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Phenotypic Screening and Antibiogram of Infectious Ulcerative Keratitis Cases in Tripoli Eye Hospital, Libya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study to investigate the most prevalent pathogens that cause corneal ulcer in Tripoli-Libya, and to recognize the antibiogram of the isolated pathogens
Study Design: This study was a prospective study performed in eye hospital (Zawyet-Aldahmany eye hospital).
Place and Duration of Study: It was conducted at the microbiology laboratory of the hospital over a year from December 2018 to January 2020, The overall size of the sample enrolled in this study was 50 inpatients in the admission department, which was taken according to the incidence of the corneal ulceration cases admitted to the hospital.
Methodology: The corneal scrapings and swabs were collected and subjected to microbiological examination. Demographic data and risk factors were collected for each patient. The antibiotic susceptibility of the bacterial microorganisms was determined by the Kirby-Bauer disk diffusion method, to identify the most appropriate antibiotic for each bacterial isolate.

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Results: 60% of the patients were males, the mean age of the patients was 46.5 years, unilateral right eye keratitis was involved in (54%) of cases, trauma was the major risk factor (24%), followed by systemic disease (14%). Concerning the seasonal distribution of the cases between months, our study revealed that summer and winter seasons in Libya (January, July, August, and September) involved most of the cases, and interestingly, fungal keratitis (*Aspergillus*) was higher in (July, August), which is the summer season in Libya. The culture positive rate was (80%), of which (36%) were bacterial, and (22%) were fungal pathogens, and the other (22%) were mixed fungal and bacterial cultures. The most common bacterial isolate was *(26%) P.aeruginosa* and (16%) *staphylococcus aureus,* while *Aspergillus* (40%) was the most common fungal isolate, followed by *Penicillium* and *fusarium* species. Our results showed that *P.aeruginosa* was the responsible microorganism for rapid keratitis progression in three cases who were using cosmetic lenses promptly. Concerning antibiotic sensitivity, all gram-negative bacteria and positive isolates were susceptible to old-generation fluoroquinolones such as ciprofloxacin and levofloxacin, while ceftazidime showed resistance to penicillins.
Conclusion: In conclusion, with early suspicion and microbiological diagnostic procedures, appropriate therapy can only reduce the incidence of this threatening ocular case.

Keywords: Antibiotic susceptibility; corneal ulceration; eye keratitis.

1. INTRODUCTION

The unique structure of the human eye as well as exposure of the eye directly to the environment render it vulnerable to a number of uncommon infectious diseases caused by a wide range of microorganisms. The surface of the eye is protected naturally with mechanical and immunological functions to defend itself against the environment. The defence mechanisms are native and acquired, both generalized and specific [1]. Because the cornea is one of the first lines of defence, the outer most layer in the eye, it can be irritated and become inflamed, gradually leading to painful keratitis. Scientifically, the cornea is defined as the most anterior part of the eye, a transparent part located in front of the iris and pupil. It is the most densely innervated tissue of the body, and most corneal nerves are sensory nerves, derived from the ophthalmic branch of the trigeminal nerve [2].

Corneal ulcer is defined as loss of corneal epithelium with underlined stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon, typical symptoms are red eye, foreign body sensation, pain, sensitivity to light, watery eyes, and blurred vision. The cornea can be invaded by various microorganisms like viruses, fungi, protozoa, and bacteria, but bacteria are most concerning due to rapidly progressive vision-threatening keratitis with irreversible visual sequelae [3]. Bacterial keratitis, or corneal ulcer, is an infection of the corneal tissue caused by varied bacterial species. It can be an acute, chronic, or transient infectious process of the cornea with a variable predilection for topographical, anatomical, or geographical domains of the cornea [3]. It can present as insidious progressive ulceration or rapidly deteriorating suppurative infection of any part of corneal tissue [4].

Bacterial infection is the most common cause of infectious keratitis. The both gram positive and negative bacteria may infect the cornea and cause corneal ulceration, but it has been studied in considerable detail that the most common causes of bacterial keratitis are *Staphylococcus aureus, Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* [5]. However, *Pseudomonas aeruginosa* is the most frequent and the most pathogenic ocular pathogen, it has the ability to diffuse rapidly through the cornea, leading to necrotic lesions that should be urgently treated to avoid corneal perforation in less than 24 hours [6]. The treatment of keratitis depended on its cause. Antimicrobial agents are used to treat infectious keratitis [7], while trauma, contact lens wear, reduced immunity, systematic disorders, and corticosteroids usage are the most predisposing factors for Non-infectious keratitis [8].

The whole microbiological study was hopefully employed for the ideal planning and proper selection of the optimal treatment for the corneal ulcer patients, and that was aimed at the ideal management of infective corneal ulceration in the study area. Therefore, the main objectives of this study were to investigate (isolate and identify) the most prevalent pathogens that cause corneal ulcers in clinically diagnosed cases in Tripoli eye hospital and recognize the antibiotic sensitivity

pattern (Antibiogram) of the isolated pathogens for the best selection of therapy.

2. MATERIALS AND METHODS

2.1. Study Design

This study was a prospective study performed in an eye hospital (Zawyet-Aldahmany eye hospital), which is a large hospital that covers a lot of ocular cases number in Tripoli, Libya. It was conducted at the microbiology laboratory of the hospital over a year, from December 2018 to January 2020, The overall size of the sample enrolled in this study was 50 inpatients in the admission department, which was taken according to the incidence of corneal ulceration cases admitted to the hospital.

