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Acanthamoeba and Some Associated Bacteria as a Predisposing Cause for Infectious Keratitis in Giza Governorate, Egypt

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Abstract: Infectious keratitis is the most devastating complication of contact and non-contact lens wearers that may result in permanent visual loss. Acanthamoeba is an opportunistic protozoon; some of its species can cause keratitis infrequently, separately or in association with some types of bacteria. Accuracy of the used diagnostic methods are considered to be important in early diagnosis and treatment of the infection. In the present work infectious keratitis patients resistant to treatment were investigated aiming to identify the causative pathogen as well as some predisposing factors especially in contact lens wearers (CLW). The data cleared that infection by Acanthamoeba was high in keratitis infected patients wearing contact lenses (40%) in comparison with non-contact lens wearers'(NCLW) patients (23.3%). There is strict association between Acanthamoeba infection and infection by *Staphylococcus epidermidis* and *Staphylococcus aureus*. Infection by Acanthamoeba as well as bacteria was high in contact lens wearers(CLW) patients (9% and 17%) in comparison with non-contact lens wearers keratitis infected patient as it was 10% and 20% respectively. High incidence for *Staphylococcus aureus* (38%) than that of *Staphylococcus epidermidis* (42.85%) while mixed infection by both bacteria species was recorded in 14.29% of the cases only. Acanthamoeba association was more with *Staphylococcus aureus* than *Staphylococcus epidermidis* in CLW infected patients. The condition was different in NCLW infected patient. Moreover no record for infection by Acanthamoeba alone was diagnosed in the examined patients using suitable cultures. PCR appeared more sensitive in diagnosis of bacterial keratitis in comparison with the traditional culture technique. At the level of the present study, diagnosis of Acanthamoeba by specific culture technique appeared more sensitive than diagnosis using PCR technique. The study concluded that contact lenses wear is considered as one of the main causes of infectious keratitis especially if associated with poor hygienic measures.

Key words: Acanthamoeba · Keratitis · *Staphylococcus aureus* · *Staphylococcus epidermidis* · PCR · Contact lens wearers

INTRODUCTION

Infectious keratitis, the most serious form of keratitis, is a frank infection of the cornea. It may result in permanent visual loss due to corneal scaring or perforation. It requires prolonged antimicrobial therapy and sometimes combined with corneal surgery for successful treatment [1].

Contact lenses, an excellent form of vision correction, are still compromising a significant risk factor for keratitis [2]. Bacterial keratitis remains as one of the most important potential complications of contact lens users. However, Acanthamoeba and fungi can be also other causes [3]. Sight threatening corneal infection by bacteria or Acanthamoeba is the most important complication of contact lenses wear [3].

Epidemiological studies over the past 20 years have shown rise in the rate of infectious keratitis associated with lenses wear; also bacterial and acanthamoebic keratitis in the developing world, appeared to be 10 times more common than in the developed countries. Moreover, bacterial sensitivity to various antimicrobials varies from

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place to place and even from time to time. Therefore, accurate identification of microorganisms involved in ocular infections and the emergence of acquired microbial resistance dictate the need for continuous surveillance to guide empirical therapy [4].

Acanthamoeba is an opportunistic protozoon of free-living amoebae that can be pathogenic to humans. Some species of Acanthamoeba can cause keratitis. Acanthamoeba grows and multiplies as trophozoites and encysts under unfavorable conditions. Cysts are double-walled, highly resistant dormant stages that remain infective for several years, which facilitates spreading and colonization of new ecological niches [5]. In recent years; particular attention was given to Acanthamoeba with the exponential increase in the incidence of reported Acanthamoeba keratitis. This increase is primarily due to infection in contact lens wearers, especially those using home-prepared saline that are recognized to be at highest risk [6]. Acanthamoeba keratitis is commonly confused with other conditions such as herpes simplex viral keratitis, which delays treatment [7].

