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The antimicrobial efficiencies of contact lens solutions

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The antimicrobial efficiencies of 34 commercially available contact lens solutions has been tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus luteus* and *Candida albicans*. A standard inoculum of 10^6 organisms ml^{-1} was placed in a sample of a contact lens solution and samples taken at various times up to 48 h. These were placed in a recovery medium and the presence or absence of growth noted after 48 h incubation at 37°. Of 14 solutions used to soak and disinfect lenses only 4 inactivated all four test strains within 1 h, 7 within 4 h, while 6 solutions allowed growth of one or more test organisms even after 24 h contact. Of the remaining 20 solutions, with their various functions such as cleaning and wetting of lenses, 13 failed to inhibit one or more test strains after 24 h contact. Some form of control of the manufacture and presentation together with minimum standards of antimicrobial efficiency would seem to be desirable.

Contact lenses are widely used to correct visual defects. Most lenses are manufactured either from polymethyl methacrylates (hard lenses) or from the hydrophilic polymethacrylates (soft lenses). Lenses are inserted and removed at least once daily, with a consequent danger of causing corneal lesions which might subsequently become infected. Various solutions are commercially available for cleaning, disinfection and wetting of lenses (Richards, 1972; Holden, 1972) and their preservation is considered necessary to reduce the risk of causing corneal infections. However, it is believed (Sibley & Yung, 1973; Browne, Andersen & Charvez, 1974) that the lenses, while soaking in the solutions, could take up preservatives which would subsequently be released into the eye causing irritation after prolonged contact, this being particularly true of soft lenses. To minimize this effect the preservative concentrations used are much lower than those for the more usual ophthalmic formulations.

The number of contact lens solutions available in the United Kingdom is expanding rapidly, at least 34 now being available. At present, there are no control requirements either for the manufacture and presentation or for the performance of these solutions. Inspection of the stated formulations led us to believe that some of these solutions might be inadequately preserved and so provide a potential hazard to the contact lens wearer. We are at present investigating various aspects of the interactions between ophthalmic preparations and contact lenses, and as a part of this study a survey of the antimicrobial efficiency of the commercially available contact lens solutions has been made.

MATERIALS AND METHODS

Contact lens solutions

Thirty-four of these were purchased from various retail outlets. A list of their stated purposes and the contained preservatives is given in Table 1. The solutions

Table 1. *The stated purpose and preservative content of commercially available contact lens solutions.*

Soaking solutions

A. Ch. 0·3% + Th. 0·004% + EDTA 0·1%
B. Bk. 0·004% + C. hex. 0·006% + EDTA 0·1%
*C. Th. 0·0025% + C. hex. 0·0025% + EDTA 0·1%
*D. Th. 0·002% + C. hex. 0·003% + EDTA 0·1%
E. Ch. 0·4% + EDTA 0·1%
F. Bk. 0·001% + EDTA
G. Bk. 0·004% + Ch. 0·4%
H. PMN 0·001% + Bk. 0·004%
*I. C. Hec. 0·005% + Th. 0·001% + EDTA 0·1%
J. Bk. 0·01%

Wetting solutions

K. Th. 0·004% + EDTA 0·1%
L. Bk. 0·004% + C. hex. 0·006% + EDTA 0·1%
*M. Th. 0·0025% + C. hex. 0·0025% + EDTA 0·1%
N. Th. 0·004% + EDTA 0·1%
O. Bk. 0·004% + EDTA
P. Bk. 0·004%
Q. Bk. 0·004%

Cleaning solutions

R. Ch. 0·5%
*S. Th. 0·004% + EDTA 0·1%
T. Zephiran + EDTA
U. Bk. 0·002%
V. Th. 0·001% + EDTA
*W. Th. 0·002% + EDTA 0·1%

Cleaning and soaking solutions

X. PMN 0·001%
*Y. Th. 0·001%
Z. Phenoxyate

Soaking and wetting solutions

AA. C. hex. 0·005%
BB. Th. 0·004% + EDTA 0·1%

Rinsing solutions

*CC. Th. 0·001% + C. hex. + EDTA 0·1%
DD. Th. 0·001% + EDTA 0·1%

Others

EE. (Ocular lubricant) Ch. 0·5%
FF. (Wetting, Soaking, Cleaning) Bk. + EDTA
GG. (Cleaning and Wetting) Bk. 0·004%
HH. (Cushioning) Th. 0·002% + EDTA 0·05%

