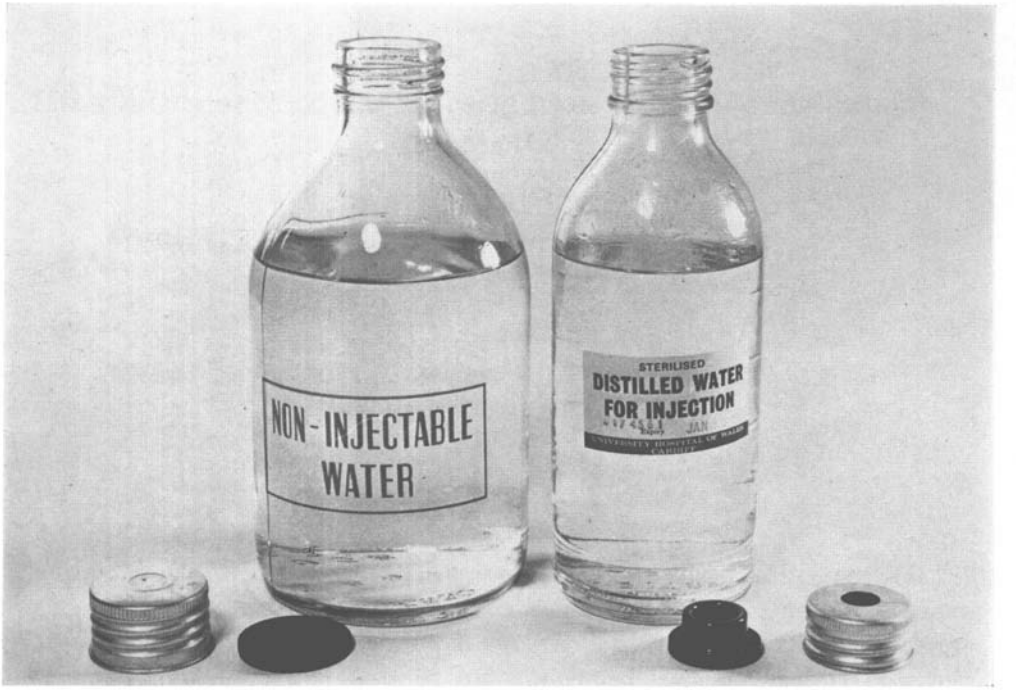
EXPLANATION OF PLATE

The two types of rubber plug used to produce a seal.



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