Standard microbiological techniques for diagnosing infectious keratitis rely on culturing the organism in nutrient media. Although culture results are highly specific, yet have low sensitivity, generally yielding results in less than 60% of cases, but such technique is considered to be time consuming [8]. Nowadays, PCR technology is preferred as a highly sensitive and rapid diagnostic tool that can be used for accurate identification of specific organisms. PCR can be used as a potential utility for improving the diagnosis of ocular infection by different pathogens [9].

For the previous reasons, the present study aimed to spot some light on the role of Acanthamoeba as well as some associated bacteria as a primary or secondary cause of keratitis in contact and non-contact lenses users in Giza Governorate, Egypt using conventional as well as PCR technique for diagnosis.

MATERIALS AND METHODS

Study Population: This study was performed at the Corneal Unit, Research Institute of Ophthalmology (RIO) from (2010-2012) with 100 patients clinically diagnosed as infectious keratitis. These patients were classified into two main categories: CLW and NCLW. In the first group contact lenses was considered as the predisposing cause of keratitis, while in NCLW group patients were allocated into subgroups according to the predisposing cause such as: ocular trauma, dry eyes, entropion, systemic risk factors and poor hygiene subgroups. Moreover; 20 healthy controls were selected as a control group.

Inside each group; the studied parameters included age, sex, demographic data and socioeconomic status as demonstrated in (Table 1). Medical history including predisposing risk factors; [trauma; dirty water splash; contact lenses wear; associated eye diseases e.g. blepharitis or entropion or dry eye ; systemic diseases]; duration and type of symptoms, previous diagnosis and treatment, presenting ocular findings and final visual outcome, intraocular pressure, treatment and sequelae (Table 1).

Specimen Collection: Patients with suspected keratitis were investigated for the presence of bacteria, or Acanthamoeba by using a common protocol that involves collection of corneal scrapings for smears and cultures. All cases underwent corneal epithelial biopsy with removal of a central sheet of corneal epithelium, approximately few millimeters in diameter, from the

Table 1: Demographic Characteristics of Examined Cases (No. = 100)

Demographics	Particulars	N0	No. of CLW	No. of NCLW
Sex	Male	30	10	20
	Female	70	60	10
Age in years	11-20 years	30	25	5
	21-30 years	40	25	15
	31-40 years	20	10	10
	41-50 years	5	5	
	51-60 years	5	5	
Residence	Rural	30	10	20
	Urban	70	60	10
Occupation	Agricultural workers	20	5	15
	Business/Professionals	40	30	10
	Students/Children	40	35	5
Total		100	70	30

Table 2: Sequences of used Primer Sets

Organism	Primer sequence	Product size	Reference
Staphylococcus epidermidis	16S rRNA 91E-F: GGA ATT CAA AKG ATT TGA CGG GGG C 13B-R: CGG GAT CCC AGG CCC GGG AAC GTA TTC AC	478 bp	[12]
Staphylococcus aureus	Sa 442f: GTC GGG TAC ACG ATA TTC TTC ACG Sa 442 r : CTC GTC TGA CCA GCT TCG GT	156 bp	[11]
Acanthamoeba	26S RNA: F: GGA GCT CCC ACG GGA GGC C R: TGG ACC GCG TGA GGC TGC GGC T	126 bp	[13]
Universal primer for bacteria	F27-6: GGA GGA AGG RGG GGA TGA CG R28-6: ATG GTG TGA CGG GCG GTG TG	241 bp	[14]

affected eye using a sterile cotton-wool swab or a blunt Kimura spatula under the magnification of slit lamp after instillation of topical anesthetic (4% xylocaine).

The received corneal samples were divided into three parts one for Acanthamoeba culture, the second is for conventional microbiological culture and the third was stored at -20°C in phosphate buffered saline for subsequent PCR analysis.

Acanthamoeba Culture: Acanthamoeba was cultivated as the swabbed cotton-bud was placed onto the surface of a co-seeded non-nutrient agar plate overlaid with a thin layer of live *Escherichia coli.* Plates were incubated at 30°C and examined daily microscopically from 7 up to 14 days. Acanthamoeba were characterized according to cyst (wrinkle double walled) and Trophozoite (acanthopodia and pseudopodia) morphology [5].