2.1.1 Selection of case

i. Cases clinically diagnosed with (corneal ulceration) by ophthalmologists are collected.
ii. All the patients included in the study are Libyan patients attending eye emergencies, causalities, and further admission departments in Zawyat-Aldhmany eye hospital (Tripoli/ Libya) for a limited period.
iii. A clinical history was recorded including the following information:

- ✓ Date of collection.
- ✓ Name of the patient.
- ✓ Sex of the patient.
- ✓ Registration number for the sample.
- ✓ Side of eye infected.
- ✓ Other illness.
- ✓ Prescribed Treatment (antibiotic used previously).

To meet the aims of the study, the study has inclusion and exclusion criteria, Patients included in this study, were patients with clinically evident corneal ulcers and loss of the corneal epithelium with underlying stromal infiltration suppuration associated with signs of inflammation with or without hypopyon. Patients who had received antimicrobial therapy but were not responding to treatment were also included in this study.

Patients with suspected (or confirmed) viral keratitis, *acanthamoebae* keratitis, pre-existing blinding, corneal perforation or any other ocular diseases were excluded from this study.

2.2 Sample Collection

An ophthalmologist thoroughly examined all patients using a slit lamp and biomicroscope. A corneal scraping was taken under aseptic conditions using sterile 26-gauge needles on a syringe after using topical anesthesia. Scrapings were taken from the leading edge and the base of the ulcer, directly inoculated onto solid appropriate media, and incubated at the proper temperature. Furthermore, a corneal swab was taken by rubbing the ulcerated area of the cornea with a sterile cotton swab and inoculated into a solid media at 35°C–37°C, which was then sent to the microbiology laboratory in the hospital for further investigations.

2.3 Microbial Culture

Culture plates are incubated in different environments (aerobic/anaerobic /CO2/atmosphere) a daily monitoring of culture media is essential, colony morphology and description is observed for 24-48 hours.

Each corneal ulcer sample (both corneal scrap and swab) was plated onto a culture media, including Chocolate agar, Blood agar, Sabrouad's dextrose agar within 2 hours of sampling, and morphologically identified by smearing on the slide for bacterial gram's stain, and further fungal staining.

a. **Mannitol salt agar:** is a selective medium for the isolation of coagulase-positive staphylococci. Phenol red mannitol agar serves as an indicator for differentiating *staphylococcus aureus* or other mannitol fermenting bacteria from non-fermenting microorganism.
b. Blood agar: this agar is derived from seaweed with the addition of 5 to 10 % of red blood cells, which provides nutrients as well as an index of hemolysis; it is standard media for the isolation of aerobic bacteria at 35°C. It is also supporting the growth of saprophytic fungi and Nocardia at room temperature.
c. **Chocolate agar:** it is incubated with 10% carbon dioxide to isolate facultative organisms. It is prepared by heat denaturation of blood at 56°C to provide human and diphosphopyridine nucleotide for the growth of fastidious microorganisms such as *Haemophilus*, *Niesseria* and *Maroxella*.

d. **Nutrient broth:** liquid culture media helps in the isolation of microorganisms [9].
e. **Brain heart infusion broth:** it is a liquid culture media that increases the chance of the isolation of the bacteria in pure bacterial and mixed infections [9].
f. **Sabouraud's dextrose agar:** it's a fungus culturing media, fungus grows on Sabouraud's dextrose agar within 24 hours to maximum of two weeks' time, so prolonged incubation at 25% C is essential at least for two to three weeks before the culture is considered negative.

2.4 Tests for Identification: The following Tests were Performed where Necessary to Confirm the Identity of the Bacteria

Oxidase test: used to determine if a bacterium produces certain cytochrome c oxidase as *P.aeruginosa. P.aeuriginosa* possess the enzyme cytochrome oxidase, which catalyses the transport of electron donor compounds (NADH) to the electron acceptors (usually oxygen). The oxidase test utilizes the reagent N, N, N –tetra-methyl-p-phenylenediamine dihydrochloride, the oxidized reagent forms the colored compound indophenols blue in oxidase positive isolates.

Catalase test: catalase is an enzyme produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites. It mediates the breakage of hydrogen peroxide into water and oxygen, helping the aerobic strains of bacteria to survive. It is used to differentiate between two common gram-positive cocci, streptococci and staphylococci, which have similar colony appearance as staphylococci contain the enzyme catalase and streptococci do not.

Coagulase slide test: it is useful to differentiate potentially pathogenic *staphylococci spp (staph.aureus)* from the other gram positive bacteria. Coagulase, or (clumping factor) bound to the cell wall of *S.aureus*, causes cell agglutination when fibrinogen from plasma is added.

2.5 Staining Methods

I-Gram staining: Gram staining classifies the bacteria into two major groups based on the cell wall of the bacteria.

I. **Potassium hydroxide wet mount preparation: kOH mount 10%**is prepared as a useful test in helping identify of fungi. KOH helps in loosening the corneal stromal lamella and exposing more fungal filaments, which are obviously clear under conventional microscope.
II. **Lactophenol cotton blue stain:** it is formulated with lactophenol, which serves as a mounting fluid, and a cotton blue .it is an acid dye that stains the chitin present in the cell walls of fungi.

