*International Journal of Pharmaceutics*, 1 (1978) 165–171



# MICROBIAL CONTAMINATION IN RESIDUES OF OPHTHALMIC PREPARATIONS

V.J. HARTE, M.T. O'HANRAHAN and R.F. TIMONEY

*School of Pharmacy, Faculty of Science, Trinity College, 18, Shrewbury Road, Dublin 4 (Ireland)*

(Received December 19th, 1977)
(Accepted January 3rd, 1978)

## SUMMARY

A survey was conducted to assess the incidence of microbial contamination in residues of eye-drops and eye ointments returned to the hospital pharmacy from the wards of a Dublin hospital. Contamination was found in 42.6% of the residues. A stricter adherence to the recommendations described in the British Pharmaceutical Codex on the distribution and mode of use of ophthalmic preparations should lead to a lowering of the incidence of contamination. Greater flexibility in the choice of antimicrobial agents when formulating multi-dose eye-drops is recommended. The usage of ophthalmic solutions with no self-sterilizing capacity should be carefully monitored.

## INTRODUCTION

Pharmaceutical preparations for ophthalmic use are formulated as eye-drops, eye ointments and eye lotions. The hazards associated with using contaminated products are emphasized by the following selected examples. An outbreak of eye infections at a Birmingham hospital was shown to be due to the use of contaminated solutions (Ayliffe et al., 1966). Eight cases of eye infection occurred in Sweden in patients who had been previously treated with an antibiotic ointment (Kallings et al., 1966). The contaminating micro-organism in both instances was identified as *Pseudomonas aeruginosa*. Subsequently the British Pharmacopoeia (B.P.), the British Pharmaceutical Codex (B.P.C.) and the United States Pharmacopoeia (U.S.P.) specified that all preparations intended for ophthalmic use must comply with tests for sterility.

The most frequently used products are eye-drops and eye ointments. These are widely used from multi-dose containers and, thus, although they should be sterile at the time of issue they may become contaminated during use. Aqueous eye-drops unless in single-dose form must therefore contain a suitable antimicrobial substance which is intended to re-sterilize the preparation if contamination is introduced during use. The following substances are recommended in the B.P.C. for this purpose; phenylmercuric nitrate (PMN) 0.002%, phenylmercuric acetate (PMA) 0.002%, benzalkonium chloride (BAC) 0.01% or chlorhexidine acetate 0.01%. Such antimicrobial agents are not usually included in eye ointments because of their limited effectiveness in oily systems. This paper reports the

results of a microbial examination of the 'dregs' of eye-drops and eye ointments which had been returned from ward-use in a Dublin hospital. The techniques employed in the examination of these two classes of products differ substantially. The results and discussion are therefore presented separately.

## EXAMINATION OF EYE-DROP RESIDUES

### *Materials and Methods*

#### *Materials*

Nutrient Agar (Oxoid Ltd.)
L-cysteine (B.D.H. Biochemicals)
Lecithin (Sta-Sol Lecithin Concentrate U.F., Staley Mfg. Co.)
Polysorbate 80 (Croda Chemicals Ltd.)
Andrade Peptone Water (Oxoid Ltd.)
Nutrient Broth (Oxoid Ltd.)
Sodium Chloride B.P. (Evans Medical Ltd.)
Cetrimide (B.D.H. Laboratory Reagent Grade)
Agar (Davis Gelatin Ltd.)

All water used was freshly distilled from a Boroglass still, and all the experimental work was carried out using a laminar flow cabinet (Centronic Europe Ltd.).

#### *Methods*

The dropper bottles containing the residues were collected once weekly from the hospital pharmacy. In most cases the bottles contained a residue in excess of 2 ml. Sterile water (2 ml) was added to each bottle which was then shaken to dislodge any of the preparation adhering to the glass surface of the bottle and dropper. A sample (1 ml) was added to a sterile petri dish. Sterile water (4 ml) was then added to each container and after agitating a 1 ml volume was transferred to a second sterile petri dish. A suitable nutrient medium (20 ml) was added to each petri dish. The plates were incubated at 37°C and examined daily during the following 7 days. Most of the residues examined contained one of the recommended antimicrobial agents and thus the possibility existed of a 'carry-over' bacteriostatic effect. This could be expected to be partially eliminated by the dilution procedure described above. Specific neutralizing agents were included in the medium whenever this was possible. Lecithin (0.5%) and Polysorbate 80 (3%) were added to the nutrient agar when the preparations being examined contained B.A.C. L-Cysteine (0.025%) was included in the medium when the preparation being tested contained either PMN or PMA.

Colonies which were present after incubation were examined, subcultured into nutrient broth and incubated. After 24 hr a Gram-stain was carried out (Preston and Morrell, 1962) and the micro-organisms were examined microscopically.

