Isolation and Diagnosis of the Bacteria Causing Corneal Ulceration Associated With Ocular Myiasis Infection

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Abstract

The current study was conducted to isolate and diagnose the bacteria that company cases ocular myiasis as complications. The bacteria were collected using a cotton swab for 30 individuals who came to the Diwaniyah Teaching Hospital due to their infection with ocularmyiasis. The swabs were cultivated on many culture media, such as blood agar and MacConkey, and mannitol salt and EMB medium for diagnosis of Ecoli. The results of the culture were positive for all samples under study. Various types were isolated; including rod and coccobacilli gram-negative in addition to gram-positive cocci, fermented and non-fermented mannitol species, and the identification of the species was confirmed using the VITEK2 system, the antibiotic susceptibility test was also checked using the same test and the results were as the following Ecoli in 5 cases, Pseudomonas spp in 4 cases, Kelbssilla pneumonia in one case, S aureus in 4 cases, S.epidermides in 5 cases in addition to many other different species.

Keywords: *Ocular myiasis, secondary infection, corneal ulcer, VITEK 2 compact system, Sphingomonas paucimobilis*

Introduction

A corneal ulcer is one of the most common serious infections in the world [1]; it can be defined as a sore in the cornea [2]. The etiological agent might be bacteria, viruses, fungi, or parasite like *Acanthamoeba spp*, besides many non-infectious agents such as chemical toxic, autoimmune diseases, neurotrophic and other [3]. One of the major reasons for corneal ulceration or keratitis is waring contact lances in developed countries, while the reason might be trauma in the developing world [4]. Corneal ulcer generally occurs as secondary to prior infection as ophthalmomyiasis cases (the invasion of the eye by living fly larvae of order Diptera). It usually occurs in shepherds and farmers in rural areas, but it has been documented in urban areas as well as in patients without close contact with animals. Other risk factors that have been identified include eye infections, eye wounds, advanced age, weakening, lack of diet and treatment, and poor hygiene. Cases of ophthalmomyiasis have been recorded in many nations around the world [5]. the causative agent might be the sheep botfly or *Oestrus ovis*, which is the most common cause of human ophthalmomyiasis, besides many other species have also been described, such as the human bot fly (*Dermatobia hominis*) latrine fly (Fannia), house fly (Musca domestica), and old screw worm *chryosoma bezzina*., in addition to many other species that cause the infection accidentally when infecting the skin, the clinical feature of the infection varies from severe foreign body sensation and redness, rhinorrhoea, chemises, and swelling. Conjunctivitis may be imitated by these initial signs and symptoms of external ophthalmomyiasis[6]. Some studies assume that corneal ulceration is one of the reasons that lead to ophthalmomyias is because of the foul-smelling caused by ulceration that attracts insects [7], but the more comprehensive hypothesis is that ulceration occurred as a result of infection with the lesion because the eye was injured by the insect In addition to a change in immunity levels [8]. In general corneal

ulcer still one of the emergency defects that might lead to blindness. Since ocular myiasis is self-limited and spontaneously healing, as such as rare disease the corneal ulcer that is caused by is also neglected despite the possibility of becoming a serious problem that can lead to blindness.

The aim of the present study was isolation and characterization of bacteria responsible for corneal ulceration which occurs as a complication of ocular myiasis, besides, to study antibiotic sensitivity testing.

Material and Methods

Diagnosis

According to the symptoms which include itching, burning, lacrimation, rhinorrhea, foreign body sensation, and swelling, the history of the patient is very beneficial in diagnosis as the patient might recently be in close contact with an animal or not, besides, to see the larvae in the eye by an ophthalmologist.

Bacterial sample collection

To study the secondary bacterial infection and diagnose the etiological bacterial species about 30 samples were collected from all patients who give positive for ocular myiasis infection; this was accomplished by using cotton swabs. Then samples transformed to the lab immediately and streaked on Petri dishes contain Blood, MacConkey, and Mannitol salt agar which were prepared already, the dishes incubated at 37 C for 24-48 hours. then the growth was identified using gram stain and then depended on the VITEK 2 system.

Results and Discussion

Corneal infections almost are the second most significant cause of blindness in the world, it usually occurs as a secondary infection. Since ocular myiasis is classified as a rare disease in addition to the possibility of it being cured automatically, there were no serious studies about its complications like corneal ulcerations. The present study was carried out in Diwanyia province in Iraq. For six months on ophthalmomyiasis infected people to identify the bacteria that cause the corneal ulceration that occurs as a secondary infection.