Bacterial Culture: In the same time scrapings were performed for bacteriological examination; direct smear examination was performed. Corneal scrapings were inoculated onto the surfaces of a variety of media including sheep blood agar, chocolate agar and Wilkins chalgren anaerobic agar and brain heart infusion broth, allowing for the growth of bacteria. All media were incubated aerobically in a CO_2 atmosphere, while the Wilkins anaerobic agars were incubated anaerobically; at 37°C. Culture was examined daily and discarded after 5 days if no growth was obtained. The anaerobic agar plates were examined only after 5 days. If positive bacterial growth was obtained on the different culture media, standard biochemical tests were performed and further identification was done up to the species level using the API STAPH and API 20 E systems [10].

Molecular Examination

Sample Collection, DNA Extraction and Amplification: Corneal scrapings for PCR were obtained by sterile swabs, which were then placed into a sterile micro centrifuge tube, capped and immediately transferred to –20°C for storage until processing.

DNA extraction was performed using Gene Jet Genomic DNA purify kit (Cat.No.00005743,Sigma Product) according to manufacturer's instructions. In this study, DNA was amplified by PCR in a DNA thermal cycler (Perkin Elmer Cestus, Norwalk, CT) using oligonucleotide primers specific to amplify specific sequence for the detected organism synthesized by (Invitrogen life technologies, Carlsbad, Ca. USA) as shown in (Table 2). Amplicons were visualized on 2.5% Agarose gel Microkit from Qiagen, stained with Ethidium bromide and observed using a UV transilluminator& samples run with DNA ladder from Solis Biodyne.

Control samples for all the strains used (*S.aureus, S.epidermidis and* Acanthamoeba) were laboratory isolates from Microbiology and Parasitology laboratories at Research Institute of Ophthalmology.

PCR Program for *Staphylococcus aureus*: Amplification was done by using forward primer (Sa 442) and reverse primer (Sa 442), product size 156bp[11]. The amplification performed in the thermal cycler involved a total of 35 cycles. Initial denaturation 95°C for 10 min each cycle consisted of denaturation at 95°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 1 min. and a final extension at 72°C for 5 min and was stopped at 4°C for 24 hours.

PCR Program for *Staphylococcus epidermidis*: Amplification was by using 91E forward primer and 13R reverse primers, product size 478bp [12]. The amplification performed in the thermal cyclerinvolved a total of 40 cycles, Initial denaturation at 95°C for 9 min each cycle consisted of denaturation at 94°C for 1 min, annealing at 69°C for 1 min- and extension at 72°C for 5 min and was stopped at 4°C for 24 hours.

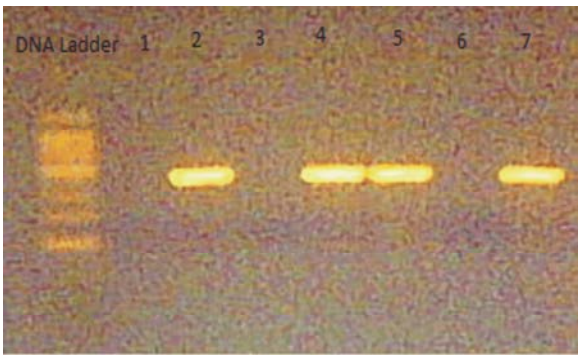

Fig. 1: Agrous gel showing PCR product of *staphylococcus epidirmidis* that amplify 478-bp amplicon

