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Clinicobacteriological study of chronic dacryocystitis in Egypt

Sahar Negm[1*], Amal Aboelnour[2], Tarek Saleh[3], Mohamed Yasser[4] and Olfat Hassanin[4]

Abstract

Background: Empirical antibiotic treatment is a common practice to manage chronic dacryocystitis in many healthcare settings in spite of the probability for changing in the types of microbial isolates.
The aims of this study were to find out the current clinicomicrobiological profile of adult cases with chronic dacryocystitis and to determine the antibiogram of the isolated organisms to the commonly prescribed antibiotics.

Results: Of the 25 samples obtained, 15 (60%) yielded a positive culture, 12 (48%) showed single bacterial isolate while 3 (12%) had mixed (two types) bacterial isolates.
A total of 18 different strains of microorganisms were obtained from 25 cases, with 12/18 (66.7%) Gram-positive, 5/18 (27.7%) Gram-negative isolates, and 1/18 (5.5%) was fungal isolate. Coagulase-negative staphylococci were the most frequently found Gram-positive bacteria (22.2%), while *Klebsiella* species was the predominant of Gram-negative bacteria (16.6%). The majority of the isolated bacterial strains were sensitive to gatifloxacin (88%) and amikacin (88%) while the main resistance of the bacterial isolates, recovered from chronic dacryocystitis, was to cephalexin (59%).

Conclusion: There is a continuous possibility of changing the type of pathogens responsible for dacryocystitis as well as their susceptibility to antibiotics. Microbiological study with microbial culture and antibiotic sensitivity test has to be done to all cases of chronic dacryocystitis for a better choice of antibiotic prophylaxis and treatment options, and to guard against the emergence of more drug-resistant strains.

Keywords: Antibiotic susceptibility, Aminoglycosides, Nasolacrimal duct, Staphylococci

Background

Dacryocystitis is an infection of the nasolacrimal sac, frequently occurs because of obstruction of the nasolacrimal duct that can affect patients of any age (Bharathi et al. 2008). Obstruction of the nasolacrimal duct leads to stagnation of tears and mucous secretion in the lacrimal sac and the lacrimal drainage system which ends with dacryocystitis with clinical presentations include pain, redness, swelling over the inner side of the lower eyelid, and epiphora with or without purulent discharge (Bharathi et al. 2008). The etiology of nasolacrimal duct obstruction may be primary idiopathic stenosis (Mills et al. 2007), usually in elder female and middle age (Hartikainen et al. 1997). It may be secondary, related to a malformation of the tear duct, injury, eye infection, neoplasm, or trauma. However, simple stenosis with epiphora may be tolerable by a large number of patients for many years (Hartikainen et al. 1997).

The chronic form of dacryocystitis is associated with chronic tearing, thickening of the lacrimal drainage system, and accumulation of germs, usually the majority of patients harbor multiple microorganisms. It is a constant threat to the cornea and orbital tissue. Complications of dacryocystitis include fistula, corneal ulcer, and orbital cellulitis; moreover, it causes social embarrassment due to long-lasting epiphora (Badhu et al. 2006; Mandal et al. 2008; Kebede et al. 2010; Huber-Spitzy et al. 1992; Das et al. 2008a; Chaudhry et al. 2005).

Although dacryocystitis is a common problem, there have been few studies on the adult lacrimal duct obstruction LDO, from a microbiological investigation point of view during the past 20 years. These studies demonstrated that, *Staphylococcus epidermidis* and *Staphylococcus aureus* are the most commonly isolated bacteria in lacrimal sac infections in the adult (Hartikainen et al. 1997). However, there is a probability for

* Correspondence: saharnegm1@hotmail.com
[1]Department of Microbiology and Parasitology, Research Institute of Ophthalmology, El Ahram Street, (RIO), Giza 12611, Egypt
Full list of author information is available at the end of the article

changing in the types of microbiological isolates in dacryocystitis. Some recent reports have indicated more common isolation of Gram-negative organisms, while other studies have noticed increased numbers of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. This information could affect the treatment of dacryocystitis markedly (Mills et al. 2007). *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* have been found to be common pathogens in children, and *S. epidermidis*, *S. aureus*, *S. pneumoniae*, and *Pseudomonas aeruginosa* are the most frequent microorganisms in adults (Eslami et al. 2018).