2.6 Antibiotic Sensitivity Test

It is a term used to describe the susceptibility of bacteria to antibiotics. It is carried out to choose the most proper antibiotic treatment for each bacterial infection. For samples that appeared a positive and developed a colony in culture media, The disk diffusion method was performed to investigate the antibiotic susceptibility of each bacterial species.

2.7 Disk diffusion method (Kirby-Baüer Method)

It is a test of the antibiotic sensitivity of the bacteria; Suspension of the isolate is made at a proper turbidity, and then spread evenly onto an appropriate agar (Muller-Hinton Agar) in Petri dish. The plate is allowed to dry, and disks containing the proper antibiotics are impregnated onto the surface of agar. Following overnight incubation, the diameter of zone of inhibition around the disk is measured. This test is recommended by CLSI M100-S26, using the following antibiotic disks: Vancomycin (30 µg), Rociphen (30 µg), amoxicillin (30 µg), amikacin (30 µg), gentamicin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), Ofloxacin (5 µg), levofloxacin(15µg) , tetracycline (30µg) and tobramycin (10 µg)standard table of antibiotic susceptibility was used to determine if the strain is resistant, intermediate, or susceptible to the antibiotic used.

2.8 Statistical Method

Descriptive statistics were used to summarize all the results, using a computer-based Statistical Package for the Social Sciences version 20 (SPSS Inc, Chicago, IL). The qualitative variables were presented as bar charts, pie charts, and percentages. The nonparametric test Chi-square was used appropriately to compare proportions. A confidence interval of 95% was used, and a $P \leq 0.05$ was considered statistically significant.

3. RESULTS

3.1 Patients' Demographics

The demographics of the corneal ulceration patients in this study were collected over a period of one year, by following their profiles directly to help in improving disease management and outcome. Concerning the gender, of 50 patients who were enrolled in this study, 30 were males (60%), While 20 were females (40%). the ratio of males to females is 1.5:1. All the patients were Libyan, their ages ranged from (7 years to 85 years), and the mean ages were 45.6±2 years.

Since corneal ulceration is either bilateral (involving both sides of the eyes), or unilateral (only one eye is infected), our results shows that All the patients had unilateral involvement of infectious keratitis, the right eye was involved in 27 patients (54%), and the left eye was involved in 23 patients (46%). The corneal ulceration was marked by the presence of hypopyn in 20 patient-infected eyes (40%).

