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Microbiota associated with human eye infections in Taïz city, Yemen

M. A. Abdel-Sater[1,2,*], A. H. Al-Zubeiry[2] and K. A. Badah[2]

[1]Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt

[2]Department of Microbiology, Faculty of Science, Taiz University, Taiz, Yemen

*Corresponding author: e-mail: masater59@yahoo.com
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Abstract: A total of 200 specimens from infected eyes were collected and studied microbiologicaly during April 2005 to January 2006 from patients admitted in different hospitals in Taïz City. Eye infections were more frequent during April, August, September and December. 139 cases (69.5%) were infected by bacteria (126 cases) or fungi (7) or a mixture of bacteria and fungi (6). Eye infections in relation to sex revealed that female cases were higher than those of males. Infection in relation to age group indicated that those of age between 21-30 years were the most susceptible. Also, infections in relation to occupation of patients revealed that, the farmers and those who have no definite work were highly susceptible. A total of 7 fungal species related to 4 genera were identified of which *Aspegillus* species were the most prevalent. Ten species and 4 unidentified species of bacteria appertaining to 10 genera were recovered. Species of *Staphylococcus*, *Pseudomonas aeruginosa* and *Escherichia coli* were the most common agents of eye infections. Five species of fungi and 4 species and 1 unidentified species of bacteria were isolated from the mixed cases.

Key words: Keratitis, fungi, bacteria, eye diseases, microorganisms.

Introduction

Fungal eye infections are rare. However numerous fungi and bacteria are reported to infect the eye either by direct introduction through trauma or surgery, by extension from infected adjacent tissues, or by hematogenous dissemination to the eye (Manzouri *et al*. 2001, Sherwal and Verma 2008, Ibrahim *et al*. 2011)). The vast majority of cases of fungal keratitis are due to septate filamentous saprophytic fungi (Labelle *et al.* 2009, Chandel *et al*. 2011, Deorukhkar *et al.* 2012), however Zygomycetes such as *Rhizopus* spp. (Schwartz *et al*. 1978) or *Absidia* (Marshall *et al*. 1997) may also be implicated.

Several fungi have been implicated as causes of dacryocystitis or chronic granulomatous dacryocystitis, including species of *Acremonium*, *Aspergillus*, *Candida*, *Paecilomyces*, dermatophytes, *Rhinosporidium seeberi*, and *Sporothrix schenckii* (Mahajan 1992, Mislivec *et al*. 1993, Levin *et al*. 1996, Wilson and Ajello 1998).

Bacterial eye infections have been reported frequently worldwide (Dannevig *et al*. 1992). Bacterial conjunctivitis may be caused by *Staphylococcus aureus* or *Moraxella* spp. (Van Bijsterveld 1972), *Mycobacterium tuberculosis* and *Treponema palladium* (Smolin *et al.* 1984). Dannevig *et al*. (1992) and Buznach *et al*. (2005) noticed that, the most common causes of bacterial conjunctivitis in children were *Haemophilus influenzae*, *Streptococcus pneumonia*, *Staphylococcus aureus* and *Moraxella catarrhalis.*

Chaudhry *et al*. (2005) identified one hundred eighty-eight adult patients with chronic dacryocystitis. The study was predominated by female subjects (65.4%). Of the cultures from the nasolacrimal sac, 183 (97.3%) were positive for bacteria, with an average of 2.3 (1 to 6) microorganisms. More than 2 microorganisms were present in 66.1% of the cultures, whereas a single microorganism was recovered from 33.9% of the cultures. The most predominant causes were *Staphylococcus* species, *Haemophilus influenzae* and the anaerobic species *Propionibacterium acne* and *Peptostreptococcus* species.

Briscoe *et al*. (2005) found 39 positive cultures emerged 41 bacterial isolates, as 2 patients grew 2 species of bacteria. The most common isolates were related to *Pseudomonas*, *Staphylococcus aureus*, *Enterobacter*, *Citrobacter*, *Streptococcus pneumoniae*, *Escherichia coli* and *Enterococcus*. Uncommon Gram negative bacteria were also cultured, *Alcaligenes* sp. in 2 cases (5%) and one case of *Stenotrophomonas maltophilia* (2.5%), but no anaerobic bacteria were isolated.