This study aims to identify the microbial etiology and to demonstrate the antibiogram of microbial isolates to commonly used antibacterial agents, and to find out the current clinicobacteriological profile of chronic dacryocystitis in adults.

Materials and methods

A prospective study to 25 patients with chronic dacryocystitis who were submitted to external dacryocystorhinostomy (DCR) for treatment of nasolacrimal duct obstruction (NLDO) was carried out, and microbiological analyses to 25 lacrimal sac contents were done. The study was carried out during the period from March 2012 to March 2014, in the Research Institute of Ophthalmology (Microbiology and Immunology Unit together with Oculoplastic Unit), and had an agreement of the medical research committee.

Patients

Patients attending the oculoplastic clinic were first examined by an ophthalmologist, and dacryocystitis was clinically defined, examined, and diagnosed as chronic dacryocystitis, based on their history, signs, and symptoms; epiphora for long period, and presence of mucoid or mucopurulent material coming out on pressure over the sac area or during lacrimal drainage system irrigation. Patients presenting with tenderness, erythema, and swollen lacrimal sac area were excluded from the study; they were diagnosed as acute dacryocystitis.

Inclusion criteria

All patients who were submitted to external DCR for chronic dacryocystitis due to primary acquired NLDO.

Exclusion criteria

Cases younger than 20 years were not included in this study. All cases with a history of topical or systemic antibiotic administration 1 week ago before surgery and before sample collection were excluded. Acute dacryocystitis cases, cases with a past history of infection, inflammatory nasal or sinus disease, endonasal surgery, and maxillofacial surgery were excluded.

All patients diagnosed as dacryocystitis due to a cause other than primary acquired nasolacrimal duct obstruction through detection of obstruction on syringing and probing or on performing anteroposterior and lateral dacryocystography using lipiodol or detected with endonasal endoscope, canalicular obstruction; canalolithiasis; lacrimal system tumor; previous trauma to the ocular and nasal regions; bony deformity; abnormal intranasal anatomy, were excluded from the study in addition to advanced deviated nasal septum, middle turbinate (MT) hypertrophy, or concha bullosa, and nasal polyps.

All patients who had a history of any lacrimal operative procedures in the past were excluded from the study.

Collection of samples

Standard operating procedures were followed to all cases during sample collection. External-DCR were done under general anesthesia by two oculoplastic surgeons and material (lacrimal sac cultures) was obtained directly from the lacrimal sac content while making sac flap using sterile cotton wool swabs (so as to avoid the risk of sample contamination). Great care was taken to guard against any possible contamination of the specimens. It was sent promptly to the microbiology laboratory for immediate processing. The specimens were cultured and results were analyzed.

Bacterial isolation and sensitivity test

Inoculation of specimens was done following standard procedures (Cheesbrough 2006). The material of lacrimal sac content was inoculated directly onto the different media: blood agar, chocolate agar, MacConkey's agar, Sabouraud's dextrose agar, and brain heart infusion broth. The sample obtained was also used to make direct Gram's staining. The inoculated media were all incubated aerobically at 37C for 24 h, examined daily, and finally declared as culture negative after 5 days if no organism had grown. Chocolate agar plates were incubated in CO2 to ensure 5–10% CO2.

Significant microbial growth was considered when positive culture was obtained for the same organism in more than one type of solid media and, or there was heavy growth in one solid medium at the site of inoculation and, or if positive culture in one medium gave the same finding of direct film by microscopy. Positive growth culture was followed by bacterial identification according to colony morphology, Gram staining, pigments, and biochemical reactions. Antimicrobial susceptibility tests were applied to all bacterial isolates according to the standard disc diffusion method (Hudzicki 2013).

The following antibiotic disks were used: Gentamicin (10 μg), Amikacin (30 μg), Tobramycin (30 μg), gatifloxacin

(5 μg), ciprofloxacin (30 μg), levofloxacin (5 μg), ceftazidime (30 μg), cephalexin (30 μg), cefotaxime (30 μg), and chloramphenicol (30 μg). The results of the sensitivity test were recorded as sensitive or resistant.

Standardized bacterial suspension for bacterial inoculation in antibiotic sensitivity test was prepared by choosing 4–5 isolated colonies with the same shape and size, and by adding them to 5 ml of brain–heart infusion broth to form a homogenous suspension, allowing to incubate at 37 °C until it is slightly visibly turbid (about 4 h). The turbidity of the suspension was adjusted to the optical density of a 0.5 McFarland tube (0.14–0.15 nm). The plates were inspected for inhibition zones after 24 h of incubation. Sensitivity pattern interpretation was done using the specific chart according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI) (Wayne 2017).