* For use with soft lenses.
Ch.—Chlorbutol.
Th.—Thiomersal.
PMN—Phenyl mercuric nitrate.
EDTA—Ethylene diamine tetra-acetic acid.
C. hex.—Chlorhexidine gluconate.
Bk.—Benzalkonium chloride.

were of unknown age but their antimicrobial efficiency was always tested within one month of purchase.

Test organisms

Pseudomonas aeruginosa (NCTC 6750), *Staphylococcus aureus* (NCTC 6571), *Micrococcus luteus* (NCTC 8512) and *Candida albicans* (No. 3153) London School of Hygiene and Tropical Medicine were used. The organisms were maintained by subculturing on nutrient agar slopes (Oxoid) every two weeks.

Preparation of suspensions of known viability

A loopful of surface grown stock culture was streaked onto the surface of a nutrient agar slope and incubated at 37° for 48 h. A loopful of the surface growth was then transferred to 100 ml of tryptone soya broth and shaken at 120 cycles min^{-1} at 37° for 24 h. A second liquid subculture was made where 1 ml of the first liquid subculture was transferred to 99 ml of tryptone soya broth and incubated with shaking

for exactly 24 h. 1 ml of this culture was then filtered through a 0·45 μm millipore membrane filter and washed with a minimal salts medium, M9 (Tyrrell, Moss & Davies, 1972).

The filter was transferred to a tube containing 20 ml of M9 medium which was agitated to suspend the cells. The extinction of a suitable dilution of the suspension was determined at 600 nm for *P. aeruginosa* and 470 nm for the other three organisms, and by reference to calibration curves of extinction against viability the viable count was estimated. The initial suspension was then diluted to give a viability of 5×10^7 organisms ml^{-1}.

Recovery of microorganisms

The recovery medium used was of the following formula (% w/v): Tween 80 (Honeywells and Atlas) 3, 90% lecithin (BDH Ltd.) 0·2, sodium thioglycollate (BDH Ltd.) 0·1, tryptone soya granules 30·0, distilled water to 100%.

The ability of the recovery medium to inactivate the preservatives included in the solutions and to allow growth of viable organisms was tested with all solutions and with all organisms. 0·5 ml of a suspension containing about 20 organisms ml^{-1} of one of the strains was added to each of two tubes containing 9 ml of recovery medium and 0·5 ml of a contact lens solution. After mixing they were incubated for 48 h at 37°. In all cases growth occurred within 48 h showing that the medium allows the growth of an inoculum as low as 10 organisms in the presence of the contact lens solutions.

Testing of antimicrobial efficiency

A challenge of 10^6 organisms ml^{-1} was considered realistic for the assessment of antimicrobial efficiencies of contact lens solutions. Therefore 0·2 ml of a suspension containing 5×10^7 organisms ml^{-1} was added to 9·8 ml of contact lens solution in a test tube. The contents were mixed and the tubes placed in a water bath at 25°. Two 0·5 ml samples were withdrawn immediately and after 0·25, 0·5, 1, 2, 4, 24 and 48 h and added to each of two tubes containing 9·5 ml of recovery medium. The solutions were mixed and incubated at 37° for 48 h in the dark and the absence or presence of growth was then noted. If growth occurred in only one of a pair of tubes for a given contact time, the growth was streaked on tryptone soya agar plates (Oxoid) and the organism identified to check whether or not growth was due to contaminants.

RESULTS AND DISCUSSION

The efficiencies of all the solutions tested are given in Table 2, for soaking, wetting and soaking and wetting, cleaning and cleaning and soaking, rinsing and other solutions.