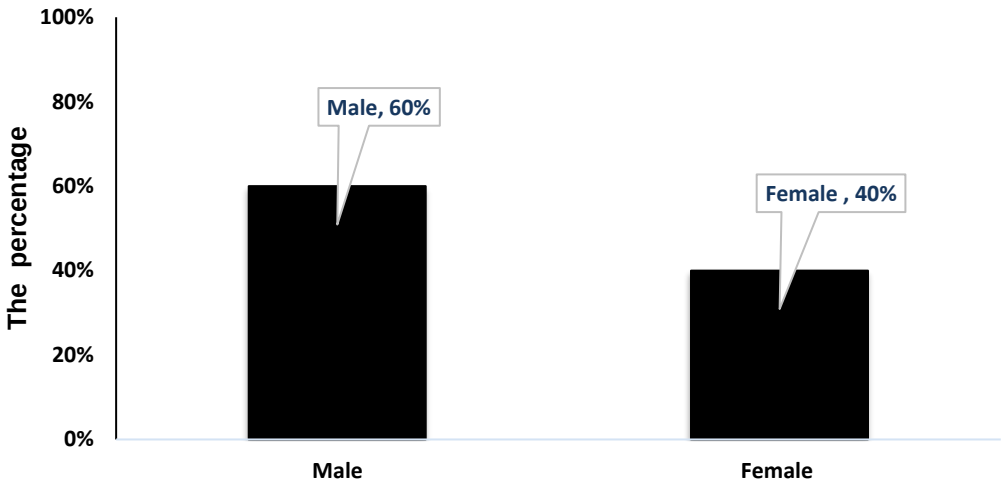

Fig.1. the gender of ulcerative keratitis patient

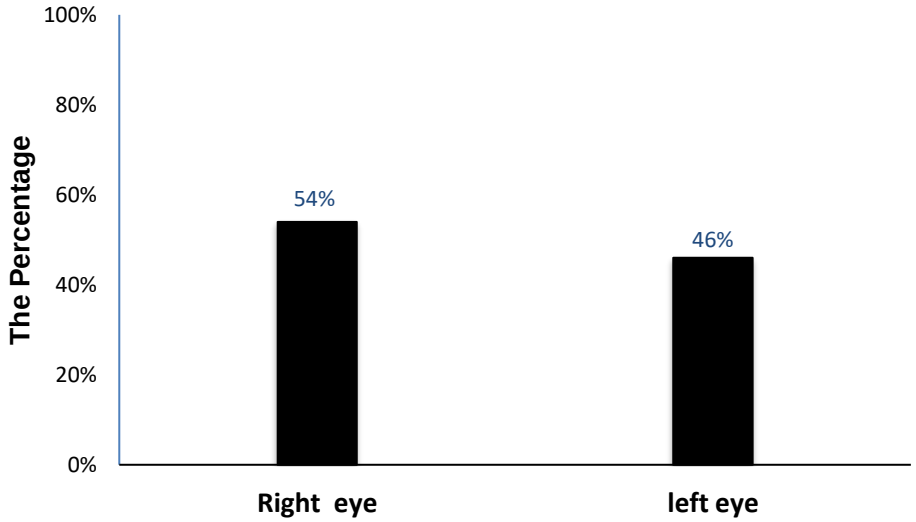

Fig. 2. The side of the infected eye

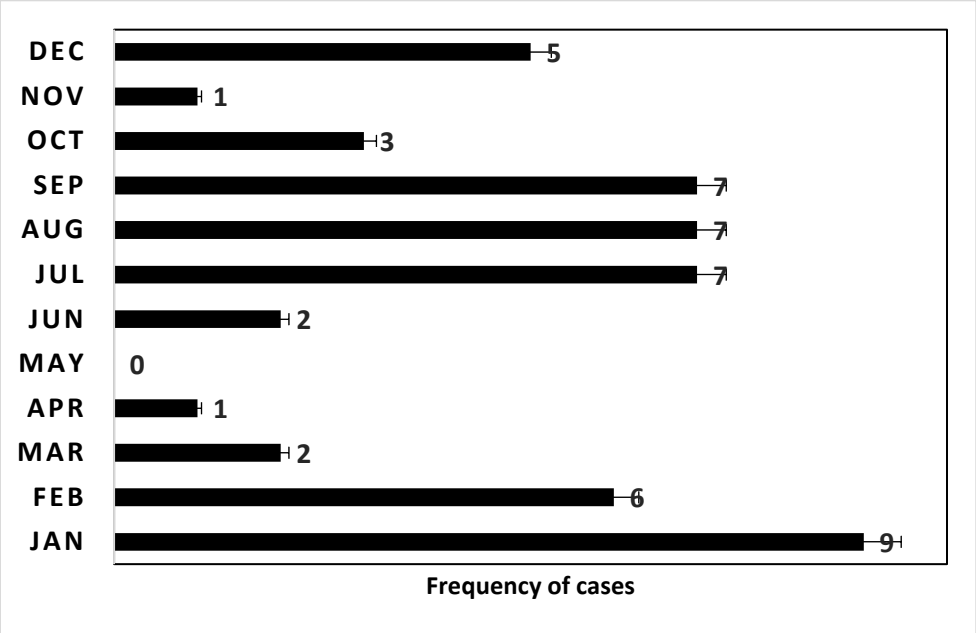

Fig. 3. Seasonal Distribution of ulcerative keratitis cases

Table 1. The predisposing factors of corneal ulceration

Predisposing factor	Number of Cases	Percentage%
Trauma	12	24%
systemic disease	7	14%
ocular surface disease/surgery	2	4%
contact lenses	5	10%
Unknown risk factor	21	42%
systemic diseaseand ocular disease	3	6%
Total	50	100%

Collecting the date of admission for each patient, revealed that the seasonal Distribution pattern of ulcerative keratitis patients between months was variable, according to the climate influence of each season, but remarkably, January, July, August, and September (the summer, and winter seasons in Libya) were the months that revealed highest number of cases. Interestingly, the incidence of fungal keratitis (*Aspergillus*) was higher in (July and August), which is the summer season in Libya.

3.2 Predisposing Factors

An identifiable risk factor for microbial keratitis was found in 29 patients (58%). The most common risk factor was ocular trauma (24%), followed by systemic disease (14%) as (diabetes mellitus, and hypertension, hypothyroidism), contact lense wear (10%), and previous surface ocular disease or surgery (4%). A total of 3 patients (6%) had more than one predisposing factor for ulcerative keratitis for instance, (systemic disease and previous ocular disease or surgery). Materials that caused trauma were plants, wood in agriculture regions, or explosive materials. Traumatic keratitis was highly related to a fungal positive culture in 7 (14%) patients. While contact lens wear contributed to pseudomonas keratitis in 3 (6%) cases.

3.3 The Isolated Microorganism from Ulcerative Keratitis Patients

Concerning the evaluation of laboratory microbiological isolation, among 50 cases of infectious keratitis patients that were inspected over a year, (80%) of the cases showed a positive culture and smear. The mean criteria for positive culture were the growth of three colonies on solid media.

Al-Shakarchi [10] of cases that shows a positive culture result, there were bacteria (36%), either

isolated bacteria (13 case), or polybacterial culture (5 cases), and [11] were fungal isolates (22%), and the rest were mixed fungal and bacterial isolates (22%). In 10 patients who undergo excessive antibiotic treatment before taking microbiological samples, no isolation was seen and it showed a negative culture result.

Table 2 number and percentage of growth

No of growth	Cases	Percentage
No growth (-)	10	20%
Growth (+)	40	80%

Table 3. The causing microorganisms of ulcerative keratitis

Microorganism	Frequency	Percentage %
Bacterial	18	36%
Fungal	11	22%
Mixed bacterial and fungal culture	11	22%
Negative culture result	10	20%
Total	50	100%

3.3.1 Bacterial isolates

The bacterial isolates predominate a high proportion among the isolates, since (36%), 18 cases, of the positive culture results were bacterial, and 11 (22%) were mixed bacterial and fungal according to gram staining results.

According to frequency of bacterial isolates, *P.aeruginosa* was the major bacterial isolate, since *P. aeruginosa* was found in 13 cases (26%),as isolated microorganism or mixed culture, followed by *staphylococci species*, such as *staphylococcus aureus* which was found in 8 cases (16%),*Staphylococcus epidermidis* which were found in 4 cases (8%), *Niesseria* was found in 2 cases (4%), while the least proportion with one case (2%) for each, was for *Enterococci, klebsiella,* gram positive *bacilli* and *streptococci species*.

