

Profile of Microbial Keratitis at a Referral Center in Iraq

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Abstract

One hundred and five (105) corneal scraping samples were taken from patients suffering of microbial keratitis referred to Ibn Al-Haitham Eye Teaching Hospital in Baghdad. After culturing on the related selective media, results showed that 73 of the samples were positive for occurrence of microorganisms; of these, 68 (64.76%) were belonged to bacterial and 5 (6.85%) to fungi. After the bacterial isolates were identified by cultural, microscopic and biochemical examinations, the following species and percentages were recorded: *Pseudomonas aeruginosa* 29 (39.73%), *Staphylococcus aureus* 22 (30.14%) and *Staphylococcus epidermidis* 17 (23.28%). While the fungal isolates were belonged to *Aspergillus niger* 3 (4.12 %) and *Alternaria* spp. 2 (2.73 %) according to their identification through the cultural and microscopic examinations, in addition to the types and shapes of fungal spores. Upon such findings, *P.aeruginosa* was the predominant bacteria and *A.niger* the prevalent fungi. Regarding to the patients gender, it was found that cases of microbial keratitis in the samples were more abundant in males 41 (56.16%) than in females 32 (42.85%). Patients group of age (41-59) years was the most microbial keratitis infected group with a percentage of (30.20 %), while the lowest infected group was (≥ 60) year with a percentage of (17.80 %).

Keywords: Microbial keratitis, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger*.

Introduction

Microbial keratitis (MK) is characterized by a defect of the corneal epithelium with inflammation of the underlying corneal stroma caused by replicating micro-organisms including bacteria, viruses, fungi and protozoa.[1] Microbial keratitis is a potentially sight threatening disorder that requires urgent identification and eradication of the causative agent(s).[2] In the developing countries, microbial keratitis is one of the leading causes of monocular visual disability.[3] Microbial keratitis can cause corneal opacity with deteriorated visual acuity, or even may lead to corneal perforation and loss of the eye. Microbial keratitis is a medical emergency; rapid to initiation of aggressive treatment is needed halt the disease process and limit the extent of corneal scarring and loss of vision.

The spectrum of microorganisms responsible for MK varies with regard to the geographical locations according to climatic conditions, predisposing factors, and demographic characteristics of the patients.[4] The spectrum of microorganisms responsible for MK may also vary with time, therefore treatment of MK starts with an appropriate

initial therapy that depends on local contemporaneous information regarding the predominating causative organisms.[5]

Risk factors for infectious keratitis due to non-viral pathogens include trauma to the eye, overnight or extended wear of conventional contact lenses or chronic ocular surface disease (including blepharitis), prior ocular surgery, systemic diseases (diabetes mellitus, rheumatoid arthritis) and use of topical corticosteroids or traditional eye medicines. Around 10 % of individuals with infectious keratitis may not exhibit any risk factors. [6]

The leading predisposing factors for microbial keratitis in Iraq were corneal abrasions, and ocular surface disorders (dry eye, trichiasis, old scars, or exposure keratitis) most of them were sequels of cicatricial trachoma.[7] Bacterial culture and smear examination using corneal scrapings is the conventional method to detect causative pathogens of corneal ulcer.[8]

This study was aimed to identify the microbial causatives of eye keratitis infection and comparing between bacterial and fungal occurrence in such infection.

Materials and Methods

This cross sectional prospective study was conducted in Ibn Al-Haitham Teaching Eye Successive new patients attended the hospital from October (2013) to March (2014) were enrolled in this study if they had corneal inflammation presumed to be microbial. Patient's age and gender were documented. Hospital/ Baghdad, Iraq

Examination of each infected eye was performed under Slit Lamp recording size and depth of the ulcer and of the stromal suppuration. Height of hypopyon if present was also recorded. A sterile syringe needle was used to scrape the area of infection.