Al-Hussaini *et al*. (2010) examined 115 cases of eye specimens and noticed that keratitis was mostly due to bacteria (50 casas) followed by fungi (30 cases) and mixed fungal and bacterial keratitis (5 cases). *Staphylococcus* and *Aspergillus* species were the most dominant etiologic agents.

To our knowledge, no report has been carried out on the microbial etiologic agents of eye infection in Taïz city, Yemen. So, the aim of the work is to characterize the fungi and bacteria causing human eye diseases and to study the prevalence of these eye

diseases in relation to sex, age and occupation of patients.

Materials and Methods

A- Clinical Diagnosis and Sampling of Infected Eye Specimens

Patients: This study was carried out during 10 month period from April 2005 to January 2006, including 200 cases (91 males and 109 females) with eye infection diagnosed by ophalmologists and admitted to one of the following hospitals: Al-Gumhoury, Al-Thawra, Al-Askary, Al-Gabaly, Al-Taawoon and Al-Noor Cityl in Taïz City with age ranged from one month to 90 years. The history for each case was recorded in a questionnaire including age, sex, occupation and antibiotic therapy.

Sampling of specimens: Specimens from the external ocular surface were collected using cotton swabs. Two swabs for each eye from the conjunctival sac were collected. Cotton swabs were used to scrub the anterior lid margins and ulcerated area (Blepharitis). Lid and canaliculus were compressed to express purulent material, which was carefully collected on a sterile spatula (dacryocanaliculitis). If pressure on lacrimal sac expresses material into conjunctival sac, it was collected as for the conjunctival swab (dacryocystitis). Swabs were used to collect corneal material. Corneal scrapes were collected with a surgical blade and directly inoculated onto the plates (Thomas 2003). Swabs were placed in sterile tubes containing Amies transport media (Cheesbrough 1992). The tubes were kept at 2-8°C and transported to the microbiology laboratory for culturing in the same day.

B- Isolation of Fungi and Bacteria

Culturing of eye specimens: The materials collected were inoculated directly into the following four culture media: (1) Blood agar which was used to grow a wide range of pathogenic bacteria. Its composition is as follows (g/l): peptone, 1; liver extract, 2.5; yeast extract, 5; sodium chloride, 5; agar, 20; pH 7.4. After autoclaving, 7% defibrinated sheep blood was added per liter of the medium. After inoculation, the plates were incubated at 37°C for 24- 48 hr for bacteria, then left for a week to detect slow growing bacteria or fungi (The Oxoid Manual 1982). (2) Chocolate agar was used to detect *Haemophilus influenzae* and other pathogens. Its composition is similar to blood agar medium except that the medium was heated at 80°C for 30 min., so the red blood cells were lyzed and the medium becomes brown in color. Inoculated plates were incubated at 37°C in a candle jar for a week (The Oxoid Manual 1982). (3) Sabouraud's dextrose agar was used to isolate pathogenic fungi from the clinical specimens. The composition of this medium is (g/l): peptone, 10; dextrose, 40; agar, 20; pH 5.2-5.6. Chloramphenicol (250 mg/l) and cyclohexamide (0.5 g) were also added to inhibit the bacterial and saprophytic fungal growth. The inoculated plates were incubated at 27°C for two weeks (Cheesbrough 1992). (4) Czapek's glucose agar was used to isolate saprophytic fungi. Its composition is as follows (g/l): KH_2 PO_4, 1; $MgSO_4.7H_2O$, 0.5; glucose, 10; agar, 15; pH 5.6. Rose bengal (1:15000) and chloramphenicol (250 mg/l) were also added as bacteriostatic agents. After inoculation the plates were incubated at 27°C for one week.

Inoculation procedure: All plates were inoculated by lightly streaking of cotton swabs over the agar surface in rows of C-streaks taking care not to penetrate the agar surface (Kwon-Chung and Bennett 1992, Benson and Lanier 1992). Growth on the C-streaks is considered to be significant, while growth off the C-streaks is likely to be caused by contamination. Scrapes were directly inoculated in the center of the plates.