3.3.2 Gram positive Bacteria

On the further subdivision of the Gram positive organisms identified in the positive bacterial cultures, the common organisms isolated included: *staphylococcus aureus, Staphylococcus epidermidis, streptococcus pneumonia, Enterococcus species, gram positive bacilli*, mixed growth of *staphylococci* and *streptococci*.

3.3.3 Gram negative bacteria

Pseudomonas bacteria was the commonest gram-negative bacteria, isolated from 13 cases of ulcerative keratitis patients (26%), followed by *Niesseria (4*%) and *klebsiella* (2%) isolates.

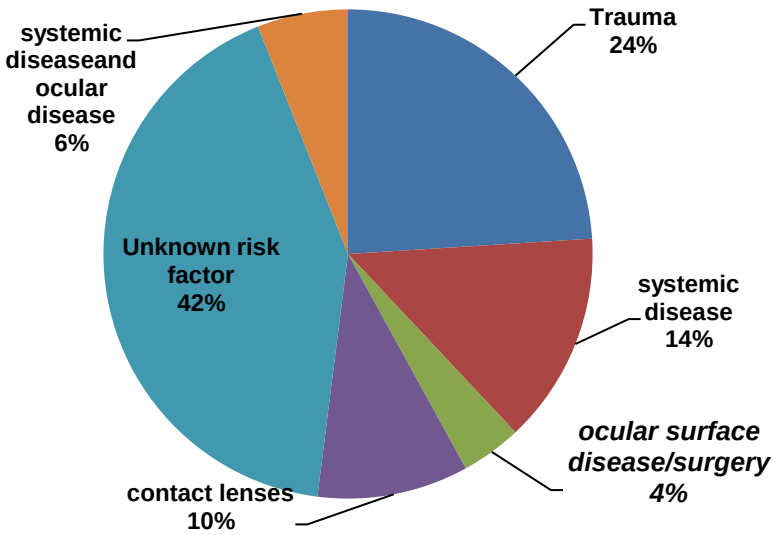

Fig. 4 the percentage of predisposing factors of ulcerative keratitis

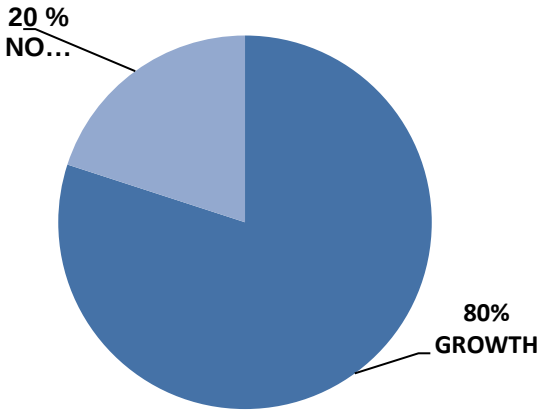

Fig. 5. Percentage of positive results (growth)

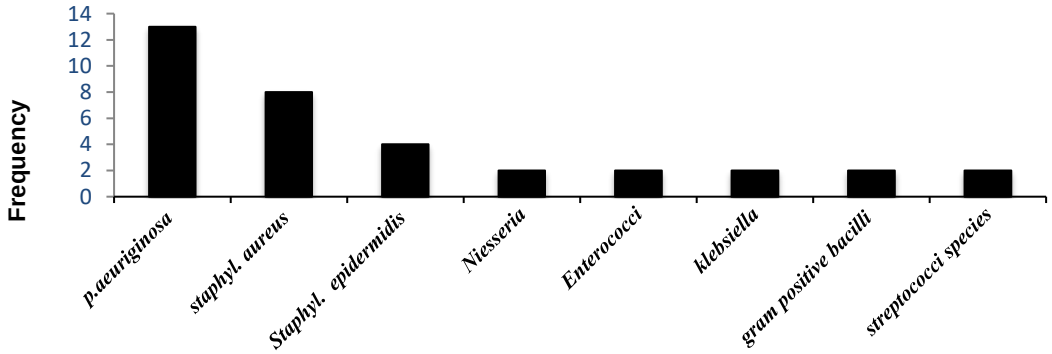

Fig. 6. The type of bacteria in the samples

Table 4. The gram-positive bacteria in the samples of ulcerative keratitis patients

Gram positive bacteria	Number of isolates
Staphylococcus aureus	6
Staphylococcus epidermidis	2
Streptococci pneumonia	1
Gram positive bacilli	1
Enterococci spp	1
Mixed staphylococci and streptococci	3
Total	14

3.3.4 Contact lens wearer and *P.aeruginosa*

During contact lens wear, many microbes can enter the eye, one of the most dangerous microbes is *P.aeruginosa.* in this study this opportunistic pathogen was isolated from Vajpayee et al. [12] cases of patients, and among those patients, three patients were using cosmetic contact lenses promptly, which lead to a rapid progression of serious visual outcome.

Table 5. the Gram-negative bacteria in the ulcerative keratitis patients

Gram negative bacteria	Number of isolates
Pseudomonas aeruginosa	13
Niesseria	2
Klebseilla pneumonia	1
Total	16

3.3.5 Fungal isolates

The most common fungus isolated was *Aspergillus* in 20 eyes, isolated or in a mixed culture, and only one case of *Penicillium* and *fusarium* were isolated.