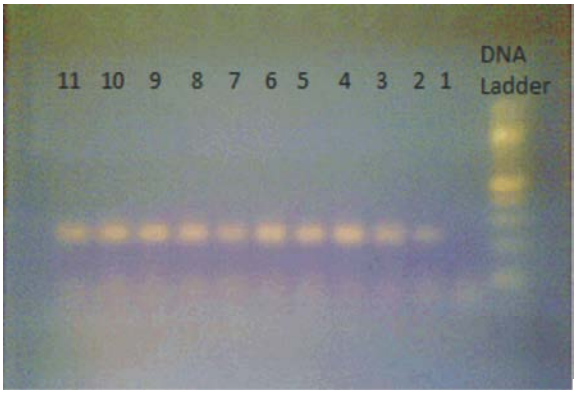

Fig. 2: Agrous gel showing PCR product of *staphylococcus aureus* that amplify 156-bp amplicon

Fig. 3: Agarous gel showing PCR product of Acanthamoeba of 26s RNA that amplify 126-bp amplicon

PCR Program for Acanthamoeba: Acanthamoeba amplification was performed by using 26sRNA forward primer (E2164C08) and 26sRNA reverse primer (E2164C09), product size126bp in a thermal cycler (Perkin Elmer)[13].PCR program involved a total of 35 cycles. Initial denaturation at 95°C for 10 min each cycle consisted of denaturation at 95°C for 1 min, annealing at 61°C for 1 min- and extension at 72°C for 1 min and a final extension at 72°C for 5 min and was stopped at 4°C for 24 hrs.

PCR amplified products were electrophoresed on 2.5% (w/v) Agarose gel, stained with Ethiduim bromide solution (0.5 µg/ml) and visualized under UV light using a Chemi-doc Image Analyzer (Biorad, Madrid, Spain) (Fig: 1, 2 & 3).

Statistical Analysis: To evaluate the diagnostic value of PCR in comparison with the culture method; statistical analysis was done for calculating the sensitivity using Medcalc program [15].

RESULTS

The results displayed in Table (3) revealed that infection by Acanthamoeba was high in keratitis infected patients wearing contact lenses (40%) in comparison with non-contact lens wearers' patients (23.3%). These data revealed strict association between Acanthamoeba infection and infection by two types of gram positive bacteria as the bacterial organism single or mixed were isolated from all Acanthamoeba infected cases and in the same time no record for infected cases by Acanthamoeba only in the examined patients using suitable cultures. Infection by Acanthamoeba as well as bacteria was high in CLW patients (40% and 82.86%) in comparison with NCLW keratitis infected patients as it was 23.3% and 60% respectively (Table 3). High incidence for *S.aureus* (42.85%) in the CLW examined patients than that of *Staphylococcus epidermidis* (25.71%) while mixed infection by both bacterial species was recorded in 14.29% of the cases only. The data revealed in NCLW examined patients higher incidence for *S.aureus* (26.6%) versus that of *S.epidermidis* which was (16.66%) using suitable cultures. However, mixed infection by both bacteria species was recorded in 16.66% of the cases only. It is important to be mentioned that patients wearing contact lenses were in age ranged from 11-40 years old. Most of them suffered severe pain and photophobia.

Aiming to inspect the relation between Acanthamoeba infection and the type of bacteria isolated from keratitis infected patients, data in Table (4) cleared that Acanthamoeba was isolated from all patients having mixed infection by both-types of *S.aureus & S. epidermidis*. In contact lenses wearing patients, the percentage of association between Acanthamoeba and

Table 3: Incidence of Acanthamoeba and Gram Positive Bacteria Diagnosed in the Examined Keratitis Patients Using Suitable Cultures

		Types of isolated bacteria							
		S. aureus only		*S. epidermidis* only		*S. aureus&* *S. epidermidis*		Total	
History of the Examined Patients	Types of the isolated pathogens	No.	%	No.	%	No.	%	No.	%
CLW (No. =70)	Bacteria	30	42.8	18	25.7	10	14.29	58	82.86
	Acanthamoeba	14	20	7	10	7	10	28	40
NCLW (No. = 30)	Bacteria	8	26.6	5	16.6	5	16.66	18	60
	Acanthamoeba	3	10	2	6.66	2	6.66	7	23.3
Total (No=100)	Bacteria	38	38	23	23	15	15	76	76
	Acanthamoeba	17	17	9	9	9	9	35	35

• No cases were recorded infected by Acanthamoeba only.