Corneal scrapings were taken from the margins of the ulcers and the suppuration. The scraping was divided into two portions; the first (for bacterial detection) was inoculated onto each of blood agar (Himedia, India), chocolate agar, brain heart-infusion agar (Salucea/Germany) and MacConkey agar (BDH/England) and incubated at 37°C for 24-48 hours. The second portion (for fungal detection) was inoculated onto Sabaroud agar (Biolife /Italy) and incubated at 28 °C for 5-7 days. After incubation, positive microbial cultures were further identified to the species as follows.[9]

-Bacterial identification

The suspected isolates were identified based on their morphological and biochemical characteristics. The morphological characterization involved culturing the isolates on blood agar plates for description of colonies appearance. Following that, smears from the colonies were Gram stained to study the isolate cell shapes and Gram reaction. Biochemical characterization of the isolates was based on the results of indole, methyl red, Voges-Proskauer, citrate utilization, catalase test, oxidase test, motility test, urease test, coagulase test and DNase test according to the 9th ed. of Bergey's Manual of Determinative Bacteriology.[9]

In culture positive cases, most bacteria (*Staphylococcus* spp. and *Pseudomonas* spp.) were identified by API system (bio Merieux).[10]

-Fungal identification

A loopfull from each suspected fungal culture was transferred onto a glass slide, then lacto phenol-cotton blue stain was added before examined under 40X magnification power of the compound light microscope for hyphae and spore morphological identification. [11]

Results and Discussion

A total of 105 samples were collected from patients suffering from microbial keratitis referred to Ibn Al-Haitham Eye Teaching hospital in Baghdad for the period from October (2013) to March (2014).

-Isolation of bacteria

Out of the 105 ulcers' tested samples, 73 (69.52%) were positive for microbial (Bacteria and fungi) occurrence after propagating on blood agar for bacteria and Sabaroud agar for molds. The rest 32 (30.48%) were reported as negative when showed no microbial growth despite extending their incubation periods.

Identification of bacterial isolates

After subjecting the suspected bacterial isolates to the microscopic, cultural and biochemical examination, results showed that out of the 73 microbial isolates, 68(64.76%) were belonged to the bacteria; in which 29 (39.72%) of them were *Pseudomonas aeruginosa*, 22 (30.13%) *Staphylococcus aureus* and 17 (23.28%) *Staphylococcus epidermidis*.

The prevalence of different microorganisms responsible for microbial keratitis varies with geographical location and time.[12] Positive culture in MK was estimated to be about 56%.[13] Adversely, another study found that positive culture occurs in only 25% of cases.[14] Different variation in the results in this regard to various factors such as size of the specimens, fixation of microorganisms on the solid surfaces (intraocular lens, lens fragment), consequent decrease of cells in the vitreous aqueous humor, taken antibiotics by patients before collection of clinical material or present of fastidious microorganisms such those causing endophthalmitis.[15] Such studies reported that out of 111 cases of corneal scraping

72(64.9%) were found to give positive cultures. Gram-positive isolates were the prevalent in a percentage of (81.1%) and *Staphylococcus* spp. was the most common genus, while *Pseudomonas* spp. was the most abundant genus among Gram-negative isolates.[16] Previous study conducted in Ibn Al-Hiatham Teaching Eye Hospital/Baghdad at 2003, found that out of 86 patients referred positive cultures were obtained in 54 cases; 41 of them were caused by bacteria with *Pseudomonas* spp. (19 cases) as the most common bacterial isolates, followed by *Staphylococcus* spp. (17 cases). Fungal growth, on the other hand was detected in 13 cases.[7] Similar studies found that significant growth of microorganisms was obtained in 30.16% of the specimens they examined. of these, bacteria were the most occurred when accounted for 61% of the cases, with *P. aeruginosa* as the most common species.[17] *Pseudomonas* spp. were seen among over half (53.84%) of the cases of bacterial isolates. while *Staphylococcus* was found in 13 of the cases (20%), *Streptococcus pneumoniae* was found in 12 cases (18.46 %), other *Streptococcus* spp. in 2 (3%) cases and *Klebsiella* spp. was found in 5(7.69%) of patients.[18] *Pseudomonas* spp. was found to be the prime isolated among the bacterial isolates in Ghana and South India.[19] While in other study, coagulase negative staphylococci were the most common isolate, and they related the reason for the variation in the microbial causatives of keratitis to different climatic conditions, socioeconomic standards, culture and occupation that are seen in these geographical areas.[20] *P. aeruginosa* was the most common dominant organism isolated in the studies.[21,22] They found that *Staphylococcus* spp. and *Streptococcus* spp. isolates were the next most frequent pathogens, and *P.aeruginosa* was associated with the presence of contact lenses. *Staphylococcus* spp. were also found to be the most common isolated pathogen.[23] According to epidemiology of bacterial keratitis, *S. pneumoniae* was the predominant organism,[24] while *Staphylococcus* spp. was the most common organism in the prospective clinical and microbiological in another study in Switzerland.[25]