C- Identification of Fungi and Bacteria

The following references were used for identification of fungal species (based on macro- and microscopic characteristics): Ellis (1971), Kwon-Chung and Bennett (1992), Cheesbrough (1992) and Moubasher (1993). Bacteria were identified using standard microbiological methods (Culture characteristics, hemolysis on blood agar, staining, motility, fermentation on culture media and biochemical tests).The following references were used for identification of bacterial isolates: Baird-Parker (1979), MacFaddin (1989), Staley *et al*. (1989), Cheesbrough (1992) and Singleton (1997).

Results and Discussion

A total of 200 specimens from infected eyes were collected and examined microbiologically. 139 (69.5% of total specimens) have either fungal (7 cases, 5.1 %) or bacterial (126, 90.7 %) or mixed infections (6, 4.3 %) (Tables 1 & 4). In agreement with the current results, Al- Hussiani *et al.* (2010) reported that keratitis cases due to bacterial infections were more common (50 out of 115 cases, 43.5%) than those due to fungal infections (30 cases, 26.1%). These results approach to those obtained by Khanal *et al*. (2005) where etiologic agents were found in 303 (67.8%) samples of which 145 (47.8%) had pure fungal growth, 103 (34%) had pure bacterial growth and 55 (18.2%) had mixed fungal and bacterial infection. Basak *et al*. (2005) studied 1198 patients with supprative keratitis over a three-year period,. Cultures were positive in 807 patients of which, 509 patients had pure fungal infections, 184 had pure bacterial infections and 114 had mixed infections.

Seasonal variations

Some regular fluctuations were seen during the months of study with the highest rate of infection being recorded in April followed by September, August and December. Infections were more frequent during the summer months. The highest number of bacterial eye infections was recorded during August, but those of fungal infections were recorded in May. The highest eye infection by polymicrobial was observed in November 2005. These months are usually characterized by high to moderate air temperature and more outdoor activities of farmers. Similar observations have been reported by Abdul-Rahman (2004) in Sana'a, who showed regular fluctuations with the highest infection being recorded during June 2002. Cases of infections were mostly more frequent during the period from April to August (Table 1). In this respect, the majority of mycotic keratitis cases was found in winter months or in spring and late fall to early winter in south Florida (Rosa *et al*. 1994). In Egypt, Alghalibi (2000) showed that, the density of etiological agents increase during the period from July to December. In India, most of the corneal ulcer cases were seen in the months of December and January, which are the harvesting seasons (Srinvasan *et al*. 1991) and in September and October (Panda *et al*. 1997). Mycotic keratitis has been reported to occur in onion harvesters in Taiwan (Lin *et al*. 1999). Butler *et al*. (2005) indicated that the peak incidence occurred in the winter months of June to August. Also, between February and May 2003 an epidemic of acute hemorrhagic conjunctivitis affected more than 200. 000 people in five geographic regions of Brazil (Moura *et al*. 2006).

Table 1: Monthly variations in eye infections.

Months	Total cases	Positive (%)	Fungal cases	Bacterial cases	Mixed cases (fungal & bacterial)
April 2005	5	100	0	5	0
May 2005	12	42	3	2	0
June 2005	8	63	0	5	0
July 2005	15	67	1	8	1
August 2005	41	73	1	29	0
September 2005	33	85	0	26	2
October 2005	20	60	0	12	0
November 2005	35	63	1	18	3
December 2005	22	73	1	15	0
January 2006	9	67	0	6	0
Total	200		7	126	6

Incidence of eye infections in relation to sex and age

Eye infections in relation to sex revealed that the total female cases were higher than those of males (54.5% versus 45.5%) (Table 2). The males were far more susceptible to fungal infection than females (85.7% versus 14.3%). In bacterial and mixed cases, males and females were equally affected. In this respect, males, particularly those older than 30 years who are engaged in agricultural activities or come from an area of endemic infection seem to be at greatest risk (Silva *et al*. 1988). Ormerod (1989) studied bacterial keratitis in 142 patients aged 65 years and over, between 1977 and 1984 at several centres in USA and found an equal sex distribution. Abdul-Rahman (2004) showed that females were slightly more affected than males with total eye infection (51.5% versus 48.5%), and bacterial eye infection (55.4% versus 44.6%). But in fungal and polymicrobial eye infections males were more susceptible than females. Isabelle *et al*. (2007) studied 1043 children and adults with purulent bacterial conjunctivitis of which 539 were males (51.7%) and 504 were females (48.3%).