Table 4: Rate of Association between Acanthamoeba and Isolated Bacteria in Keratitis Infected Patients

		Acanthamoeba / Types of isolated bacteria			
Patient history	Isolated Organism	Acanthamoeba / *S.aureus*	Acanthamoeba / *S.epidermidis*	Acanthamoeba / Mixed	Total
CLW (No= 70)	No. infected	14 / 30	7 / 18	7 / 10	28 / 58
	%	46.66%	38.88%	70.0%	48.28%
NCLW (No = 30)	No. infected	3 / 8	2 / 5	2 / 5	7 / 18
	%	37.5%	40.0%	40.0%	38.88%
Total (No= 100)	No. infected	17 / 38	9 / 23	9 / 15	35 / 76
	%	44.74%	39.13%	60.0%	46.05%

Table 5: Relation between Infectious Keratitis and Some Predisposing Factors

			Keratitis +ve		Infection by Bacteria		Infection by Acanthamoeba	
History	Predisposing Risk Factor	No. examined	No.	%	No.	%	No.	%
Contact lens wear		70	58	82.86	58	100%	28	48.27
Non-Contact	Ocular trauma	10	8	80	8	100	4	50%
Lens Wearers	Dry eye	5	2	40	2	100	2	100
	Entropion	5	3	60	3	100	1	33.3
	Systemic risk factors	3	1	33.3	1	100		
	Poor hygiene	7	4	57.14	4	100	1	25
	Total	30	18	60	18	100	8	44.4

S.aureus infected patients was (46.66%) while it was isolated from (38.88%) of *S. epidermidis* infected patients. The condition was different in NCLW infected patients as Acanthamoeba association with *S. aureus* was (37.5%) in comparison with association with *S. epidermidis* (40.0%).

Aiming to define the relation between some predisposing factors in occurrence of infectious keratitis; the data in Table (5) cleared that contact lenses appeared as an important predisposing factor as its incidence reached up to (82.86%). In NCLW ocular trauma was from the main predisposing factors for the disease (80%) followed by entropion (60%), dry eye (40%), while (57.14%) suffering from the disease accompanied with poor hygienic conditions as a predisposing factor. Acanthamoeba infection was recorded in (48.27%) of bacterial keratitis infected patients wearing contact lenses. The parasite was not recorded separately in any of the diseased cases. The parasite was isolated in (50%) of ocular traumatic patients; all infected patients suffering from dry eye and in (33.3%) of cases with entropion (Table 5).

On rescreening of all collected samples using PCR; data in Table (6) revealed that PCR appeared to be more accurate than traditional culture methods in diagnosis of bacterial causes of infectious keratitis. Percentage of positive samples reached up to (94%) in comparison with (76%) after specific cultivation of the samples concerning *S.aureus* and *S.epidermidis* or even the mixed samples. Also, PCR results appeared higher than that after bacterial cultivation for each type of bacteria alone or even in samples containing mixed infection. Concerning diagnosis of Acanthamoeba in the same samples; culture methods

Table 6: Differences between Diagnostic Efficacy of Culture Methods and PCR Technique (No. =100)

History of Examined Patients	Type of isolated Pathogen	Types of Isolated Bacteria: *S. aureus* only No.+ve	%	*S. epidermidis* only No. +ve	%	*S. aureus*& *S.epidermidis* No. +ve	%	Total No. +ve	%
Diagnosis by culture in	Bacteria	38	38	23	23	15	15	76	76
CLW& NCLW (No=100)	Acanthamoeba	17	17	9	9	9	9	35	35
Diagnosis by PCR in	Bacteria	42	42	28	28	24	24	94	94
CLW& NCLW (No=100)	Acanthamoeba	15	15	7	7	8	8	30	30
Sensitivity of PCR	Bacteria		91.3		84.8		72.7		
	Acanthamoeba		89.4		81.8		90		

proved the infection in 35% of these samples while this percentage decreased to 30% using PCR technique in the same samples (Table 6).