Detection of fungal agents

Two specimens of the material of lacrimal sac content were used for fungal detection. Wet preparation in 10% KOH was done using the first specimen. Inoculation of plain Sabouraud's dextrose agar medium (SDA) was done using the second specimen. The inoculated Sabouraud's dextrose agar plates were incubated at 27 °C, examined daily, and discarded at 14 days if no growth was seen in order not to miss slow-growing fungi. True growth was detected on the streak lines of inoculation, and any growth outside that lines was considered contaminations. Fungal growth was identified microscopically by lactophenol cotton blue stain, grossly identified from above, by colony morphology, and by pigment production on reverse (Cheesbrough 2006).

Antifungal susceptibility tests for fungal isolates were done using disc diffusion method: voriconazole (1 μg), fluconazole (25 μg), itraconazole (10 μg), ketoconazole (50μg) metronidazole (50 μg), and amphotericin B (20 μg). The results of susceptibility were recorded as sensitive or resistant.

Results

Samples were obtained from 25 adult patients with chronic dacryocystitis and sent to the microbiology laboratory for evaluation. Female predominance was noticed among dacryocystitis infections. The results of the aerobic and anaerobic (bacterial) cultures are presented in (Table 1). Of the 25 samples, 15 (60%) yielded a positive culture for different types of bacterial pathogens and 10 (40%) yielded no growth. Of the 15 positive culture samples, 12 (48%) showed single bacterial isolate while 3 (12%) had mixed two types of bacterial isolates. Single eye was infected in all patients. There were 18 total culture isolates (Table 2), with 12/18 (66.7%) Gram-positive, 5/18 (27.7%) Gram-negative isolates, and 1/18 (5.5%) were fungal isolates. The most common organisms were Gram-positive bacteria. Nine samples were Gram-positive bacteria accounting for 80% of the 15 positive cultures samples and 36% of all the samples. Three of the 15 positive culture samples had two different Gram-positive bacterial isolates. By far, the most common bacterial isolates were coagulase-negative staphylococci CNS and *Streptococcal pneumococci* where each were recovered in 4 (16%) of all the samples accounting for 22.2% of all the isolates. *Staphylococcus aureus* was isolated from 3 samples (12%), and they represented 16.6% of the isolates. Gram-negative bacteria were isolated from 5 samples (20%), and they represented 27.7% of the isolates. The majority of Gram-negative bacterial isolates were Klebsiella and Pseudomonas, each represented 16.6% and 11% of the isolates respectively. Fungal organisms (*Candida*) were detected in 1 (4%) sample, which accounted for 5.5% of the isolates.

The highest antibiotic susceptibility of all Gram-positive and Gram-negative bacterial isolates from chronic dacryocystitis was to gatifloxacin and amikacin, both 15/17 and 88.8%, while the lowest susceptibility was to cephalexin 9/17, (53%). The majority of the Gram-positive organisms were sensitive to gatifloxacin (91.6%) followed by amikacin (83.3%), while the majority of the Gram-negative organisms were sensitive to amikacin (100%) followed by gatifloxacin and cefotaxime (80%) each.

Discussion

The lacrimal excretory system is connected with the conjunctiva and nasal mucosa via its mucous membrane lining sharing their colonies of normal bacterial flora. Obstruction of the nasolacrimal duct will lead to the

Table 1 The number and percentage of the eyes (specimens) with positive growth and no growth

Growth pattern	Total no. of eyes	Percentage
Total no. of eyes (specimens)	25	100
Total no. of eyes (specimens) with negative cultures	10	40
Total no. of eyes (specimens) with positive cultures	15	60
Total no. of eyes (specimens) with one bacterial isolate	12	48
Total no. of eyes (specimens) with two bacterial isolates	3	12
Total no. of microbial isolates	18	72

Table 2 The number and percentage of bacterial isolates from dacryocystitis cases

Name of the bacterial isolate recovered	Number of organisms	% of positive cultures ($n = 15$)	% of all organisms ($n = 18$)	Total no. of positive samples	% of all culture samples ($n = 25$)
Total Gram-positive cocci:	12	80	66.7	9	36
Staphylococcus aureus	3	20	16.6	3	12
Coagulase-negative Staphylococci	4	26.6	22.2	4	16
Streptococcus pneumonia	4	26.6	22.2	4	16
Streptococcus viridans	1	6.6	5.5	1	4
Fungal organisms: *Candida species*	1	6.6	5.5	1	4
Total Gram-negative bacilli	5	33.3	27.7	5	20
Pseudomonas species	2	13.3	11	2	8
Klebsiella species	3	20	16.6	3	12
Total Gram-positive and Gram-negative	18	120	100	25	72

stagnation of tears, mucoid secretions, and desquamated cells. This constitutes a favorable condition for secondary bacterial infection (Bharathi et al. 2008).