Identification of fungi

After culturing the 73 specimens of keratitis patients on Sabouroud agar for 7 days at 28°C, grown fungi were identified according to their morphology and spores. Results indicated that fungal infection was represented by 5 (6.85%) of the total specimens. Colonies of *Aspergillus niger*, which represented 3 (4.12%), were initially covered with a white, fluffy, aerial mycelium, and as colony matured, their surfaces were covered with black spores, while the reverse of the colony remained as light tan in color. The microscopic characteristics showed that hyphae were hyaline and distinctly septate, and the conidiophores were long. *Alternaria* sp. 2 (2.73%) were dark greenish surface with gray periphery, black on reverse side. The microscopic featured hyphae distinctly as septet and yellow brown in color, while the conidia were dark brown, multicelled, with both transverse and longitudinal septa, drumstick shaped, arranged in tandem long chains. From the results mentioned above, it can be concluded that *A. niger* was the most prevalence fungus isolated from the mycotic keratitis patients. While fungi were isolated in 39% of the cases, with *Fusarium* spp. as the highest (14.04%) followed by *Aspergillus* (7.02%).

A study was found that out of the 396 cases he tested, positive fungal growth was appeared in 74 of the cases (18.7%), and the most common fungi isolated were *Aspergillus* spp. 42 (56.8%) followed by *Fusarium* spp. 20 (27%), *Pencillium* spp. 4 (5.4%), *Scopulariopsis* spp. 2 (2.7%), *Geotrichum* spp. 1 (1.4%), *Alternaria* spp. 1 (1.4%) and *Candida* spp. 4 (5.4%).[26] In studying the management of fungal keratitis in Iraq with the eye drop amphotercin B (0.1%), they found that out of 129 patient specimens of suppurative keratitis, 22 of them had culture proven fungal keratitis (17%).

Again here, the most common fungi isolated were *Aspergillus* spp. (10 cases) followed by *Fusarium* spp. (8 cases), *Scopulariopsis* spp. (2 cases), and one case for each of *Penicillium* sp. and *Candida* sp. [26] In another research, investigation found that the prevalence of fungal keratitis in Iraq and identify types of fungi responsible for corneal

ulceration in 100 patients. They found that fungal growth was detected in 16 cases, and the most common fungus isolated was *Aspergillus* spp. (9 cases) followed by *Fusarium* spp. and *Candida albicans* (3 cases for each), while *Scopulariopsis* sp. was isolated from only one case.[27] Fungal infections was accounted as 32.50%, with *Aspergillus* spp. was the most abundant (56.42%), followed by *Curvularia* spp. (17.95%), *Cladosporium* spp. (7.70%), *Candida* spp.(5.13%), *Fusarium* spp.(5.13%), *Alternaria* spp.(5.13%) and *Penicillium* spp. (2.57%). They concluded that fungal infection is a life threatening condition, which needs early diagnosis and treatment to save the patient's eye.[11] Reports found that from a group study of 485 cases, 191(39%) were diagnosed as mycotic (fungal) keratitis. Microscopic examination revealed presence of fungal elements in the corneal scrapings of 62.3% and 60% of the subsequently fungal culture-positive cases, respectively. They found also that the *A.niger* was the most common fungus isolated, followed by *Curvularia* spp. in the culture-proven cases of fungal keratitis.[28]

Patients with culture positive were of different age groups but more during active age groups (20-60 years). Males had a tendency to get ocular ulcer more than females when 41 (56.16%) of the patients were males and 32 (42.85%) females.

