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Original Research Article

Investigation of Some *Moraxella lacunata* virulence Factors and Detection of *Toxoplasma gondii* in Patients with Eye Infections in Hilla City

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Abstract

This study based up-on 100 eye swab samples were collected from patients complaining of ocular disease who were attended to Al-Hilla Teaching Hospital during period ranging from November 2015 to January 2016. Those patients belong to age groups between two years to sixty years .Only 12 isolates of *Moraxella lacunata* was isolated from patients with ocular infection.

The results obtained in this study explain all bacterial isolates sensitive entirely to Neomycin ,Gentamicin ,Ceftriaxone and Piperacillin while the results show the bacterial isolates less sensitive to Clarithromycin and Ceftazidime antibiotics.

Besides, the results of this study depicted all bacterial isolate inability to produce CFA/I in the presence D – mannose and positive for CFA/III. Also, study the effect of lysozyme on bacterial isolate . The results showed that the isolated bacteria can withstand in thepresence of different concentration of lysozyme.

Key Words : *Moraxella lacunata*, Lysozyme, CFA/I and CFA/III.

التحقق من بعض عوامل الضراوة لبكتيريا *Moraxella lacunata* والتحرِّي عن طفيلي *Toxoplasma gondii* في المرضى المصابين بالتهابات العيون في مدينة الحلة

الخلاصة

في هذه الدراسة تم الحصول على 100 مسحة من المرضى الذين يعانون من اصابات في العيون المراجعين لمستشفى الحلة التعليمي للفترة من نوفمبر / 2015 ولغاية يناير / 2016 والذين تراوحت اعمارهم من 2 سنة الى 60 سنة ,حيث تم عزل وتشخيص 12 عزلة فقط من بكتيريا ال *Moraxella lacunata*.

تم دراسة تأثير بعض المضادات الحياتية على هذه جميع العزلات البكتيرية وقد اشارت النتائج الى ان جميع العزلات كانت حساسة تماماً 100% لل Neomycin , Gentamycin , Ceftriaxone , Piperacillin وبدرجة أقل لمضاد Ceftazidime , Clarithromycin.

كما اظهرت نتائج هذه الدراسة ان جميع العزلات كانت موجبة لعامل الالتصاق الثالث بينما كانت جميع العزلات سالبة لانتاج عامل الالتصاق الاول. كذلك تم دراسة تأثير اللايسوزايم على العزلات البكتيرية حيث أظهرت النتائج ان هذه العزلات تستطيع البقاء والمقاومه في تراكيز مختلفه من اللايسوزايم .

الكلمات المفتاحية : Moraxella lacunata، عامل الالتصاق الاول، عامل الالتصاق الثاني ,اللايسوزايم.

Introduction

Moraxella lacunata bacteria is isolated from eyes of patients suspected of infection as major ocular bacterial agent. Ocular bacterial infection present with varying in clinical symptoms and signs, these include pus discharge, visual impairment and conjunctival hyperemia. The infection by this bacteria may be acquired from external environment or from systemic infection by blood, which acts as transport media , also because this bacteria presented as a normal flora on the eyelid and conjunctiva for this any limited protect environment facilitated external or internal infection [1].

Many predisposing factors facilitated infection with, *Moraxella lacunata* especially patients who are alcoholic, diabetic or debilitated person, as well as contact lenses use help to increase keratitis infections [2].

There is little information about this species of *Moraxella* when compared to other *Moraxella* spp. particularly *Moraxella catarrhals*. However little is known about its virulence factors which are studied mostly by molecular technique [3].

Furthermore, the ocular infection can occur by parasite agent such as *Toxoplasma gondii*. The Toxoplasmosis can be transmission congenitally or postnatally, also may occur ocular lesion during some years after the acute infection[4].

Toxoplasma gondii is infected about more than third of world's population, also its answerable for the majority of uveitis infection (intra ocular infection), about 50% rate of posterior uveitis infection in many countries occur by acquired *Toxoplasma* [5,6].