The incidence of eye infection in relation to age indicated that the age group of 21-30 years were more susceptible (24.5% of total patients) followed by age group of 11-20 and 41-50 years (14.5% each). The bacterial cases were also high in age group of 21-30 years, while moderate in groups of 11-20, 31- 40 and 41-50 years. Regarding fungal infection, the age group 31-40 years showed the highest percentage while age groups less than one year, 11-20, 21-30, 41-50 and 71-80 years showed moderate infection. The rest age groups of 1-10, 51-60, 61-70 and 81-90 years were not infected by fungi (Table 2). Age groups 1-10 and 21-30 years were predominated by mixture of fungi and bacteria (33.3% each), while, age groups of 31-40 and 51-60 years were represented by 16.7 % (Table 2). Musch *et al*. (1983) showed a bimodal age distribution for infective keratitis with two distinct peaks of

incidence, one around the age of 30, and another at 70 years. Infective keratitis in the elderly population was studied in the United States (Ormerod 1989) and southern India (Kunimoto *et al*. 2000). The most susceptible persons with keratitis were adults over 30 years of age (83.2% of total cases) with the highest number of cases being found among patients 41-50 years of age (20.8% of total cases). The disease was less frequent among children less than 10 years of age. Also, Alghalibi (2000) found that within the different age groups, the number of cases of bacterial ulcers was generally greater than that of fungal or mixed cases and the age groups 41-50 and 51-60 comprised the highest number of microbial keratitis cases. Andreou and Rose (2002) showed the ages between 19 and 85 years had either single or multiple dacryoliths.

Abdul-Rahman (2004) observed that the most susceptible age group was 11-20 years (16.0% of total cases) and the disease was less frequent among people more than 81 years age. Within the different age groups the number of bacterial cases was generally greater than that of fungal or mixed cases and the age group 11-20 years was the most susceptible to bacterial infection, whereas age group 41-50 years was the most susceptible to fungal infection. More recently, Al- Hussaini *et al.* (2010) observed that the most susceptible persons to keratitis were adults of age 31- 70 years. Also, keratitis in general showed a correlation with sex of patients where males were more often contracted the disease than females.

Table 2: Incidence of eye infections in relation to sex and age of patients.

Age group (year)	Total cases				Fungal cases				Bacterial cases				Polymicrobial cases			
	M	F	Total	%	M	F	Total	%	M	F	Total	%	M	F	Total	%
< Year	5	5	10	5	1	0	1	14.3	4	4	8	6.3	0	0	0	0
1--10	13	10	23	11.5	0	0	0	0	7	4	11	8.7	1	1	2	33.3
11--20	13	16	29	14.5	1	0	1	14.3	9	9	18	14.3	0	0	0	0
21--30	22	27	49	24.5	1	0	1	14.3	14	16	30	23.8	1	1	2	33.3
31--40	14	11	25	12.5	2	0	2	28.6	10	6	16	12.7	1	0	1	16.7
41--50	13	16	29	14.5	1	0	1	14.3	9	11	20	15.9	0	0	0	0
51--60	4	10	14	7	0	0	0	0	4	6	10	7.9	0	1	1	16.7
61--70	3	9	12	6	0	0	0	0	3	5	8	6.3	0	0	0	0
71--80	2	3	5	2.5	0	1	1	14.3	1	0	1	0.8	0	0	0	0
81--90	2	2	4	2	0	0	0	0	2	2	4	3.2	0	0	0	0
Total	91	109	200	100	6	1	7	100	63	63	126	100	3	3	6	100
Percentage	45.5	54.5			85.7	14.3			50	50			50	50		

M = Male, F = Female.