Gram-positive cocci were subcultured into medium containing sodium chloride (10%) in order to detect the presence of staphylococci. Gram-negative rods were subcultured onto cetrimide agar plates and into cetrimide broth in order to detect contamination by pseudomonads. A motility test was carried out in some cases by making stab cultures into

nutrient broth containing 0.2% of New Zealand Agar. All micro-organisms were inoculated into peptone water and glucose and tested for acid production.

*Results*

Two hundred and eighteen containers were examined, the details of which are presented in Table 1. Approximately 33% of the formulations examined did not have an added bactericide. Contamination was found in 44% of the residues examined. The results also show that 37% of the preparations which had a bactericide included were contaminated, whereas 58% of those with no added bactericide were contaminated. The true level of contamination could be higher than is reported here as the nutrient medium and the incubation temperature could not be considered optimal for the growth of fungi.

*Discussion*

Almost all of the eye-drops examined were prepared in the Hospital Pharmacy. The method of preparation would indicate that they were adequately sterilized. Samples of each batch were subjected to a sterility testing procedure. The possibility of contamination during storage of the unopened containers seems unlikely. The percentage contaminated is very high compared with the results of a previous survey (Hugo and Wilson, 1970) where the incidence of contamination was less than 3%.

Assuming that eye-drops were sterile at the time of issue an explanation must be sought to account for the high level of subsequent contamination. The two particular aspects which merit examination are the adequacy of the bactericide being used and also

TABLE 1

SUMMARY OF EYE-DROPS EXAMINED, THEIR COMPOSITION AND THE RESULT OF THE MICROBIOLOGICAL EXAMINATION

| Name of eye-drops | Bactericide % | | Number tested | Number contaminated |
|---|---|---|---|---|
| Atropine | B.A.C. | 0.01 | 28 | 17 |
| Homatropine | B.A.C. | 0.01 | 20 | 6 |
| Phenylephrine | B.A.C. | 0.01 | 22 | 6 |
| Polymyxin | B.A.C. | 0.01 | 7 | 0 |
| Eserine | B.A.C. | 0.01 | 4 | 4 |
| Pilocarpine | B.A.C. | 0.01 | 37 | 12 |
| Lachesine | P.M.N. | 0.002 | 4 | 0 |
| Adrenaline | Chlorbutol | 0.5 | 19 | 7 |
| Idoxuridine | B.A.C. | 0.01 | 2 | 2 |
| Chloramphenicol | P.M.N. | 0.002 | 1 | 0 |
| Pilocarpine and eserine | B.A.C. | 0.01 | 2 | 0 |
| Normal saline | None | | 72 | 42 |
| Total | | | 218 | 96 |

the mode of distribution and use of the eye-drops on the wards. It was noted that of those residues which had a bactericide included 83% contained BAC. The poor performance of this substance is unexpected in view of its wide acceptance and acknowledged effectiveness. In contrast, although no definite conclusions can be drawn because of the small number examined, it should be noted that none of the 5 formulations which contained PMN as the bactericide were found to be contaminated. Development of decreased sensitivity to BAC is a common occurrence. Gram-negative micro-organisms are not very sensitive to quaternary ammonium compounds such as BAC. These two factors may explain the high level of Gram-negative contamination. The volatility and chemical instability of chlorbutol may contribute to the high level of contamination in preparations containing this substance as a bactericide. Thus it seems reasonable to suggest that an over-reliance may have been placed on the efficacy of BAC when formulating these eye-drops. A reduction in contamination might be achieved by replacing BAC as the bactericide with either PMN or PMA where compatibiliby permits and there are no other known contraindications. Prolonged continous use of the same bactericide is probably not a good practice in a hospital environment.

Regarding the distribution and use of eye-drops the BPC states that 'when the eye-drops are used in hospital wards, a separate container should be provided for each patient and when both eyes are being treated, a separate container for each eye. They should be discarded not later than one week after first opening the container'. These recommendations were not being complied with at the time the survey was conducted. Prolonged use from the same container with the consequent risk of multiple contamination obviously increases the risk of using non-sterile formulations.

Finally, 33% of the residues did not have an antimicrobial agent included, and 58% of these were contaminated. These preparations all consisted of normal saline. The incidence of contamination may seem alarmingly high but two factors must be considered before passing judgment. Firstly, eye-drop preparations which do not contain a bactericide should be used on one occasion only, and, provided this requirement is complied with, they do not constitute a hazard to the patient. Secondly, it was learned subsequently that a considerable number of the normal saline drops are used in the ear, nose and throat (ENT) unit of the hospital. In this situation the contents would be used on one occasion only and indeed sterility would not be an absolute requirement. This survey did not differentiate between residues returned from the ENT unit and those returned from normal ophthalmic use. Neither did it seek to ascertain if the latter had been used on one or more occasions.

