

## BACTERIOLOGY OF THE HEALTHY CONJUNCTIVA\*

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THE normal bacterial flora of the mucous membranes of the human body have been described for many years and the nature of the common inhabitants is well established; the bacteriological findings in the healthy conjunctiva have been critically surveyed by Duke-Elder (1938). These latter results were obtained in the decades before the use of modern chemotherapeutic drugs for ocular infections became widespread, and it is important to know in what way, if any, such treatment is influencing the commensal organisms of the eye. It has been postulated that the selection pressure due to the use of antibiotics will favour the replacement of bacteria sensitive to these drugs, by strains or mutants which have become, or are, drug-resistant. Recent reports by Gibson (1951) and Barfoed (1953) suggest that these changes may be occurring.

In order to determine whether similar changes were happening in London, a survey of the bacterial flora found in 5,000 persons who were admitted for clean surgical procedures was undertaken. From such data it was hoped to determine which single antibiotic, or combination of antibiotics, was most likely to be of use in the pre-operative sterilization of the conjunctiva. Since most of the cultures reported below were taken during the years 1950-51, they do not reflect the effect of the aureomycin-chloramphenicol group which was not in widespread ophthalmic use at that time. Acquired antibiotic resistance was, therefore, restricted to penicillin, streptomycin, and the sulphonamides.

### Material

Patients cultured in this investigation were in-patients of two ophthalmic hospitals, to be called A and B, who had been admitted for operative procedures; they were fairly equally distributed between males and females and their ages ranged from 1 to 90 years. No attempt has been made to assess the effect of age and sex upon the flora of the conjunctiva.

Before culture the clinical cleanliness of the eye was assessed and a history of past antibiotic treatment was requested. This history may not have been reliable in many cases, since a patient had frequently had drops without being aware of their exact nature.

Cultures were taken by three technicians, one of whom worked throughout the period at A while the other two each worked for one year at B. An almost equal number of cultures was taken at the two hospitals, thus enabling a check to be made on bacteriological procedures at each centre.

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Whenever more than one culture was taken from a patient only the first was considered in this survey.

Media prepared at a central laboratory consisted of nutrient agar with 10 per cent. horse blood added.

Five thousand cases were investigated and in 95 per cent. cultures were taken from both eyes, hence the results are expressed per patient not as individual cultures.

### Methods

Cultures were taken either at the bedside or in the out-patient department before admission to the wards; before the cultures were taken all antibiotic treatment was suspended for a minimum of 24 hours, but other therapy was not discontinued. The material for culture was taken from the lower conjunctival fornix with a platinum loop, care being taken to avoid blinking; the material so obtained was plated directly upon a blood agar plate, half a plate being used for each eye.

The cultures were incubated aerobically for 18 hours at 37°C., and were examined by one of three bacteriologists; on any given day, however, all plates were examined by the same individual. No anaerobic cultures were made and the plates were not incubated in the presence of carbon dioxide.

The antibiotic sensitivity of all potentially pathogenic organisms was determined by the filter paper disk technique, the sterile disks being saturated in antibiotics of the following concentrations :

Penicillin	...	...	...	...	500 units per ml.
Streptomycin	...	...	...	...	200 µg. per ml.
Aureomycin and chloramphenicol	...	...	...	...	500 µg. per ml.
Albucid	...	...	...	...	0.5 per cent. solution.

As a routine procedure organisms were tested against penicillin, and those found resistant were retested against the other antibiotics. In the latter part of the investigation, "coliform" organisms were tested for sensitivity to polymyxin by a serial dilution method.

### Results

Table I shows the proportion of sterile cultures and pathogenic and non-pathogenic organisms at A and B and in total. There were less sterile cultures at A than at B; the percentage of pathogenic organisms was roughly equal. This difference is statistically significant and the cause for it will be discussed later.

The relative frequencies of the main species of bacteria isolated are set out in Table II. In Columns A and B only 4,000 of the 5,000 cases are considered, while all 5,000 cases are analysed in the total; since the numbers involved are so large it is legitimate to make comparisons between these groups. There is a close agreement between the frequencies of each species in these groups with two exceptions: *C. xerosis* and the "coliform" group. These organisms were significantly more frequent at A than at B, and account for the lower number of sterile cultures at A than at B.

TABLE I  
TYPES OF CULTURES OBTAINED

Series	A		B		Total	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
Cultures... ..	2,671	53·42*	2,329	46·58*	5,000	100
Sterile ... ..	1,132	42·38	1,219	52·34	2,351	47·02
Showing Growth ...	1,439	57·62	1,110	47·66	2,649	52·98
Pathogens only... ..	382	14·30	343	14·73	725	14·50
Non-pathogens only ...	811	30·66	577	24·77	1,388	27·66
Mixed Pathogens and Non-pathogens ...	333	12·46	203	8·72	536	10·72
Total showing Pathogens	715	26·77	546	23·44	1,261	25·22

\* Percentage of grand total; other percentages in these columns are of individual hospital totals.