Soaking solutions are supposed to inhibit any contaminants introduced to the solution by the lens. While the period of soaking will frequently be overnight, this is not always the case and thus the solutions need to act in much less time. Certainly four hours would seem to be the maximum time allowable for disinfection of the solutions. If this criterion is adopted then only 5 of the 10 soaking solutions are acceptable. Kohn, Gershenfeld & Barr (1963) recommending a more stringent standard for ophthalmic preparations state "an antimicrobial substance which has a sterilizing time greater than one hour may, arbitrarily, be considered too slow acting

Table 2. *Antimicrobial efficiencies of the various solutions examined.*

Time (h)	Organism	Test solution																														
		Soaking solutions										Wetting, soaking and wetting solutions										Cleaning, cleaning and soaking solutions									Rinsing and other solutions					
		A	B	C*	D*	E	F	G	H	I*	J	K	L	M*	N	O	P	Q	AA	BB	R	S*	T	U	V	W*	X	Y*	Z	CC*	DD	EE	FF	GG	HH
0·25	*M. luteus*	−	−	+	+	−	−	−	+	+	−	+	+	+	+	−	−	−	−	+	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+
	P. aeruginosa	−	−	−	+	−	−	−	−	−	−	+	−	+	+	−	+	+	−	+	−	+	−	+	+	+	−	+	−	−	+	+	−	−	+
	S. aureus	−	−	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	−	+	−	−	+	+	−	−	+
	C. albicans	−	−	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0·50	*M. luteus*	−	−	+	+	−	−	−	+	+	−	+	−	+	+	−	−	−	−	+	−	+	−	−	+	+	+	+	−	−	−	+	−	−	+
	P. aeruginosa	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	+	−	−	+	−	+	−	+	+	+	−	+	−	−	−	+	−	−	+
	S. aureus	−	−	+	+	+	−	−	−	−	−	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	−	+	−	+	+	+	−	−	+
	C. albicans	−	−	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1·0	*M. luteus*	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	+	+	−	−	−	+	−	−	+
	P. aeruginosa	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−	−	+	−	+	−	+	+	+	−	+	−	−	−	+	−	−	+
	S. aureus	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	−	+	−	+	+	+	−	−	+
	C. albicans	−	−	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2·0	*M. luteus*	−	−	+	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−	+	−	−	+
	P. aeruginosa	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	+	−	+	−	+	+	+	−	−	−	−	−	+	−	−	+
	S. aureus	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	+	+	−	+	−	−	−	+	−	+	−	+	+	+	−	−	+
	C. albicans	−	−	−	+	+	+	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
4·0	*M. luteus*	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	+	−	+	−	−	−	+	−	−	+
	P. aeruginosa	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	+	−	+	−	+	+	+	−	−	−	+	−	+	−	−	+
	S. aureus	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−	+	+	−	+	−	−	−	+	−	+	−	+	+	+	−	−	+
	C. albicans	−	−	−	+	+	+	−	−	−	−	−	+	+	−	+	+	+	+	+	+	+	+	−	−	+	−	+	−	+	+	+	+	+	+
24·0	*M. luteus*	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−
	P. aeruginosa	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
	S. aureus	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	+	+	−	−	+
	C. albicans	−	−	−	−	+	−	−	−	−	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	+	−	+
48·0	*M. luteus*	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	P. aeruginosa	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	S. aureus	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	C. albicans	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−

* For use with soft contact lenses

for use as a preservative in a multidose ophthalmic solution". Only solutions A, B, G and J satisfy this criterion, although another solution, F, is effective against all the bacterial test strains. Of great concern is that four solutions C, D, E and I did not inhibit one or more strains even after 24 h and that three of these, C, D and I, are those recommended for soft lenses. Two other solutions AA and BB have a combined soaking and wetting role, and three, X, Y and Z a combined cleaning and soaking role. Of these only X and Z inhibit all test strains within 4 h and none do so within 1 h.

Solutions with other roles such as rinsing, wetting and cushioning should not perhaps be expected to be as efficient in inhibiting contaminants as the soaking solutions. Rinsing and wetting solutions are however used over a long period and most manufacturers recognize that they should be capable of removing chance contamination that might arise during use, using such phrases as "bactericidal" and "antiseptic" to describe their antimicrobial properties. Of the seven wetting, two rinsing and one cushioning solutions, only three have inhibited all four test strains within 24 h, none of them being capable of inhibiting all strains within 4 h.