Incidence of eye infections in relation to occupation

Farmers and persons with no definite work were noticably susceptible (21.5% of total cases each). Students were represented by 20% of total cases, followed by housewives and children (Table 3). Fungal infections appeared more frequently in employees (42.9% of total cases), housewives, persons with no definite work, students and children were represented each in 14.3%. Fungal infections of eyes were not detected in farmers, retireds, policemen and drivers. From 126 bacterial cases, 32 were farmers, 29 from those of no definite work and 26 students. Housewives (11.1%) and children (10.3%), followed by employees (6.3%) and drivers (1.6%), but 0.8% of each of retireds and policemen were infected by bacteria (Table 3). Six cases were infected by mixture of bacteria and fungi and these were farmers, children and persons with no definite work (2 cases each) (Table 2).

Filamentous fungal keratitis appears to occur most commonly in healthy young men engaged in agricultural work or outdoor occupations (Rosa *et al*. 1994, Gopinathan *et al*. 2002). Purulent bacterial conjunctivitis may also cause epidemics among people in close quarters, such as in nursery, school and student populations (Martin *et al.* 2003). In agreement with the current results, Abdul-Rahman (2004) reported that the majority of eye infection in Yemen were housewives who help in agricultural activities (31.6% of total cases), followed by farmers who live in rural areas (18.9% of total cases), while students children and workers accounted for low percentages. Also, our results were basically similar to those obtained by Al-Hussaini *et al.* (2010) who noticed that most patients with keratitis were farmers (40 %) followed by housewives (16.5 %).

Table 3: Incidence of eye infections in relation to occupation of patients.

Occupation	Total cases		Fungal cases		Bacterial cases (one or two species)		Polymicrobial cases (Fungi & Bacteria)	
	No.	%	No.	%	No.	%	No.	%
Farmers	43	21.5	0	0	32	25.4	2	33.3
No definite work	43	21.5	1	14.3	29	23.0	2	33.3
Students	40	20	1	14.3	26	20.6	0	0
Housewives	28	14	1	14.3	14	11.1	0	0
Children	26	13	1	14.3	13	10.3	2	33.3
Employees	14	7	3	42.9	8	6.3	0	0
Policemen	2	1	0	0	1	0.8	0	0
Drivers	2	1	0	0	2	1.6	0	0
Retired	2	1	0	0	1	0.8	0	0
Total cases	200		7		126		6	