Women are more commonly affected by primary nasolacrimal duct obstruction. This was also the case in this study. Women have smaller lower nasolacrimal fossa and middle nasolacrimal ducts as proved by measuring the bony nasolacrimal system; this may explain the higher incidence of dacryocystitis in women (Groessl et al. 1997).

The levels of overall culture positivity might be influenced by different techniques of sample collection. Hartikainen et al. (Hartikainen et al. 1997) had collected refluxed secretion through the lacrimal punctum, or by swabbing with a sterile broth-impregnated swab the lower conjunctival cul-de-sac, and reported positive cultures in 84% samples. High positive samples were also obtained by Das et al (Das et al. 2008a) and reported 90.9% of the cases were positive for bacteria, 74.5% had single isolations while 16.3% had mixed bacterial isolations. Also Chaudhry et al. (Chaudhry et al. 2005) reported that 97.3% were positive for bacteria, 33.9% of the cultures showed a single microorganism, while > 2 microorganisms were reported in 66.1% of the cultures. On the contrary, De Angelis et al. (DeAngelis et al. 2001) analyzed the posterior lacrimal flap and found that only 41.7% of the samples were positive. In our work, external-DCR was done under general anesthesia, and material (lacrimal sac cultures) was obtained directly from the lacrimal sac content while making sac flap using sterile swabs. We had found positive growth in 15 (60%) specimens. Twelve of them (80%) showed single isolations while 3 of them (20%) showed mixed bacterial isolations. Similar positive growth results were obtained by Assefa et al. (Assefa et al. 2015) (60.8%), Kebede et al. (Kebede et al. 2010) (79.8%), and more recently Chaudhary et al. (Chaudhary et al. 2010) (76.6%). For positive culture, 85.86% showed a single isolates and 14.13% showed a mixed isolates.

In this study, as shown in Table 2, Gram-positive cocci were found in 66.7% of the isolates, and similarly Bharathi et al. (Bharathi et al. 2008), Mills et al. (Mills et al. 2007), and Hartikainen et al. (Hartikainen et al. 1997) reported 69.7%, 64.9%, and 69.2% Gram-positive cocci from patients with dacryocystitis, respectively. Also in more recent study, Shahraki et al (Shahraki et al. 2016) showed that staphylococci and other Gram-positive have the most frequency in patient's eye pus. The most common organism isolated in our study was *Staphylococcus* species, accounting for 38.8% of the isolates. A similar incidence was reported by Das et al. (Das et al. 2008b), Huber-Spitzey et al. (Huber-Spitzy et al. 1992), and Coden et al. (Codon et al. 1993), their percentage being 75%, 51%, and 49% respectively. Sun et al. (Sun et al. 2005) in a study of chronic dacryocystitis in China reported that *Staphylococci* accounted for 34.5% of isolates in their series.

Streptococcus pneumoniae represented 22% of the bacterial isolates in this study, which is higher than Huber-Spitzy et al. (2%) Coden et al. (2.3%), and Hartikainen et al. (5%). This percentage compares fairly well with results of Bharathi et al. (10%), Mandal et al. (10%), Chaudhary et al. (19.8), and Kebede et al. (23%).