TABLE II  
FLORA OF HEALTHY CONJUNCTIVA

Series	A		B		Total	
	Number	Per cent. of Cultures	Number	Per cent. of Cultures	Number	Per cent. of Cultures
Staphylococci (all) ...	726	33·90	637	34·69	1,669	33·78
Coagulase-positive staphylococci ...	—	—	—	—	397	7·94
Pneumococci ... ..	69	3·22	62	3·33	161	3·22
Haemolytic streptococci	5	0·23	8	0·43	15	0·30
Other streptococci ...	17	0·79	15	0·83	44	0·88
<i>N. catarrhalis</i> ... ..	62	2·91	51	2·74	113	2·26
<i>C. xerosis</i> ... ..	715	33·39	499	26·84	1,555	31·10
"Coliforms" ... ..	54	2·52	32	1·74	103	2·06
<i>H. influenzae</i> ... ..	12	0·56	3	0·16	18	0·36
<i>B. proteus</i> ... ..	12	0·56	—	—	19	0·38
<i>Klebsiella pneumoniae</i> ...	3	0·14	1	0·05	7	0·14
<i>Moraxella</i> ... ..	5	0·23	—	—	5	0·10

Since this discrepancy is statistically significant it is important to determine its probable cause. There seem to be three main possibilities:

(a) *Patients attending A and B drawn from different populations.*—Since both hospitals are situated in London and serve similar geographical areas it is unlikely that bacterial flora would be unlike in these groups. Furthermore, only two species of bacteria are involved and population differences would be expected to have a more widespread effect.

(b) *Techniques of taking cultures varied.*—Although every attempt was made to standardize technique, differences in the force used to draw the loop across the conjunctiva can affect the results of cultures. For example, it is known that *C. xerosis* is adherent to the conjunctival cells, so that the more cells are detached by the platinum loop the more frequently will this organism be isolated.

This seems to be the most probable reason for the greater number of isolations of *C. xerosis* at A, but it will not account for the greater number of "coliform" organisms as these are not adherent to the conjunctival cells.

(c) *Cross infection.*—While it is most unlikely that simple cross infection from conjunctiva to conjunctiva could occur, there is a means by which organisms can spread rapidly from eye to eye in ophthalmic hospitals, namely by the communal use of drops containing bacteria. The possibility that this mechanism influences the incidence of "coliform" organisms is supported by the fact that the bacteria were often isolated from several patients at the same time. It has also been our experience that "coliform" organisms are those most frequently isolated from infected eye drops.

In considering the group of organisms termed "coliform", the name has been used for Gram-negative bacteria which would grow upon MacConkey agar at 37°C. No exact differentiation into species was made and it is possible that some of these organisms may not have belonged to the genus *Escherichia*. Cason and Winkler (1954) noted that Gram-negative bacilli are being isolated with increasing frequency from the conjunctiva, and that the *Pseudomonas* and *Aerobacter* are two of the most important of these species; it is possible therefore that a number of our "coliform" organisms may belong to these genera.

A number of organisms not associated with human infections were excluded from Table II. Thus, though occasional colonies of various micrococci and *B. subtilis* were encountered, they were not considered to be true inhabitants of the conjunctival sac. It has, however, been shown that *B. cereus*, which is closely allied to *B. subtilis*, may infect the eye (Davenport and Smith, 1952).

The change in ocular flora is shown in Table III, in which the findings of workers over the past 50 years are compared. The decrease in the percentage of cultures showing organisms is most striking, particularly where Pillat's figures are concerned; but these should be treated with reserve, as they show a much higher incidence of pathogenic organisms than do any other series. Whether or not all the changes shown in Table III can be attributed to antibiotics is doubtful. It is probable that both changing hygiene

TABLE III

COMPARISON OF REPORTED INCIDENCE OF VARIOUS STRAINS OF BACTERIA IN THE HEALTHY CONJUNCTIVA (EXPRESSED AS PERCENTAGES)

Author		Rymowicz	Pillat	Von Pellathy	Gibson	Barfoed	Smith
Date		1902	1922	1932	1951	1953	1954
Organism	<i>C. xerosis</i> ...	94	100	51	30	32	31
	Staphylococci ...	87	94	68	62	43	34
	Pneumococci ...	9	41	18	—	0·8	3·22
	<i>Moraxella</i> ...	6	22	9·5	—	—	0·10

and the geographical area from which the population studied was drawn may be of importance. The part played by climate is suggested by a report from Queensland (Gibson, 1951), where 62 per cent. of healthy conjunctivae contained staphylococci, whereas in this series there were only 34 per cent., it being known that staphylococci are more prevalent in hot climates.

**Relation of Clinical Condition to Bacterial Flora.**—Although the eyes which showed minor stickiness and were classified as dirty were, in fact, less frequently sterile than the clinically clean cases, attempts to relate the pre-cultural condition of the eye to the bacterial flora were not highly successful. The relationships are set out in Table IV which shows that antibiotics markedly increase the percentage of sterile cultures in both clinically clean and dirty eyes.