Thirty samples from thirty individuals who visited the hospital due to infection with ophthalmomyia sis, samples have been collected with cotton swabs according to S. Ballim et al [9,10]. even the ophthalmologist didn't notice keratitis signs.

These samples have transported to the lab and soon streaked on already prepared blood, MacConkey, and mannitol salt agar culture media, the results of growing bacteria are explained in Figures 1, 2,3,4 below

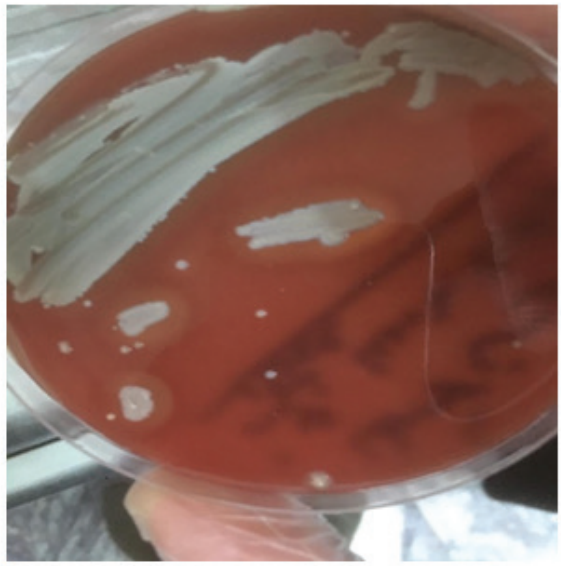

Fig 1 growing bacteria on blood agar

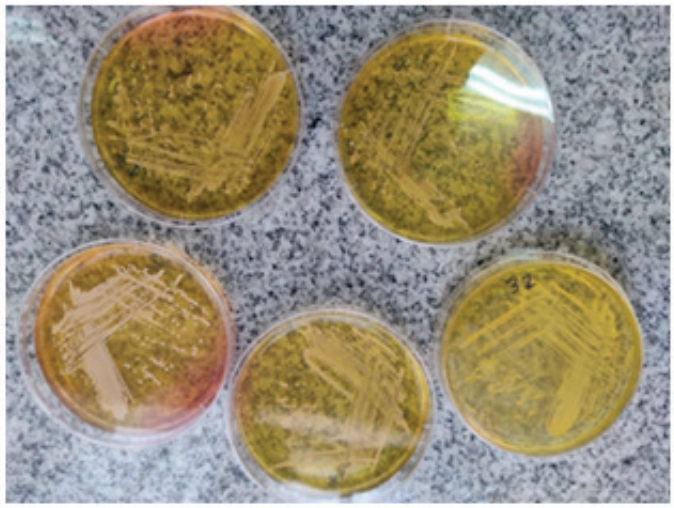

Fig2. Growing bacteria on mannitol salt agar that ferment mannitol.