Fungal eye infections

A total of 7 species represented by 13 isolates were isolated using Sabouraud's dextrose agar and Czapek's dextrose agar at 27°C (Table 4). *Aspergillus* (represented by 4 species) with *A. flavus* and *A. oryzae* were the most common where the former was isolated from 4 cases and the latter from 3 cases. *Candida albicans* was the second most common fungus associated with 2 cases. *Cochliobolus lunatus* and *Penicillium griseofulvum* were also isolated but less freqently each from one case (Table 4). In this respect, Thomas *et al.* (2007) reported two cases of fungal endophthalmitis due to *C. albicans. C. albicans* was reported also as the most common cause of endogenous endophthalmitis or keratitis (Forster 1994, Tanure *et el*. 2000), and has been infrequently isolated in most previous studies in tropical countries (Dunlop *et al*. 1994, Srinivasan *et al*. 1997, Leck *et al*. 2002 and Gopinathan *et al*. 2002), possibly due to the predominance of livelihoods, such as agriculture, which carry a higher risk for the occurrence of trauma-related keratitis caused by filamentous fungi than for keratitis due to *C. albicans* (Santibanez *et al*. 2005 and Buchanan *et al*. 2006). *Fusarium solani* and *Aspergillus flavus* constituted up to one-third of cases of traumatic infectious keratitis in the study of Liesegang and Forster (1980). *Penicillium* spp. have also caused endogenous endophthalmitis in an intravenous drug abuser (Swan *et al*.1985). *A. flavus* was also recovered from 6 out of 65 cases (Kalavathy *et al*. 1986). Fungi were found to account for 5% (Brook and Frazier 1998) to 14% of eye infections (Ghose and Mahajan 1990). Ocular trauma, diabetes mellitus and prolonged topical medications appeared to predispose to infections by fungi (Rosa *et al*. 1994, Tanure *et al.* 2000). Fungi such as *Alternaria* spp., *A. fumigatus* and other *Aspergillus* spp., *Candida* spp., dermatophytes, *Fusarium* spp., *Penicillium* spp., *Scopulariopsis* spp., and *Sporothrix schenckii* have been reported as causes of canaliculitis (Levin *et al*. 1996, Ruggli *et al*. 1997 and Behrens-Baumann 1999). Wilson and Ajello (1998) listed some 105 species in 35 genera of fungi as causes of keratitis and other ophthalmic mycoses including species of *Fusarium*, *Aspergillus* and *Curvularia*. Also, most of the studies done exclusively on mycotic keratitis, filamentous fungi revealed that *Fusarium* spp. or *Aspergillus* spp. to be the most frequent. However in a review of 32 patients with keratitis, *Curvularia* spp. were the most frequent (Wilhelmus and Jones 2001). Dematiaceous fungi have been reported to be the third most frequent cause of mycotic keratitis (behind *Aspergillus* and *Fusarium*) in the studies of Thomas (1990), Panda *et al*. (1997), Srinivasan *et al*. (1997), Garg *et al*. (2000), Gopinathan *et al*. (2002) and Leck *et al*. (2002). Many species of *Aspergillus* have been reported to cause endophthalmitis, but *A. flavus* is probably the most common, followed by *A. nidulans* (Tong *et al*.1990), *A. fumigatus*, *A. niger*, *A. terreus* and *A. glaucus* (Stevens *et al*. 2000). *Aspergillus* was also the most common cause of fungal keratitis in Upper Egypt. It was recoverd from 46 cases, contributing 65.7% of total fungi. *A. terreus* was the most predominant species followed by *A. flavus*, *A. niger* and *A. fumigatus* (Alghalibi 2000). Also, recently, Al- Hussaini *et al.* (2010) could isolate 12 fungal species from 30 fungal keratitis in Upper Egypt of which species of *Aspergillus*, *Candida* and *Cladosporium* were the most common.

Bacterial eye infections

The microbiological analysis of specimens showed that 126 cases (63% of total cases) were due to bacteria. Of these 120 cases were due to single bacterial species whereas 6 cases were due to two bacterial species. Bacterial species were also involved with fungal species in 6 cases and all bacteria in these cases were of Gram positive type (Table 4).

Table (4): Fungal and bacterial species recovered from eye infections specimens.

Organisms	No. of positive cases	Percentage %
Pure fungi	7	5. 1
Aspergillus awamori Nakazawa	1	o.7
A. flavus Link	2	1.4
A. niger van Tieghem	1	0.7
A. oryzae (Ahlb.) Cohn	2	1.4
Candida albicans (Robin) Berkhout	1	0.7
Pure bacteria (one species)	120	86. 3
Total Gram – positive cocci	100	71.9
Staphylococcus	95	68.4
S. aureus Rosenbach	62	44.6
S. epidermidis (Winslow & Winslow) Evans	3o	21.6
S. saprophyticus (Fairbrother) Shaw, Stitt & Cowan	3	2.2
Streptococcus	4	2.9
St. viridans (group)	2	1.4
St. pyogenes Rosenbach	2	1.4
Enterococci	1	0.7
Total Gram-positive bacilli	4	2.9
Bacillus cereus Frankland & Frankland	3	2.2
B. coagulans Hammer	1	0.7
Total Gram-negative bacilli	15	10.8
Pseudomonas aeruginosa (Schroeter) Migula	7	5.0
Escherichia coli (Migula) Castellani and Chalmers	4	2.9
Alcaligenes sp.	2	1.4
Acinetobacter sp.	1	0.7
Proteus mirabilis Hauser	1	0.7
Nocardia sp. (Actinomycetes)	1	0.7
Mixed bacteria (two species)	6	4.3
S. epidermidis + S. aureus	1	0.7
S. epidermidi + P. aeruginosa	1	0.7
S. saprophyticus + S. epidermidis	1	0.7
S. epidermidis + Alcaligenes sp.	1	0.7
S. aureus + St. viridans	1	0.7
S. aureus + P. aeruginosa	1	0.7
Mixed (fungi + bacteria)	6	4.3
A. flavus + Enterococci	1	0.7
A. flavus + St. viridans	1	0.7
A. oryzae + St. viridans	1	0.7
Cochliobolus lunatus Nelson and Hasis + *S. saprophyticus*	1	0.7
Penicillium griseofulvum Thom + *S. aureus*	1	0.7
Candida albicans + S. epidermidis	1	0.7
Total	139	