Materials and Methods

Specimens collection:

Sterilized swabs were used to collect the specimen from patients suffer from eye infection ,this process occur under help of physician to prevent any contamination occur.This swab immediately placed in transporter media tube to prevent swab dryness and maintained moist until be transported to laboratory, the swab transported back to laboratory through 30 minutes of initial collection. Each collected specimen was inoculated in blood culture broth and incubation for 24 hours to determine aerobic isolate, only aerobic isolates are chosen for culturing on blood, Mac Conkey and chocolate agar and incubation for 24 hours at 37C°, then chose the isolate appear growth on blood agar and chocolate agar for bacterial diagnosis , while any growth appear on Mac Conkey agar neglected. For toxoplasmosis infection collecting blood sample from patients and diagnosis by using Minividus kit, serum was obtained from patients who are negative for bacterial growth , to show the presence of *Toxoplasma gondii* as causative agent.

Isolation and identification of *M. lacunata*:

Laboratory diagnosis can be performed by depending on the procedures recommended by [7].For diagnosis, from each positive culture take a single colony , and then identification by depending on morphological properties (colony shape, color production, edge and texture),for detection morphology properties of bacterial cell using gram stain and to differentiate between $GV^-$and GV^+[8].

Antibiotics susceptibility test:

Susceptibility test was done according to Kirby-Bauer method [9].

Detection of colonization factors:

Bacterial isolates tested for producing colonization factors (CFA/I & CFA/III) depending on procedure done by[10].

Detection the ability of bacteria to resist lysozyme: In this study investigated the capacity of *Moraxella lacunata* to resist lysozymethis test done by depending on some steps :

1- preparation fresh bacteria from bacterial isolate by inoculated bacterial isolated in B.H.I broth and incubation for 24 hours at 37Cº.

2-Placed (100µl) from bacterial broth in APP and added (100µl) from 1%concentration of lysozyme and incubation at 37Cº for different hours (1,2,3,4,5,6,7 hours and over- night).

3- The mixture was transferred after each hour incubation and cultivation on B.H.I agar at 37Cº for 24 hour , any growth appear on culture media this indicate the bacterial isolatehave abilityto resist lysozyme solution.

Results and Discussion

Isolation of *Moraxella lacunata*:

According to the data obtained in this study, only 12 isolates of *M.lacunata*are isolated from eye infection as show in Table (1), and the results showed that all the patients are free from ocular toxoplasmosis by detect the level of IgG and Ig M as shown in Table (2). The isolation rate is (12%) higher than that obtain by Mahdi [11]and Masri [12]. However, the presence of this bacteria in eye infection is not recorded previously in Hilla city.

Table 1: Prevalence of *M. lacunata* and other etiological agents associated with eye infection

NO. of Eye swabs	Number of isolates		
	***M . lacunata* isolates**	Other bacteria	Negative culture
100	**12**	76	12

Table 2 : The level of *Toxoplasma* IgG & IgM in patient's samples

Isolate No.	*Gender*	*IgM concentration*	*IgG concentration*
1	♂	0.11	1.12
2	♂	0.17	3.14
3	♂	0.09	4.16
4	♀	0.06	5.22
5	♀	0.21	4.23
6	♀	0.23	6.18
7	♂	0.20	3.70
8	♂	0.14	2.92
9	♀	0.19	4.33
10	♂	0.24	3.81
11	♀	0.07	5.66
12	♀	0.01	5.07

Antibiotic profile among *Moraxella lacunata*:

All the bacterial isolates obtained from eye infection underwent to antibiotic susceptibility testing by using Kirby-Bauer disc diffusion method. These antibiotics selected such as Ceftriaxone,Gentamicin, Ceftazidime, Clarithromycin ,Neomycin and Piperacillin. The results depicted complete sensitivity 100% toward Neomycin, Gentamicin, Ceftriaxone and Piperacillin and appear less sensitivity 67% toward Ceftazidime and Clarithromycin.As depicted in Figure(1).