Table 6. Fungal isolates and their percentage

Fungi	Frequency	Percentage
Aspergillus	20	40%
Penicillium	1	2%
Fusarium	1	2%
Other M.O	28	56%
Total	50	100%

3.4 Antibiotic Susceptibility

Concerning the susceptibility of bacterial isolates to different antibiotics, AST results shows that all gram positive cocci species were susceptible to vancomycin, followed by ofloxacin, and ciprofloxacin, and the highest resistance was to amoxicillin, ceftazidim, whilemost gram negative bacilli isolates were sensitive to vancomycin, gentamicin, fluoroquinolones (ciprofloxacin and ofloxacin), Notably *(P. aeruginosa*), the most frequently isolated gram negative bacilli was susceptible to ciprofloxacinfollowed by levofloxacin, tobramycin, amikacin, gentamicin, and the highest resistance was seen to antibiotics amoxicillin, ceftraixone, ceftazidime, and tetracycline. There was significant association between young age and ocular trauma ($P \leq 0.05$).

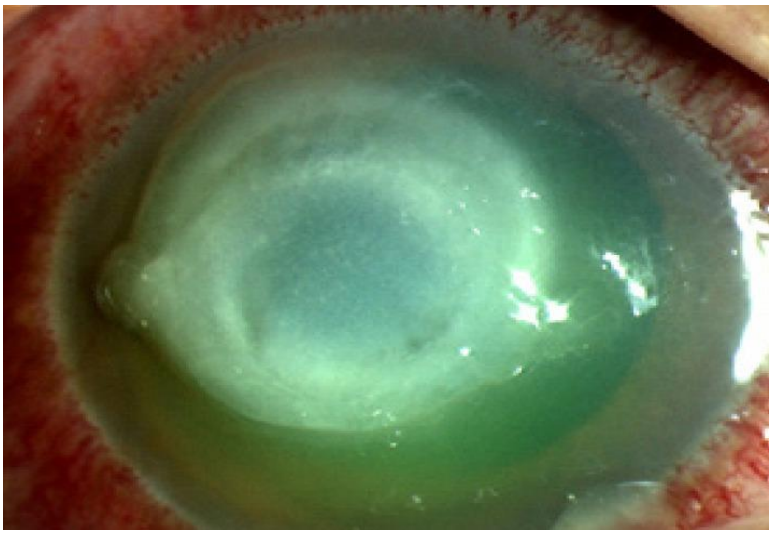

Fig. 7. An eye infected with *p. aeruginosa*

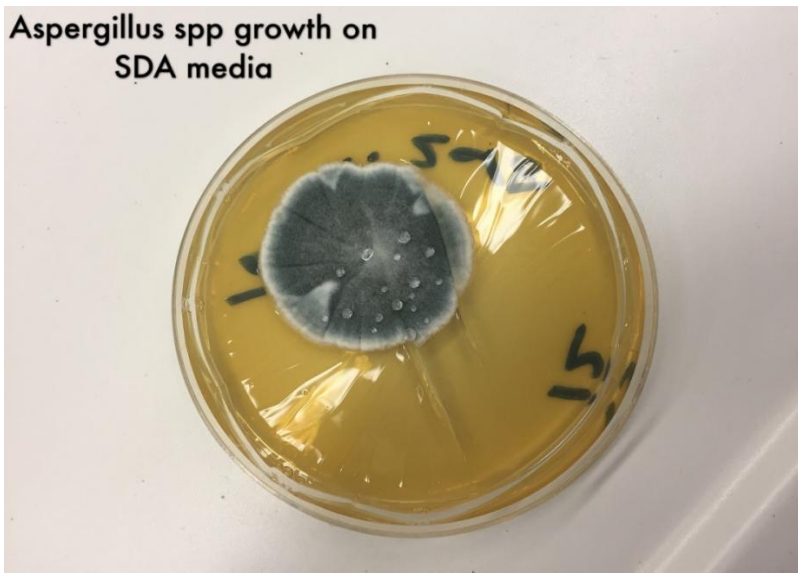

Fig. 8. Aspergillus fungi on SDA media (my work)

Table 7. The antibiotic susceptibility of isolated bacteria from ulcerative keratitis patients

	CN	VA	CIP	AK	OFX	TOB	LEV	TE	AMC	C	CAZ	CRO
P. aeruginosa	S	R	S	S	S	S	S	R	R	R	R	R
S. aureus	S	S	S	S	S	S	S	S	R	S	R	R
S. epidermidis	S	S	S	S	S	S	S	S	R	S	R	R
streptococci	S	S	S	S	S	S	S	R	R	S	R	R
Niesseria	S	S	S	S	S	S	S	R	R	R	R	R
Gram positive bacilli	S	S	S	S	S	S	S	S	R	S	R	R
klebsiella	S	S	S	S	S	S	S	R	R	R	R	R
Enterococci	S	S	S	S	S	S	S	R	R	S	R	R

Chloramphenicol(C),Ciprofloxacin(CIP),Gentamicin(CN),Tetracycine(TE),Ofloxacin(OFX),Vancomycin(VA),Aknamycin(AK),Amoxicillin (AMC), Tobramycin(TOB), (S= Sensitive, R= Resistant).