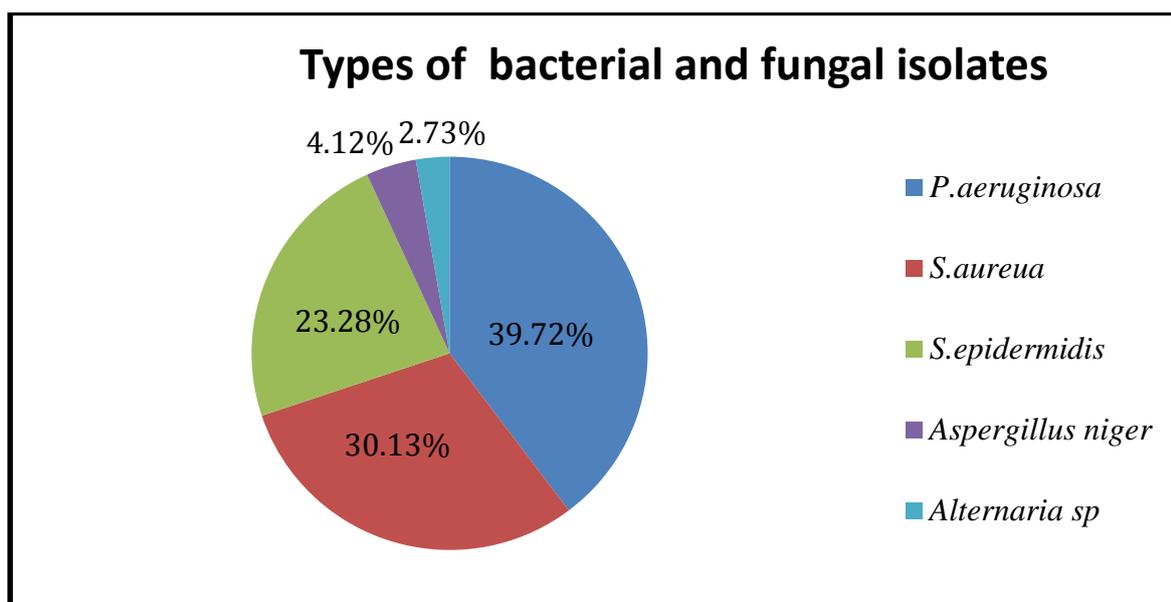
The relatively high percentage of males suffering microbial keratitis as compared with that of females came almost closely to some other similar studies. Research found that men (68%) were more affected by fungal keratitis than women (32%), [28] and some other reports found that out of 214 eyes patients, males comprised about two-third (66.2%) compared to the females who represented only the remaining one-third (33.8%), while age disease distribution of the patients showed that the disease was common among the age 30-60 years patients.[17] Some studies viewed that from 188 patients infected with microbial keratitis, 121 (64.36%) were males and 67 (35.73%) females, and regarding age group, 24 patients (12.76%) were <30 years of age, 43 (22.87%) aged between 30-60 years and 121 (64.36%) were >60 years. They referred the