Concerning the sensitivity of PCR in diagnosis in comparison with the culture methods. PCR revealed higher positive diagnostic rates for bacterial infection pathogens. The sensitivity of PCR in comparison with culture methods reached to (91.3%), (84.8%) and (72.7%) for diagnosis of *S.aureus* and *S. epidermidis* and mixed bacterial infections respectively (Table 6).

DISCUSSION

Severe infectious keratitis remains a leading cause of ocular morbidity worldwide[16].Contact lenses wear is now the major predisposing factor for corneal infection; as in the past several years there has been a steady increase in contact lens wearers [2].

Multiple organisms have been reported from the microbial keratitis seen in association with contact lenses wear. Nearly all contact lens wearers with infectious keratitis cases had mixed microbial populations. The most common microbial contaminant isolated in descending prevalence were bacteria (77%) fungi (24%) & protozoa (20%) [2].

Bacterial keratitis is one of the most important causes of corneal opacifications. It is the second common cause of legal blindness world-wide after cataract with disparities amongst populations living in both western and in developing countries. It is estimated to be 10–20 times higher with the use of extended wear disposable contact lenses [17]. In general, Gram-positive bacteria are more frequently recovered in temperate climate regions specifically *S. aureus*, of culture proven infections [3]. Acanthamoeba keratitis is a potentially blinding corneal infection that may aggressively affect both eyes. Contact lenses wear remains the main risk factor in transmitting Acanthamoeba trophozoites and cysts to the cornea [5]. Many physio-pathological effects have been reported due to contact lenses increased the risk, the most important is an induced hypoxia and hypercapnia of the cornea [3]. Moreover; inhibition of normal corneal epithelial cell shedding; corneal epithelial thinning; increased binding of bacteria to corneal epithelial cells, increased internalization of bacteria through expression of membrane lipid rafts on corneal epithelial cells, reduced tear exchange and disruption to the normal lid/cornea/tear resurfacing mechanism [18]. In the present study, keratitis infection in all of CLW and NCLW had mixed microbial populations. The most common microbial contaminant isolated in descending prevalence were bacteria 76% and Acanthamoeba 35%. This was in agreement with Al-Mujaini *et al*. [17].

Patients in this study presented with poor visual acuity, redness, watering with ocular pain disproportionate to the degree of keratitis. Various clinical signs were noted through slit-lamp bio-microscopic examination by an ophthalmologist. Anterior stromal infiltrates were present in all patients; corneal scar with oedema was another finding. Ring infiltrate was present in some patients and was the more often presenting feature. The less specific signs such as satellite stromal infiltrate diffuse stromal infiltrates and endothelial plaques were seen in many of the patients. More than half of the patients had anterior chamber reaction with hypopyon ranging from trace to 3.5mm. Radial keratoneuritis, (specific for the diagnosis of Acanthamoeba keratitis) was also seen in some patients.

In the present study, incidence of keratitis was higher in CLW (82.86%) than in NCLW (60%) with overall percentage reached to 76%. All of these patients were infected by bacteria or accompanied with Acanthamoeba infection. These data were in agreement with Radford *et al*. [19] & Green *et al*. [20] who reported that mostly infectious keratitis cases were contact lenses wearers'. On the contrary, Lalitha *et al*. [21] in their study revealed that the major risk factors were association of ocular trauma and poor water supply.

The positivity rate of bacterial keratitis in the present study was 82.86% in CLW keratitis patients and 60% in NCLW keratitis patients caused by gram positive cocci *S. aureus & S. epidermidis* using suitable culture methods. However mixed bacterial infection was of a lower incidence. The incidence was (42.85%, 25.71%) in CLW keratitis patients and (26.6% & 16.66%) in NCLW keratitis patients from the total positive isolates. This was also obvious in NCLW patients with infectious keratitis as *S. aureus and S. epidermidis* presented the higher incidence among patients. The same was reported by Bourcier *et al*. [22] & Al-Mujaini *et al*. [17]. However, there was no record for isolation of fungi in the examined patients.