## MICROBIAL EXAMINATION OF EYE OINTMENT RESIDUES

Eye ointments are usually prepared on a basis consisting of a mixture of paraffin and wool fat. Some formulations are prepared on a basis described as 'plastibase', which is a mixture of paraffin and polythene. When testing ointments for sterility direct inoculation does not permit uniform dispension of the sample in the nutrient medium due to their oily semi-solid properties. Two methods are commonly used to test ointments for sterility. The first method, which is recommended in the B.P. and the B.P.C., involves the addition of a sample of the ointment (1 g) to a sterile, equal parts mixture of arachis oil

and Polysorbate 80 (20 g), previously heated to 40–45°C. The resulting uniform mixture is then inoculated into the nutrient medium and incubated. The second method utilizes membrane filtration of a solution of the ointment in a suitable organic solvent. A number of workers have investigated the suitability of various solvents for this testing procedure (Sokolski and Chidester, 1964; Tsuji et al., 1970; Hambleton et al., 1972; Hambleton and Allwood, 1973; Hart and Ratansi, 1974). Despite some conflicting results the most favoured solvent is isopropyl myristate. It is shown to have a low degree of toxicity towards bacterial cells, but its toxicity increases if it is sterilized by a heat method. Thus it is recommended that isopropyl myristate should be sterilized by filtration if it is to be used for this purpose. It is an excellent solvent for ointments prepared on a paraffin-wool fat basis but does not dissolve those formulated on plastibase.

## *Materials and Methods*

### *Materials*

Isopropyl Myristate (donated by Leo Laboratories Ltd. Dublin).
Nutrient Broth (Oxoid Ltd.)
Polysorbate 80 (Croda Chemicals Ltd.)
Tryptone Soya Broth (Oxoid Ltd.)
Laminar Flow Cabinet (Centronic Europe Ltd.).

### *Methods*

The ointment residues were collected once weekly from the hospital pharmacy. The plastic caps of the tubes were swabbed with alcohol (70%) prior to removal of the sample. Approximately 1 g of the ointment was added to 50 ml of filtration-sterilized isopropyl myristate which was previously heated to 40°C. The resulting solution was filtered through a sterile membrane filter (pore diameter 0.22 μm). A sterile wash medium consisting of nutrient broth plus Polysorbate 80 (0.5%) was passed through the membrane to remove any residual oily layer. The membrane was then removed aseptically from the filter holder and transferred to pads soaked in tryptone soya broth. The pads were incubated at 32°C for 7 days and then left at room temperature for a further 7 days. The colonies present were examined and identified using the same procedure as for the eye-drops.

## *Results*

A total of 45 ointment residues were examined, details of which are presented in Table 2. Contamination was present in 36% of those examined. The contaminants isolated included Gram-positive cocci (25%), Gram-positive rods (12.5%), Gram-positive filamentous organisms (31.2%), Gram-negative bacteria (18.8%) and fungi (12.5%).

## *Discussion*

Most reports on sterility testing of eye ointments refer to unopened containers. The reported incidence of contamination has shown wide variation. Vander Wyk et al. (1958)

TABLE 2

SUMMARY OF EYE OINTMENTS EXAMINED, THE NUMBER TESTED, AND THE RESULT OF THE MICROBIOLOGICAL EXAMINATION

| Ointment | Active ingredient(s) | Number tested | Number contaminated |
|---|---|---|---|
| Atropine | Atropine sulphate | 6 | 2 |
| Florinef | Neomycin sulphate<br>Gramicidin<br>Fludrocortisone acetate | 1 | 0 |
| Graneodin | Neomycin sulphate<br>Gramicidin | 3 | 0 |
| Betnesol-N | Betamethasone sodium phosphate<br>Neomycin sulphate | 5 | 2 |
| Betnesol | Betamethasone sodium phosphate | 3 | 1 |
| Erythromycin | Erythromycin | 2 | 0 |
| Dispersa | Idoxuridine | 2 | 1 |
| Scheroson F | Hydrocortisone caproate<br>Chloramphenicol | 1 | 0 |
| Achromycin | Tetracycline HCl | 2 | 0 |
| Chloromycetin | Chloramphenicol | 14 | 7 |
| Chloromycetin Hydrocortisone | Chloramphenicol<br>Hydrocortisone acetate | 3 | 2 |
| Hydrocortisyl | Hydrocortisone acetate | 1 | 1 |
| Pimafucin | Natamycin | 1 | 0 |
| Neo-Cortef | Neomycin sulphate<br>Hydrocortisone acetate | 1 | 0 |
| Total | | 45 | 16 |