Gram-negative organisms represented 27.7% of all isolates; the most common isolated species was Klebsiella species (16.6%) followed by *Pseudomonas* species (12%). Similarly, Coden et al. (Codon et al. 1993) observed Gram-negative organisms in 27% of all isolates, reporting *Pseudomonas aeruginosa* in 9% of the isolates. Huber-Spitzy et al. (Huber-Spitzy et al. 1992) reported Gram-negative organisms accounting for 26% of the isolates, the most frequent species being E. coli (12%). Bharathi et al. (Bharathi et al. 2008) observed Gram-negative organisms in 29% of the isolates, the predominant isolated species being Pseudomonas species (10%) followed by E. coli (4.7%). Hartikainen et al. (Hartikainen et al. 1997) reported that Gram-negative organisms represented 17% of

Table 3 Antibiogram of bacteria isolated from lacrimal sac specimens in chronic adult dacryocystitis

Antibiotic tested Name of organism	CAF	CL	CAZ	CTX	CN	AK	TOB	GAT	CIP	LEV
Staphylococcus aureus	3/3 (100%)	2/3 (66.6%)	2/3 (66.6%)	2/3 (66.6%)	2/3 (66.6%)	2/3 (66.6%)	2/3 (66.6%)	3/3 (100%)	2/3 (66.6%)	2/3 (66.6%)
Staphylococcus epidermidis	3/4 (75%)	2/4 (50%)	3/4 (75%)	3/4 (75%)	¾ (75%)	4/4 (100%)	4/4 (100%)	4/4 (100%)	3/4 (75%)	3/4 (75%)
Streptococcus pneumonia	2/4 (5o %)	3/4 (75%)	2/4 (50%)	2/4 (50%)	2/4 (50%)	3/4 (75%)	2/4 (50%)	3/4 (75%)	2/4 (50%)	2/4 (50%)
Streptococcus Viridans	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1(100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Total Gram-positive	9/12 (75%)	7/12 (58%)	8/12 (66.6%)	8/12 (66.6%)	8/12 (66.6%)	10/12 (83.3%)	9/12 (75%)	11/12 (91.6%)	8/12 (66.6%)	8/12 (66.6%)
Klebsiella	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)	2/3 (66.6%)	2/3 (66.6%)	3/3 (100%)	2/3 (66.6%)	2/3 (66.6%)	2/3 (66.6%)	2/3 (66.6%)
Pseudomonas	1/2 (50%)	1/2 (50%)	2/2 (100%)	2/2 (100%)	1/2 (50%)	2/2 (100%)	1/2 (50%)	2/2 (100%)	1/2 (50%)	1/2 (50%)
Total Gram-negative	2/5 (40%)	2/5 (40%)	3/5 (60%)	4/5 (80%)	3/5 (60%)	5/5 (100%)	3/5 (60%)	4/5 (80%)	3/5 (60%)	3/5 (60%)
Total	11/17 (65%)	9/17 (53%)	11/17 (65%)	12/17 (70.5%)	11/17 (65%)	15/17 (88.8%)	12/17 (70.5%)	15/17 (88.8%)	11/17 (65%)	11/17 (65%)

Number of the susceptible bacterial isolates/number of the tested isolates (% of the susceptible isolates) against common antibacterial agents by disc diffusion method
CAF chloramphenicol, CL cephalexin, CAZ ceftazidime, CTX cefotaxime, CN gentamycin, AK amikacin, TOB tobramycin, GAT gatifloxacin, CIP ciprofloxacin, LEV levofloxacin

all the isolates, the most commonly isolated species being Haemophilus influenza (4%). However, recently, Briscoe et al. (Briscoe et al. 2005) recovered 61% of the bacterial isolates as Gram-negative bacilli with the predominance of *Pseudomonas aeruginosa* (22%) from pus samples of dacryocystitis.

In this study, fungal organisms were detected in 5.5% of the positive cultures; the predominant fungus was Candida species. Brook and Fraiser (Brook and Frazier 1998) reported that fungal species constituted 5% of the isolates in their series.

The spectrum of aerobic and facultative organisms recovered in this study is also consistent with results, reported by Brook and Fraiser in the USA, as *Staphylococcus*, *Pseudomonas*, and *Streptococcus* species were also among the predominant pathogens.

The analysis of the antibiogram of bacterial isolates (Table 3) showed that among the cephalosporins, the first generation of cephalosporins, cephalexin (58%), showed lower efficacy against Gram-positive isolates when compared with the third generation of cephalosporins, ceftazidime (66%), and cefotaxime (66%). Gram-negative isolates were also more sensitive to the cefotaxime (80%) followed by ceftazidime (60%) and cephalexin (40%). Similarly, Briscoe et al. (Briscoe et al. 2005) reported that ceftazidime and cefuroxime (third generation of cephalosporins) showed sensitivity (95%) and (50%) respectively while cephalexin showed (14%).