Cleaning solutions are used to remove protein and lipid deposits and other materials. They do not, therefore aim to inhibit bacteria, but again they are all formulated with a preservative system to cope with chance contamination during use. But as can be seen from Table 2, they are not well preserved, four of the six solutions allowing growth of one or more test strains even after 24 h contact.

Two other solutions, designed to be instilled into the eye to reduce irritation and aid cleaning of hard contact lenses *in situ*, still allow growth of *Candida* after 24 h and one, EE allows the growth of *Micrococcus* and *S. aureus* as well. As these are, by definition, eye drops, they appear to be inadequately preserved as presently formulated.

To check that the results obtained truly indicate the preservative efficiencies of the stated preservatives, control solutions were prepared in our laboratory containing the following preservatives (% w/v):

benzalkonium chloride 0·004, chlorhexidine gluconate 0·004, thiomersal 0·004 and chlorbutol 0·5.

Table 3. *The antimicrobial efficiency of benzalkonium chloride 0·004%, chlorhexidine gluconate 0·004%, chlorbutol 0·5% and thiomersal 0·004% in the presence of 0·1% EDTA against* Pseudomonas aeruginosa *and* Staphylococcus aureus.

Solution	*Staphylococcus aureus* Time (h)						
	0·25	0·50	1·0	2·0	4·0	24·0	48·0
Benzalkonium chloride	—	—	—	—	—	—	—
Chlorhexidine gluconate	+	+	+	+	+	+	—
Thiomersal	+	+	+	+	+	+	—
Chlorbutol	+	+	+	+	+	+	—
	Pseudomonas aeruginosa						
	0·25	0·50	1·0	2·0	4·0	24·0	48·0
Benzalkonium chloride	—	—	—	—	—	—	—
Chlorhexidine gluconate	+	+	—	—	—	—	—
Thiomersal	+	+	+	+	+	—	—
Chlorbutol	+	+	—	—	—	—	—

Each solution was prepared using 0·9% w/v saline solutions containing 0·1% EDTA and were sterilized by filtration through a 0·45 μm filter. The antibacterial efficiencies of these individual preservatives were tested in an identical manner to the contact lens solutions using *S. aureus* and *P. aeruginosa* as the two test organisms. The results are given in Table 3 and show that benzalkonium chloride 0·004% is the most efficient antibacterial agent tested having an inhibition time of less than 15 min. Chlorbutol 0·5% and chlorhexidine gluconate 0·004% were only successful against *P. aeruginosa*. Thiomersal 0·004% required more than 4 h and more than 24 h to inhibit innocula of *P. aeruginosa* and *S. aureus* respectively.

Some discrepancies arose in the performance of different solutions containing the same stated preservative content, these may be due to two reasons. Firstly, all of these solutions are packaged in plastic containers and some of the preservative may be sorbed by the plastic (Eriksson, 1967). This could result in a reduced concentration of preservative available in the solutions and would depend on such factors as the type of plastic used and the time stored. Secondly, these solutions are complex and some contain viscolizers such as hydroxyethylcellulose and polyvinylalcohol, buffering agents, electrolytes and surfactants, all of which may influence the antibacterial performance of the preservatives.

These results show a large variation in the capacity of commercially available contact lens solutions to inactivate standard innocula of four common test organisms. Of the 14 solutions that have a soaking role, only 7 will inactivate all four test strains within 4 h. Of the remaining 20 with their various functions 13 of them still allowed growth of one or more test strains after 24 h contact. It is likely, therefore, that in some cases at least wearers are introducing contaminated contact lenses into their eyes. In 1971 two cases of *Pseudomonas* ulcers causing loss of vision in contact lens wearers were reported (Golden, Fingerman & Allen, 1971).

In view of the present proliferation in numbers and range of contact lens solutions it would appear desirable that some form of control of the manufacture and presentation be introduced together with minimum standards of antimicrobial efficiency.

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