A total of 10 species and 4 unidentified species belonging to 10 genera of bacteria were recovered from different specimens of eye infections (Table 4). Of these, Gram positive cocci were the predominant found in 100 cases (71.9% of the positive cases), while only 15 and 4 cases were related to Gram negative bacilli and Gram positive bacilli, respectively. *Staphylococcus* was the most frequent infecting 68.4 % of total positive cases. It was represented by 3 species of which *S. aureus* was found in 44.6% of total bacterial cases followed by *S. epidermidis* (21.6 %) and *S. saprophyticus* (2.2%). These results are similar to those obtained by Vajpayee *et al.* (1998) who found that *S. aureus* (14/32; 43.7%) and the coagulase negative staphylococci (12/32; 37.5%) were the most common from corneal ulcer specimens. In the United States, coagulase-

negative staphylococci are responsible for about 70% of post-cataract surgery endophthalmitis, followed by *S. aureus*, viridans group streptococci, other Gram positive microorganisms, and Gram negative microorganisms (Brook and Frazier 1998 and Behrens-Baumann 1999). Similar results were reported by Al- Hussaini *et al.* (2010) who identified 7 bacterial species of which species of *Staphylococcus* were the most common agents of human keratitis in Upper Egypt. The majority of bacteria isolated from patients with infective keratitis were Gram positive organisms (72%) and the most common were coagulase negative *Staphylococcus* (16%), *Propionibacterium acnes* (14%), *S. epidermidis* (11%), and *Streptococcus pneumoniae* (9%) (Wong *et al*. 2003). Coagulase-negative *Staphylococcus* was the most common species isolated from keratitis specimens, followed by *S. aureus*, *Corynebacterium* spp., *P. aeruginosa*, *Moraxella* sp., *S. pneumonia* and fungal keratitis (Leibovitch *et al*. 2005, Ly *et al*. 2006).

Streptococcus (*S. viridans* and *S. pyogenes*) was the second most common of Gram positive cocci type causing infection. It was involved in 2.9 % of the positive cases. *P. aeruginosa* and *Escherichia coli* were the most common Gram negative bacilli isolated. These species appeared in 7 and 4 cases, respectively. In addition, 6 cases were due to two different bacterial species (mixed with either Gram +ve or Gram –ve species) (Table 4). *Pseudomonas* keratitis was associated with the wearing of hard and soft extended-wear contact lenses (Butrus *et al*. 1987, Bourcier *et al*. 2004) or poor outcomes including loss of the eye (Cruz *et al*. 1998). In agreement with the current results, Coden *et al*. (1993) reported that positive cultures of specimens from patients with dacryocystitis were obtained in 52.5%. Cultures consist of a single organism in 71%, mixed in 29%, and Gram positive organisms accounted for 64.5% of the isolates with *S. epidermidis* and *S. aureus* being the most frequent. Gram negative organisms were present in 27.3% of the isolates with *Pseudomonas aeruginosa* the most common, accounting for 8.7% overall. *Bacillus cereus* ranked second behind staphylococci in prevalence and some cases are polymicrobial in post-traumatic endophthalmitis (Thompson *et al*. 1993, O'Brien and Choi 1995 and Kunimoto *et al*. 1999). *Bacillus* endophthalmitis is a highly explosive infection of the eye that commonly results in rapid inflammation and vision loss, if not loss of the eye itself, within a few days (Callegan *et al*. 2005). *S. aureus*, *B. cereus*, *Escherichia coli*, *Neisseria meningitidis*, and *Klebsiella* spp. were also common causes of endogenous bacterial endophthalmitis (Tseng *et al*. 1996 and Romero *et al*. 1999). Chaudhry *et al*. (2005) found that 183 (97.3%) out of 188 adult patients infected by nasolacrimal sac were positive for bacteria, with an average of 2.3 (1 to 6) microorganisms. More than 2 microorganisms were present in 66.1% of the cultures, whereas a single microorganism was recovered from 33.9% of the cultures, and the majority of microorganisms were Gram positive bacteria, representing 53.7% of the overall microorganisms with a predominance of *Staphylococcus* species. Gram negative bacteria were recovered from 26.0% of the specimens with predominance of *Haemophilus influenzae*. Anaerobic microorganisms were present in 19.1% of the samples (Chaudhry *et al*. 2005). The most common isolates from purulent dacryocystitis were *Pseudomonas* (22%), *S. aureus* (13%), *Enterobacter* (10%), *Citrobacter* (10%), *S. pneumoniae*, *E. coli*, and *Enterococcus* (7%) (Briscoe *et al*. 2005), whereas from purulent conjunctivitis were *S. epidermidis* followed by other coagulase-negative *Staphylococci*, *S. aureus*, *Haemophilus*, *S. pneumoniae*, *Enterobacteriaceae* and *Acinetobacter*, and in children under 12 years old, a higher prevalence of *Haemophilus* and *S. pneumoniae* was reported (Isabelle *et al*. 2007). Also, *P. aeruginosa* followed by *S. aureus* were the most common species from corneal scrapings (Pachigolla *et al*. 2007).