highest percentage of microbial keratitis among male patients to the greater frequency subjection to corneal ulcer.[18] Moreover, some researchers found that among 100 patients clinically suspected of microbial keratitis, 67% were males and 33% females. They referred this high percentage by males to the fact that males have more chance of exposure to the accidents or trauma due to their outdoor activities.[29] Reports found that from a total of 190 patients included in their study, there were 103 (54%) males and 87 (46%) females' patients, with a range of (60–101) recorded in the mean age group of 75.5 years.[30] A study was mentioned that from a total of 156 patients, there were 102 (65.4%) males and 54 (34.6%) females with a mean age of 48 years.[30] Another reports found that out of 42 patients included in their study, 61.9% were males and 38% females, with a mean age of 44.5 years.[30] The microbial keratitis infection was found to be higher in males (60%) than in females with a mean age of 51 years.[30] Such results are similar to those of another researches, who found that microbial keratitis patients were mainly of the group (51-60) years.[20] A study of 2009 which reported that the mean age for patients with microbial keratitis was 51 year. [23] While other findings elucidated that 44.3 years was the mean age of the 85 patients suffering from bacterial corneal infiltrate. Age of those patients was in a range between 7 and 91 years.[25] In a previous study conducted at Ibn Al-Haitham Teaching Eye Hospital in Baghdad, the mean age of the patients suffering from microbial keratitis was 47.1 years.[26] Studies found that the percentage of male patients in microbial keratitis culture was 65.02% compared to 34.98% for female, distributed on the age groups as (14.7% for 21, 20.91% for (21-30), 24.38% for (31-40), 21.55% for (41-50) and 18.45% for 50) years of ages.[29]

Table (1)
Age and gender of patients with microbial keratitis.

Age group (year)	Gender		Total no. of patients	Percentage
	Male	Female		
≤ 20	9	8	17	23.27
21-40	12	9	21	28.73
41-59	13	9	22	30.20
≥ 60	7	6	13	17.80
Total	41	32	73	100.00

Table (2)
Profile of bacterial and fungal isolates.



Conclusion

According to this study *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were in this order the most important bacterial responsible for MK. While fungal isolates were belonged to *Aspergillus niger* and *Alternaria spp.*

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الخلاصة

لقد تم أخذ (105) عينة كشطات من مرضى التهاب قرنية العين المراجعين لمستشفى ابن الهيثم للعيون التعليمي في بغداد. أظهرت النتائج بعد زرع العينات على الاوساط الزرعية الاختيارية، أن 73 من هذه العينات كانت إيجابية لتواجد الأحياء المجهرية: 68 (64.76%) عينة حاوية على البكتريا و 5 (6.85%) على الفطريات.

بعد ان تم تشخيص العزلات البكتيرية بالاعتماد على الاختبارات الزرعية والمجهرية والفسولوجية امكن الحصول على الأنواع والنسب المئوية التالية:

Pseudomonas aeruginosa 29 (39.72%),
Staphylococcus aureus 22 (30.13%) and
Staphylococcus epidermidis 17 (23.28%).

اما بالنسبة للفطريات فقد تم تشخيصها اعتمادا على صفاتها الزرعية والمجهرية اضافة الى نوع وأشكال الأبواغ التي تكونها، وكانت ممثلة بالنوعين الأتيين ونسبتهما المئويتين:

Aspergillus و. (2.73 %) *Alternaria* spp. 2
niger 3 (4.12 %)

اعتمادا على ماتم الحصول عليه من نتائج اعلاه عدت قرنية العين البكتيرية، بينما كان عفن الـ *Aspergillus niger* هو السائد في التهابات القرنية التي سببتها الفطريات.

وفيما يتعلق بجنس المرضى، تبين أن حالات التهاب القرنية الجرثومية في الذكور والتي بلغت (41) حالة (56.16%) هي أكثر منها في الاناث التي تمثلت بـ (32) حالة (42.85%). وفيما يتعلق بالفئات العمرية التي شملتها الدراسة، فقد كان مرضى الفئة العمرية (41-59) عاما هم الأكثر تعرضا لإصابة قرنية العين الجرثومي بنسبة مئوية بلغت (30.20%)، فيما كانت الفئة العمرية (60 ≥) عاما هي الأقل عرضة ونسبة (17.80%).