Concerning Acanthamoeba–bacteria interaction; the selective-binding capacity of amoebae towards different species of bacteria, a feature probably involving pattern recognition and other cell-surface receptors; the growth-promoting effect of certain bacteria and the toxic effect of others; and different food preferences of different Acanthamoeba species/isolates [23]. About 95% of Acanthamoeba keratitis cases are attributed to contact lens wear [18]. The increase in the risk of Acanthamoeba keratitis among disposable contact lens users was largely attributable to repeated wear of lenses, lack of disinfection and use of saline and chlorine-based solutions [24].

In the present study, Acanthamoeba was diagnosed in association with bacteria (20% &10%) with *S. aurues & S. epidermidis* respectively in CLW keratitis patients versus (10% & 6.66%) in NCLW keratitis patients of total positive isolates. This was in agreement with the study made by Ibrahim *et al*. [24] who reported that about 50% of the eyes infected with Acanthamoeba had positive cultures for bacteria. Other studies; Kilvington & Lonnen [25] showed that 85% of contact lens systems infected with Acanthamoeba were contaminated with bacterial strains. Also Ibrahim *et al*. [24] demonstrated that bacterial species capable of supporting amebic growth may be the first step in the pathogenesis of ameba-induced keratitis by the provision of large inocula of amebae.

Other studies; Stapleton & Carnt [18] brought to light, that among the commonly isolated organisms were *S. aureus, Pseudomonas aeruginosa* in association with Acanthamoeba in culture-positive cases. This was on the contrary with Al-Mujaini *et al*. [17]; who mentioned that *P. aeruginosa* and *S. aureus* were the most frequent and the most pathogenic ocular pathogens that can cause ocular perforation in just 72 hours. They added ocular surface disease is considered to be the most common predisposing factor causing keratitis infection.

This study revealed that bacterial keratitis was associated with different predisposing factors such as contact lenses, ocular trauma, entropion and dry eye. This was in agreement with Willcox *et al*. [2] as extended wear or occasional overnight lens use; poor hygiene: (infrequent lens disinfection; omitted or infrequent case cleaning; omission of hand washing before handling lenses) and smoking were some of the predisposing factors.

It was interesting to note that the causative keratitis pathogens at the level of the present study were mainly gram positive bacteria followed by Acanthamaoba infection. This was in agreement with Stapleton & Carnt [18]. Egypt is in a region of hot climate, the disease was more common in urban than rural areas; with higher incidence among females rather than males mainly due to increased cosmetic care and also economic reasons in addition to the type of work of both genders, all these may share in development of the sequence of infection.

Diagnosis of pathogens causing keratitis (bacteria or Acanthamoeba) by culturing the suspected samples is the most accurate tool for diagnosis for these agents. However; still it is a more time consuming methods. A sensitive method, such as PCR is able to detect the specific genotypes in very short time considered useful for clinical applications. The potential utility of PCR based techniques for improving the diagnosis of ocular infection is well recognized and expanding. This was in agreement with Khattab *et al*. [15] as they mentioned that PCR detects microbial DNA in the majority of bacterial and fungal corneal ulcers and identifies microorganisms in a high proportion of culture-negative cases. Although being expensive; PCR remains a promising tool for faster and highly sensitive diagnosis of microbial keratitis.In the same time; the results obtained for diagnosis of Acanthamoeba using culture methods were still higher (35%) than that obtained after PCR (30%). In the author's opinion, this may be due to type of the used primers in relation to the genotype of the present Acanthamoeba isolated from infected patients.

In conclusion, rapid initiation of appropriate microbiological testing and treatment; continual education, both of ophthalmologists and patients, is still required to minimize the incidence and severity of microbial keratitis. Professionals and public education measures should be continual emphasize the importance of lens hygiene and the significance of the duration of contact lens wear to all contact lens wearers. Also, strategies directed towards microbial virulence characteristics may be more successful in preventing or limiting disease severity rather than attempting to modulate the host response.

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