Fig 3 different bacteria on MacConkey agar

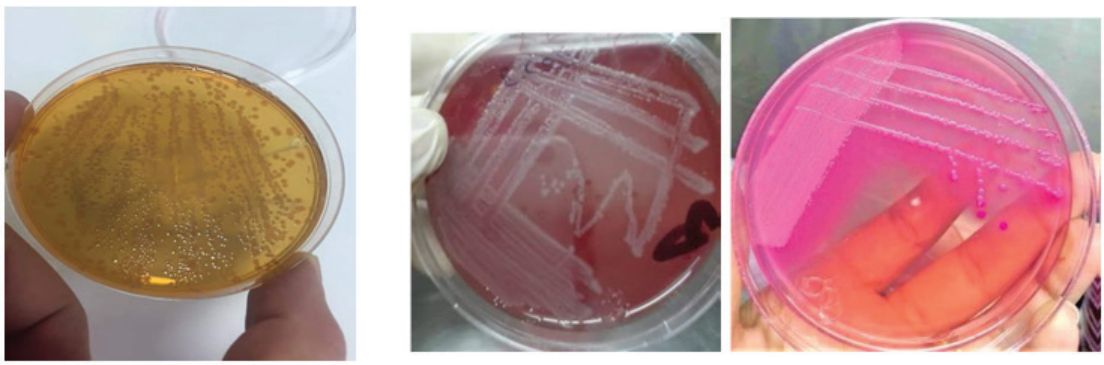

Fig 3 different bacteria on MacConkey agar

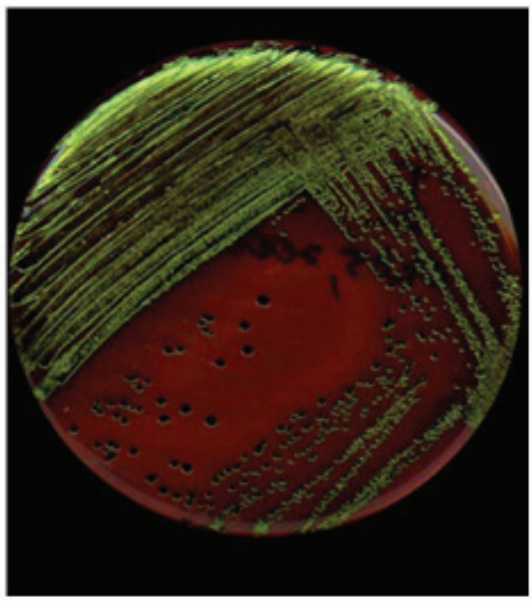

Fig 4 *Ecoli* colonies on EMB media

For further identification biochemical tests according to morphology on Gram stain and cultural characteristics on various media

Gram-positive cocci in clusters on gram stain, positive coagulase test, positive mannitol fermentation, and phenolphthalein phosphate test for Staphylococcus aureus Staphylococci with coagulase-negative staphylococci – coagulase-negative staphylococci negative and non-fermenting mannitol, Gram-negative bacteria, Pseudomonas aeruginosa Positive for catalase and oxidase, and green blush on agar plate *Ecoli* Gram-negative pink colonies on MacConkey and green with metallic shine on Eosin Methylene Blue (EMB).Indole and methyl red positive, *klebsiella pneumonia* gram-negative pink with slime layer on MacConkey agar indole, and methyl red negative. All these results are explained in the table1,2.

Table 1. Biochemical tests for gram posetive bacteria

bacteria	Staphylococcus aureus	Staphylococcus epidermidis	S.pseudintermedius
coagulase	+	-	+
Catalase	+	+	+
Mannitol fermintation	+	-	+

Table 2 Biochemical tests for gram negtive bacteria

bacteria	E.coli	klebsiella spp	Pseudomonas spp
indole	+	-	-
Methyl red	+	-	-
oxidase	-	-	+
catalase	+	+	+

Also for advanced identification and antibiotic sensitivity testing. The isolated bacteria have been sent to be diagnosed by using VITEK 2 system and the results showed many species of bacteria are represented in table 3below

Table 3 VITEK2 compact system species identification results

Bacterial species	No .of cases isolated from
Klebsiella pneumonia	1
Escheichia coli	5
Staphylococcus aureus	4
Staphylococcus pseudintermedius	3
Pseudomonas earoginosa	5
Pseudomonas alcaligenes	2
Sphingomonas paucimobilis	1
Acinatobacter baumannii	1
Acrombacter deitrificans	1

Original results that obtained from VITEK2 system had appendixes at the end of research. Most studies believe that infection with ophthalmomyisis is one of the rare, minor, limited infections that rarely occur recently, and if they do occur, their effect is limited to inconvenience. However, it may turn the eye into a pus cave full of smelly maggots which might encourages bacterial growing .but dependingon that most but not all of the isolated bacterial species return to the normal flora present in the eye and may have become pathogenic due to the flystrike that causes superficial scratches in the eye, on the other side immunologically larvae responsible for ocular myiasis contained have two antigens first one is the cuticle and second one represented by polypeptides that arise from salivary glands of the larvae and that the last is more immunogenic despite the direct contact between the larval cuticle and their hosts when host suffers from flystrike hypersensitivity reaction type I which is known as immediate type[11], occur lead to the proliferation of eosinophils and masts cells activation, beside IgE trigering and relaesing many mediators such as IL4,5,13 and Th2 cells response or simply just like anaphylaxis to bee venom[12]. These interferences can affect the normal environment of the eye to become pathogenic to the normal bacteria In addition to allowing the opportunistic types to cause ulcers in the cornea[13].

Conclusion

In general, it can be said that cases of ocularmyiasis, even if they are spontaneous cure, but what accompanies them of bacterial infections may be dangerous and lead to blindness.

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