found that 85.5% of the commercially produced ophthalmic ointments which they examined were contaminated. They found that 60% of the ointments which contained antibiotics were contaminated. In another survey of antibiotic ointments using the same testing procedure Bowman and Holdowsky (1959) found only 10% to be contaminated. Subsequent surveys by Bowman (1969) and Bowman et al. (1972), employing the membrane filtration technique, found 7% and 22%, respectively, of antibiotic ointments to be contaminated. In the latter survey the overall incidence of contamination in a broad range of ointments was 19%. It seems unlikely that the vastly different results from the surveys of Vander Wyk and Granston (1958) and Bowman and Holdowsky (1959) were due to improved production methods developed in the interval between the tests. A more likely explanation is the low sensitivity of the testing procedure. Encasement of microorganisms by a film of oil may lead to poor recovery (Tsuji et al., 1970). Although the membrane filtration method using isopropyl myristate has been widely accepted for paraffin based ointments, Hart and Ratansi (1975) only achieved a 50% recovery of an inoculation of *Ps. aeruginosa* when using this method.

The results of the present survey indicate an exceptionally high incidence of contamination when compared with other surveys except that of Vander Wyk et al. (1958). However, a direct comparison of the results is scarcely valid. This survey reports on con-

tamination in partially used products whereas other surveys have been conducted on unopenened containers. In the present work the sample tested was the first portion of the ointment extracted from the tube after it had been returned from the hospital ward. This is the portion most likely to be contaminated, a point that was confirmed by the finding that in 5 cases where the initial sample was contaminated, a sample taken after most of the contents had been ejected failed to show growth of any micro-organisms. Thus it seems probable that a different sampling procedure would indicate a lower incidence of contamination. This apparent non-uniform distribution of contamination suggests that if ointments are to be used from multi-dose tubes the first portion should be rejected prior to each application.

Many of the ointments contained antibiotics which would be expected to contribute to the maintenance of sterility. An examination of the results reveals that the incidence of contamination is exactly the same for the ointments with and without antibiotics. It is interesting to note that approximately 53% of the residues containing chloramphenicol were found to be contaminated.

ACKNOWLEDGEMENTS

The authors are indebted to the Chief Pharmacist and the relevant hospital staff for their assistance in carrying out the survey. We also wish to thank the Pharmaceutical Society of Ireland for the facilities granted.

REFERENCES

Ayliffe, G.A.J., Barry, D.R., Lowbury, E.J.L., Roper-Hall, M.J. and Walker, W.M., Postoperative infection with *Pseudomonas aeruginosa* in an eye hospital. Lancet, i (1966) 1113–1117.

Bowman, F.W., Microbial contamination of antibiotic ophthalmic ointments. J. Pharm. Sci., 58 (1969) 277–278.

Bowman, F.W. and Holdowsky, S., Sterility of antibiotic ophthalmic ointment. J. Amer. Pharm. Assoc., Sci. Ed., 48 (1959) 95–96.

Bowman, F.W., Knoll, E.W., White, M. and Mislivic, P., Survey of microbial contamination of ophthalmic ointments. J. Pharm. Sci., 61 (1972) 532–636.

Hambleton, R. and Allwood, M.C., Recovery of *Bacillus megaterium* spores from white soft paraffin. J. Pharm. Pharmacol., 24 (1972) 671–672.

Hambleton, R. and Allwood, M.C., The recovery of bacteria from white soft paraffin. J. Pharm. Pharmacol., 25 (1973) 559–562.

Hart, A. and Ratansi, M.B., The isolation and enumeration of *Pseudomonas aeruginosa* from oily cream. J. Pharm. Pharmacol., 27 (1975) 142–144.

Hugo, W.B. and Wilson, J.V., In-use contamination of eye-drops. Pharm. J., 204 (1970) 714.

Kallings, L.O., Ringertz, O and Silverstolpe, L., Microbial contamination of medical preparations. Acta Pharm. Suec., 3 (1966) 219–228.

Preston, N.W. and Morrell, A., Reproducible results with the Gram stain. J. Pathol. Bacteriol., 84 (1962) 241–243.

Sokolski, W.T. and Chidester, C.G., Improved viable counting method for petrolatum-based ointments. J. Pharm. Sci., 53 (1964) 103–107.

Tsuji, K., Stapert, E.M., Robertson, J.H. and Waiyaki, P.M., Sterility test method for petrolatum based ophthalmic ointments. Appl. Microbiol., 20 (1970) 798–801.

Vander Wyk, R.W. and Granston, A.E., A Bacteriological study of ophthalmic ointments. J. Amer. Pharm. Assoc., Sci. Ed., 47 (1958) 193–196.