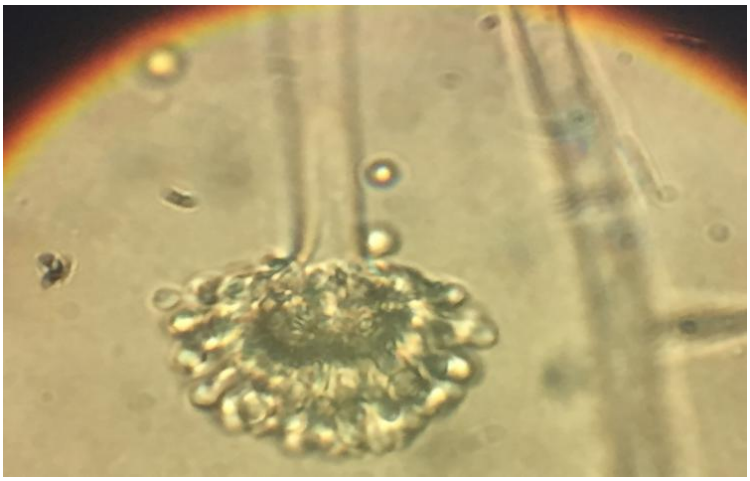

Fig. 9. Photo of *Aspergillus* hyphae and spores under microscope (my work)

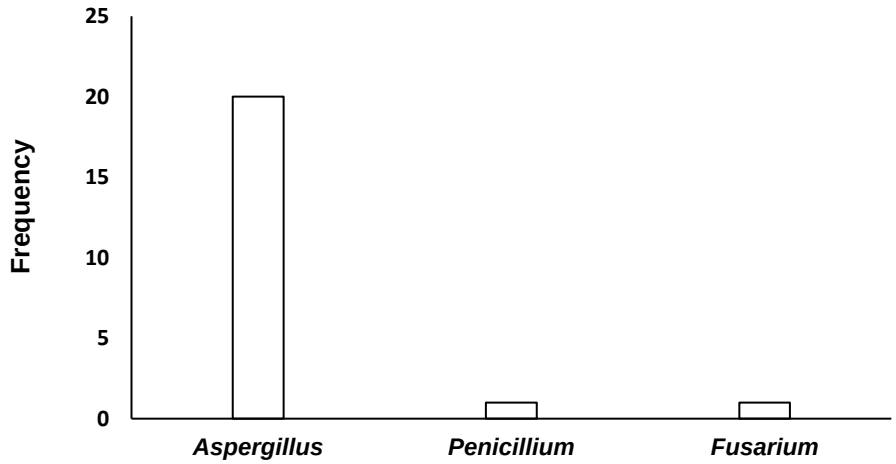

Fig. 10. The fungal isolates from the ulcerative keratitis cases

4. DISCUSSION

Corneal ulceration is no doubt a formidable ocular emergency that leads to loss of vision worldwide. This study was a prospective analysis of 50 patients who were hospitalized for corneal ulceration in Tripoli-Libya at Zaweyat-Aldehmany hospital, for a whole year to reveal the incidence and the nature of this disease, and evaluate demographic, clinical, and microbiological information of ulcerative keratitis patients. And it was the first of its kind in this region.

In this study, males predominately had a higher incidence rate of corneal ulcers(60%) than females. this is in conformity with variety of studies conducted in other regions like the Madurai study [13] and also another study done by Titiyal et al., [11], this might be explained with the fact that males spend a longer time outdoors, are more physically active than females, and are exposed to the different environments through their variable work nature, like farmers in rural regions, who are exposed to trauma daily, which is a major risk factor for corneal ulceration.

Regarding the age in this study, the age of the patients were ranged from (7 years to 85) years, majority of the cases were over 30, other study [13] reported that a higher prevalence of the middle age group to corneal ulceration.

The most common risk factor for ulcerative keratitis in this study was trauma (24%), which

shows a similar results as the most common factor for keratitis in the study of sirnivasan and his research group [13], from south India, that reported ocular trauma in (65%) of patients in contrast united states study, [14] reported a trauma history in only 8.2% of the cases.

Vajpayee et al. [12] reported that Trauma was a predisposing factor in rural areas, or low income countries, and since most of the cases in our study belong to rural regions, trauma was a major predisposing factor.

Plants, wood in agriculture regions, or explosive materials were the multifactors that result in ocular trauma and gradually corneal ulceration, but there was a one ocularly rare condition of eye injury, which is a bee sting, that caused a corneal epithelia effect. a surgical approach of removing retained bee stinger, and a conventional antibiotic therapy were used for the management of this rare case [15].

Concerning the seasonal distribution of ulcerative keratitis patients between months of the year, winter season months (January, February, and December) consist the highest number of cases, followed by the summer season (July, august).

The incidence of fungal keratitis (*Aspergillus* cases) was higher in June and August (the summer season in Libya). This may be due to the hot and humid season of the summer, that make fungal keratitis more frequent in tropical zones, and the high tolerance of *Aspergillus* spores to hot and dry weather conditions, which lead to high incidence of this fungal species in this weather. This result agrees with the results of Bharath et al. [16], who evaluated the influence of climate and geographical variation in microbial keratitis in south India, in which the incidence of fungal keratitis cases was higher between June and September.

According to the culture positive results, our culture positive rate was (80%), the predominant bacterial species was *Pseudomonas aeruginosa*. it was the most common gram negative bacteria isolated since *Pseudomonas aeruginosa* is a common inhabitant of soil, water and vegetation, and it has an invasive ability due to the adhesion to the eye surface. it can cause visual impairment in a few days.