Mixed eye infections

Five species of fungi and 4 species and 1 unidentified species of bacteria were isolated from mixed cases (Table 4). *A. flavus* together with *S. viridans* appeared in 2 cases (16.7%). Other bacterial or fungal species were isolated from one case each (Table 4). In this respect, 33% of culture positive cases were due to one organism, placing the rate of mixed infection among the highest reported (McLeod *et al*. 1996, Levey *et al*. 1997 and Schaefer *et al*. 2001). A laminated concretion (dacryolith) may develop in the lacrimal sac and is often associated with bacterial and fungal infections (Thomas 2003). mixed infection (from 1 to 6 organisms) from sever infective keratitis was identified in 25 out of the 75 culture positive cases (33%) (Wong *et al*. 2003). Laspina *et al*. (2004) found out that of the 524 (79%) positive cultures from patients with corneal ulcers, 267 were due to bacteria (51%), 136 to fungi (26%), and 121 (23%) cultures yielded both fungi and bacteria. Also, out of 172 patients with corneal ulcer, only 28 were infected with organisms of Gram +ve cocci (16 cases), Gram +ve bacilli (5), Gram -ve bacilli (5) and multiple types (2) (Butler *et al*. 2005). However, in the study of Chaudhry *et al*. (2005) more than

2 microorganisms were present in 66.1% of the cultures, whereas a single microorganism was recovered from 33.9% of the cultures. On the other hand, in a series of 110 cases of infectious keratitis studied by Sridhar *et al.* (2006) between 2001 and 2005, six cases of bacterial co-infection in keratomycosis were mixed with *Fusarium* spp. The current results are in harmony with those obtained by Al- Hussaini *et al.* (2010), who reported that only 5 cases (out of 115 cases) were caused by a mixture of bacterial and fungal agents.

Recommendation: Microbial eye infections can be preventable diseases; where many factors that enhance the disease could be controlled or reduced by: 1) Enhancing public health education for the early management of ocular surface fungal or bacterial infections, 2) Early treatment with a proper antibiotic, 3) Prevention of fungal and bacterial eye infections in Yemen requires major commitment and efforts from governmental agencies non- governmental organizations (NGO) and individuals. Ophthalmologists should also be aware of: 1) The drug of choice to be used, since an early eye infection most likely is due to Gram positive bacteria, 2) Possible diagnosis of fungal or bacterial infection of the eye and, microbiological investigations should be performed for all cases as soon as possible to allow prompt diagnosis and treatment. Further study is required to cover other agents causing eye infection in Yemen will be of a great value to the Yemeni Society.

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