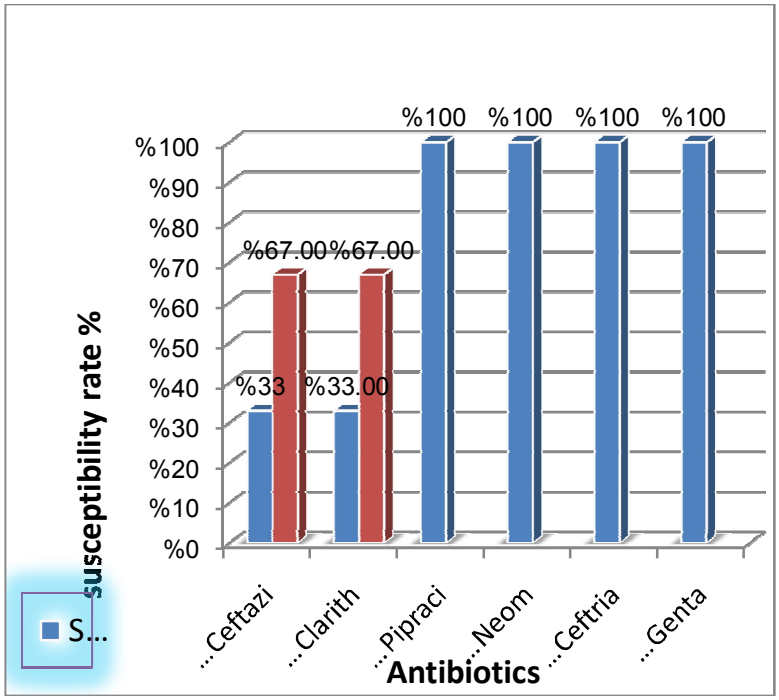

Figure 1: Sensitivity of *M.Lacunata*to some types of antibiotics
R= Resistance, S= Sensitivity.

3-Investigation of colonization factors :

In this study the present of both colonization factors antigens (type I) and (type III) investigation. The test results showed inability of the all isolated to produce CFA/I in the presence of D-mannose , whereas all isolate complete positivity toward CFA/III.This result proves the incapacity of the bacteria to produce CFA/I in the presence of D-mannose and its sensitivity toward it. As depicted in Table (3).

Table 3: Investigation of colonization factors antigen type I and type III

No. of isolates	CFA/I	CFA/III
12	-	+

The first and the essential step between a host and pathogen is adherence of the pathogen to the host epithelium by way of interaction between the pathogen adhesion and the host tissue receptors. This contact is very important to estimate the outcome of the infections process [13].The result obtained through this study was proved that all *M. lacunata* isolates isun ability of adherence to tissue containing D-mannose on their surface. This result will give remarkable explanation that this bacterial type have another mechanisms to adhere to the tissue.

Effect of Lysozyme on Bacterial Viable Count

This experiment depicted how the *M.lacunata* isolates have ability to permanence in the presence of lysozyme, resist to effect and fixed in eye. Although the eye tears produce many proteins as lysozyme, lactoferrin and IgA ,this protect the eye from the pathogenic infections due to its toxic effect on bacteria. One of these proteins is lysozyme which protect the eye from many invaders particularly the bacteria through its ability to hydrolyze the peptidoglycan presence in the cell wall [14]. However, the result showed all *M. lacunata* bacterial isolates can permanence and grow continuously in culture media containing 1% lysozyme, this prove *Moraxella lacunata* has ability to resist lysozyme and causes hard eye infection .As depicted in Table (4).

Table 4: Survival bacterial isolated in the presence of 1% lysozyme

Exposure time to lysozyme	Viable count
Without lysozyme at zero time	5×10^7
After 1 hour	3.5×10^7
After 2 hour	3.4×10^7
After 3 hour	1.7×10^7
After 4 hour	8.6×10^6
After 5 hour	8.0×10^6
After 6 hour	3.7×10^6
After 7 hour	3.5×10^6
After 24 hour	2.2×10^5

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