The predominant gram positive bacteria isolated was *S.aureus*, which is in confirmatory with study in eastern Nepal [17].

The commonest fungal pathogen was *Aspergillus* spp, followed by *fusarium* and *Penicillium*, which are the most common isolates in many studies in india [18], and Baghdad [10]. it might be due to geographic location since those filamentary fungal species are predominant in tropical and subtropical climates, and are commonly found in soil and plants, and most of the patients infected with fungal keratitis in our study were from rural background which are rich in agriculture work.

4.1 Contact Lens Related Keratitis

Contact lens usage has been a major risk factor for bacterial keratitis in reports from US [19], and Western Europe [20], where contact lens usage in our study represens (10%) as a predisposing factor of corneal ulceration.

Five cases were using cosmetic contact lenses promptly. Three cases were females who used cosmetic lenses, and *Pseudomonas aeruginosa* was the most isolated bacteria in those contact lens wearers, which agree with [21] that reported *P.aeruginosa* and *Staphylococci* species are the most common organisms cultures in contact lens, associated bacterial keratitis.

The use of contact lens can interfere with ocular defenses, leading to hypoxia and distruption of epithelial tear film [22], which eventually lead the gram negative opportunistic *p.aeruginosa* to easily invade the surface of the cornea. Infection of the cornea by opportunistic microorganisms occurs during contact lens wear since microbes can enter the eye from the wearer's lid margins, their fingers upon lens insertion (or removal), or via the contact lens, from the care solutions, or the storage conditions. Generally speaking, good hygiene and proper contact lens handling will minimize the risk of contact lens keratitis [23]. This result reveals the importance of people awareness with the hygienic contact use to avoid the hazardous outcome of *pseudomonal* keratitis .

Since many studies show that there is a high variability in microbial isolates and their antibiotic resistance, depending on geographic region and population, and with time [24], we manifest the antibiotic susceptibility pattern of our patients in this study to reveal the most suitable empirical treatment of antibiotic with each ulcerative keratitis case. The protocol that is used in Tripoli eye hospital consist of fortified gentamycin and vancomycin antibiotics in hourly dosages, vancomycin covers the gram positive

microorganisms, while aminoglycosides are active against both gram positive bacteria and gram negative isolates, or a duo therapy of subconjuctival gentamycin and vancomycin is used to produce a high corneal drug level. Also, fluoroquinolones that have a broad spectrum against gram positive and gram negative bacteria were used. Although significant resistance to fluoroquinolones has been reported from India [25] and United States, our study results shows that ofloxacin, ciprofloxacin and levofloxacin are still effective in bacterial keratitis therapy, and Fluoroquinolones appear to cover most staphylococcus species and pseudomonas. Gram positive bacteria were most commonly resistant to beta-actam antibiotics (amoxicillin, ceftazidim, ceftraixone).

Gram negative *P.aeuroginosa* was sensitive to ciprofloxacin, levofloxacin, and ofloxacin, in contrast to Subedi et al. [26], which reported the resistance of *P.aeruginosa* to ciprofloxacin. and [27] study, which reported that gatifloxacin is superior to ciprofloxacin for management of bacterial keratitis.

Antibiotic resistance should be considered precisely When a gram negative organism is a risk. Monitoring antibiotic resistance in that case is an emergency. Since *Pseudomonas* the gram negative bacteria, is typically much more aggressive both in depth and the defect, and its antibiotic resistance will gradually lead to the catastrophic effect of corneal perforation and blindness [28,29].

Moreover, this study shows that Second-generation fluoroquinolone monotherapy could be successfully recommended as the empirical treatment in microbial keratitis, since Fortified antibiotics such as fortified vancomycin are known to cause significant ocular toxicity, such as increasing epithelial defect size, compared to fluoroquinolones antibiotics that penetrate ocular tissues well and have good safety profiles with minimum ocular toxicity, and cover a broad spectrum of bacterial organisms,

5. CONCLUSION

This study clearly elucidates the importance of this sight-threatening ocular condition. The major ocular bacterial pathogens for ulcerative keratitis were *P.aeruginosa* and *S.aureus* bacteria, and *Aspergillus* was the most prevalent fungi. The overall results emphasize the importance of *P.aeruginosa* as a major risk factor for the rapid progression of the largest corneal ulcers, and the urge of public health education about this microorganism in contact lens related keratitis [30,31].

Concerning the treatment of the ulcerative keratitis, it was heartening to know that the prescription of monotherapy with fluoroquinolones and fortified aminoglycosides, as the current protocol in our country is correct and effective, and could be generally reserved. However antibiotic resistance monitoring should be continued to avoid any change in the future. It is clear that Old generation fluoroquionolnes is effective against gram negative and positive bacteria, according to the results, but it is further interesting to know the effect of newer-generation fluoroquinolones (moxifloxacin and gatifloxacin), as many studies have reported their higher efficacy, and how it will change the landscape of antimicrobial sensitivity in the future.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The Study was approved and confirmed under the rules and regulations of research in the department of microbiology and immunology, Faculty of Pharmacy, Tripoli University Tripoli-Libya. Therefore, at the beginning of the research point, ethical approval and authorization were issued while referring to the hospital. At the hospital, the regulations for research were approved by the ethical committee of the hospital and the microbiology laboratory as well.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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