TABLE IV

RELATIONSHIP BETWEEN CLINICAL CONDITION OF CONJUNCTIVA AND STERILE CULTURES

Clinical Condition	No. of Cultures	Percentage with Sterile Cultures
"Clean" ... ..	4,864	49·4
"Dirty" ... ..	136	34·56
"Clean" but previous antibiotic treatment ...	213	60·0
"Dirty" but previous antibiotic treatment ...	37	50·6

**Antibiotic Resistance of Ocular Bacteria.**—The results of sensitivity tests on potentially pathogenic organisms isolated in this survey are set out in Table V. It will be seen that of all the bacteria isolated only one was insensitive to all the antibiotics used—this was a strain of *B. proteus*.

TABLE V  
ANTIBIOTIC RESISTANCE OF CONJUNCTIVAL PATHOGENS

Resistance of Pathogens	Number	Percentage of all Cultures
Penicillin-resistant ... ..	210	4·20
Penicillin-resistant staphylococci ... ..	128	2·56
Organisms resistant to all antibiotics ... ..	1	—
Penicillin-resistant staphylococci as percentage of all staphylococci ... ..	—	14·4

Probably the most important pathogenic organism in the healthy conjunctiva is the staphylococcus; with these bacteria the increasing frequency of antibiotic-resistant strains, noted in other parts of the body, is also seen in the eye. This trend is particularly important with penicillin, to which one in six of all the staphylococci isolated was resistant, but during the period under review these penicillin-resistant strains were all sensitive to either streptomycin or aureomycin. More recent cultures than those reported here show an increasing number of strains which are becoming resistant to the newer antibiotics.

### Discussion

In general these results follow closely those of modern investigators (Gibson, 1951; Barfoed, 1953) for the conjunctival flora; the main differences being a lower incidence of staphylococci and a higher incidence of such organisms as pneumococci and coliforms. Since Gibson was reporting on the flora of Queensland, and Barfoed on that in Denmark, it is possible that these differences may merely reflect varying climatic conditions. In complete contrast are the figures given by investigators in the pre-antibiotic period (Rymowicz, 1901; Pillat, 1922; Von Pellathy, 1932; cited by Duke-Elder, 1938). Without exception these figures show a higher percentage of both pathogenic and non-pathogenic organisms (Table III), and it seems unlikely, as has been stated earlier, that this can be attributed to any one factor, even one so potent as the modern chemotherapeutic agents.

While in general the number of bacteria isolated has fallen, "coliform" organisms, which occurred in 2 per cent. of all the ocular cultures, were present more frequently in our series than in any other found in the literature except Cason and Winkler (1954). This may be due to one of two factors:—either they replace other organisms in the conjunctiva which have been destroyed by antibiotic treatment, or they may be introduced by the use of unsterile drops.

It is probable that both these mechanisms play a part, as it is known that antibiotics in the mouth cause the usual mouth streptococci to be replaced by coliforms, and there seems no reason why a similar removal of staphylo-

cocci and diphtheroids might not lead to a coliform invasion of the conjunctiva. The importance of unsterile eye drops is probably greater; this is particularly the case with fluorescein, penicillin, and cortisone, each of which provides a most suitable culture medium for Gram-negative bacilli if contaminated by them; recent outbreaks of *Ps. pyocyaneus* infection of the eye have been found to result from such contamination of eye-drops.

The antibiotic resistance encountered in this survey is lower than that found by many authors, particularly as regards staphylococci; Laurell and Wallmark (1953), reviewing the literature on this subject, quote papers in which up to 64 per cent. of staphylococci were resistant to penicillin. The figure of 14 per cent. reported here is much more in keeping with the level found in the population at large, rather than in hospital in-patients; this might be predicted since the cultures are taken soon after admission to hospital. It would be interesting to compare the sensitivities of organisms isolated from the conjunctiva before and after operation as this would provide a guide to the amount of hospital cross infection.

It seems unlikely that any single antibiotic would be satisfactory in sterilizing the conjunctiva, and combinations of antibiotics must be regarded with caution since some may be less efficacious than either component alone (Jawetz and others, 1952); apart from the risk of allergic reactions, the most useful combination would seem to be streptomycin and penicillin, and it is possible that a mixture of polymyxin and bacitracin which has a wide spectrum of antibacterial activity may be more satisfactory. Both these combinations of antibiotics are rapidly bactericidal, a point of importance in the healthy conjunctiva where the rate of multiplication of bacteria is almost certainly low and hence bacteriostatic drugs would be unlikely to produce sterility.

It is impossible to say that pre-operative cultures are no longer necessary when up to 25 per cent. of cases have potentially pathogenic organisms present, but it seems that, unless extreme care is taken in the use of eye drops in the period between culture and operation, there is a risk of replacing one pathogen by another, thus reducing the value of the pre-operative culture. To overcome this, two suggestions might be made: that cultures should be made as short a time as possible before operation, and that a combined drop of two bactericidal antibiotics with a wide range of antibacterial activity should be used if antibiotics are thought to be necessary.

### Summary

(1) The bacterial flora has changed in the last 50 years with a decrease in the frequency of isolation of both pathogenic and non-pathogenic organisms. This is probably due, in part, to the use of antibiotics.

(2) Because Gram-negative bacilli are found in the eye with increased frequency, prophylactic pre-operative drops should contain an antibiotic

active against these organisms, for example streptomycin or polymyxin.

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