Among the aminoglycosides, amikacin showed higher effectiveness toward Gram-positive and Gram-negative isolates. Similarly, Sun et al. (Sun et al. 2005) observed higher effectiveness of amikacin for both Gram-positive and Gram-negative isolates. In a more recent study of the bacteria antibiotic resistance demonstrated the highest bacterial sensitivity to antibiotics was for chloramphenicol (76.7%), gentamycin (66.7%), and amikacin (60%) (Shahraki et al. 2016).

Among the fluoroquinolones, the gatifloxacin showed increased efficacy against all pathogens more than ciprofloxacin and levofloxacin which showed lower efficacy against all pathogens. Similarly, Bharathi et al. (Bharathi et al. 2008) observed higher effectiveness of gatifloxacin against Gram-positive and Gram-negative bacterial isolates. In a study done by Lijuan et al. (Chen et al. 2018) in China, all Gram-positive bacteria showed sensitivity to vancomycin and 86.5% sensitive to gatifloxacin. Meanwhile, nearly all Gram-negative bacteria showed sensitivity to gatifloxacin (99.5%), and 92% were sensitive to tobramycin and ceftazidime. Thus, gatifloxacin was the best choice agent against all Gram-positive, Gram-negative, and anaerobic isolates.

In our study, clinicobacteriological study of chronic dacryocystitis demonstrated the increased prevalence of Gram-negative organisms particularly Pseudomonas and Klebsiella. These findings indicate that the antibiotic susceptibility test and treatment protocol before and after lacrimal surgery should be done.

The study of clinicobacteriological character of chronic purulent dacryocystitis done by Briscoe et al. (Briscoe et al. 2005) demonstrated a significant change in bacterial flora and antibiotic treatment. A higher isolation percentage of Gram-negative bacteria particularly Pseudomonas was detected and showed increasing resistance to the commonly used antibiotics. The emergence of rare highly resistant Gram-negative microorganisms may also indicate a new picture in lacrimal sac infections (Briscoe et al. 2005).

Conclusion

Clinicobacteriological study of chronic dacryocystitis in adults demonstrated predominance isolation of Gram-positive *Staphylococcal species*, along with an increased incidence of Gram-negative organisms, *Klebsiella pneumoniae* and *Pseudomonas species*, which was not part of the conjunctival flora. On the contrary, Gram-negative bacteria are a threat of post-operative infection in ophthalmic and lacrimal drainage surgeries. For this reason, antibiotic prophylaxis in lacrimal drainage surgery should be effective against Gram-negative bacteria as well. We recommend that microbiological analysis, and culture and sensitivity test are mandatory to be done to all cases with microbial infection for the selection of the effective antimicrobial treatment and to help control the increasing rate of antibiotic resistance.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors' contributions
SN made the main contributions to work design, microbiological laboratory testing of eye samples, data collection from patients' files, and other scientific data; helped in drafting the manuscript, and revising and approving the final version. She was responsible for the submission of the article as a corresponding author and resubmission after making the necessary revision and correction to the manuscript. AE has been involved in microbiological laboratory testing; microbial isolation and antibiotic sensitivity tests of the eye samples; and recording of results, data collection, drafting of the article, and revising and approving the manuscript content. TS performed the clinical work of the study including patient examination and diagnosis, obtaining samples intraoperative, supervised the immediate transport of samples to the microbiology laboratory, revised the manuscript, and gave final approval for publishing. MY shared performance of the clinical aspect of the work including patient's examination, and diagnosis and surgical interference, sample collection, and follow-up. He revised the draft of the clinical aspect of the study, read the manuscript, and gave his final approval to be published. OA shared patients' examination and diagnosis, supervised the sample transport, helped in the collection of scientific data, revised the manuscript, and gave her

final approval to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Agreement for the study, in addition to the ethical approval was obtained first from the Medical Research Committee of RIO, Egypt. All the patients who were enrolled had submitted an informed consent in advance.

Consent for publication

Not applicable (participants were fully anonymous).

Competing interests

The authors declare that they have no competing interests.

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Author details

[1]Department of Microbiology and Parasitology, Research Institute of Ophthalmology, El Ahram Street, (RIO), Giza 12611, Egypt. [2]Infection Control Unit, Department of Microbiology and Parasitology, RIO, Giza, Egypt. [3]Ophthalmology, Oculoplastic Department, RIO, Giza, Egypt. [4]Department of Ophthalmology, RIO, Giza, Egypt.

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