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عزل وتشخيص البكتريا المسببه لألتهاب اللوزتين في الانسان

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شهادة البكالوريوس في الطب والجراحة البيطرية

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Isolation and identification of bacteria that causes
tonsillitis from tonsils of students in Veterinary
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الاهداء

الى الله الذي لولاه لما وفقت لهذا .

الى منبعي الكثير العطاء والدي ووالدتي حفظهما الله .

الى اغلى البشر على قلبي اخواني وأخواتي .

الى من تعلمت على ايديهم ونهلت من علمهم أساتذتي .

الى سندي وقوتي وملاذي

من ارى التفاؤل في عيونهم

من رافقونا منذ ان كنا نحمل الحقائب الصغيرة وسعدت

برفقتهم اصدقائي

أهديهم ثمرة جهدي المتواضع

أنمار

شكر وتقدير

الحمد لله رب العالمين والصلاة والسلام على خير خلق الله سيد الاولين
والاخرين محمد (صلى الله عليه وسلم) وعلى اله صحبه اجمعين ..

أتوجه بالشكر والامتنان الى اساتذتي ومعلمتي المشرفة الفاضلة الاستاذة وداد
صالح الكرخي لاقتراحها موضوع البحث وما قدمته لي من ملاحظات
وتوصيات قيمة أوضحت الدراسة بالصورة التي انتهت بها ..

وكذلك اتوجه بالشكر الجزيل الى صديقتي (نورا ناصم علي أزهار هاشم
هندي) لما قدموه لي من مساعدة في هذا البحث ..

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الخلاصة

تضمنت الدراسة عزل وتشخيص البكتريا المسببة لالتهاب اللوزتين على اوساط زرعيه خاصة، حيث تم اخذ (44) عينه من طلاب كلية الطب البيطري/جامعة ديالى لكافة المراحل الدراسيه تتراوح اعمارهم بين (18-24) سنة، حيث تم اخذ مسحه من اللوزتين ولكلا الجنسين. تم عزل تلك المسحات وتشخيصها باتباع طرق التشخيص المختبريه وهي بطريقه الزرع على وسطي *blood agar*، *maCconky agar* وتم الحضان لمدة 24 ساعة بعد اكتمال الزرع في اليوم التالي وجد ان البكتريه نامية على *blood agar* ولم تنمو على *maCconky agar* وهذا يعني ان البكتريا المسببه لالتهاب اللوزتين هي من مجموعته *gram positive* وبعد ذلك تم اجرا *gram stain* للتأكد من المسبب ووجد ان المسبب هي بكتريا *streptococcus spp.* وكانت نسبة اصابه الاناث اكثر من نسبة اصابه الذكور بظهور بكتريا *streptococcus spp.* وتم التوصل الى ان البكتريا المسببه من مجموعته *group A streptococcus (B-haemolytic streptococcus)* وذلك عن طريق ملاحظه *clear haemolysis around colony* التي هي صفه مميزه لبكتريا *B-haemolytic streptococcus*.

Abstract

- Were taken (44) sample of students from the College of Veterinary Medicine for all grades aged (18-24 years) , where he was a tonsil swab of tonsils and both sexes.
- Those were isolated swab and diagnosed following a laboratory diagnostic methods in a way to the center of the implant bloodagar and maCconky agar were cuddling for 24 hours
- bacteria growth on blood agar and not growth on maCconky agar that mean the bacteria causes tonsillitis is gram positive and then make gramstain to ensure the diagnosis.
- Was reached that the bacteria strepto coccous spp. Is a bacteria that causes inflammation of the tonsils.
- The proportion of injured girls more than boys to appear of bacteria strepto coccous spp
- Also found bacteria streptococcous from group A (B-haemolytic streptococcous) about way the present clear haemolysis around colony on blood agar.

CHAPTER ONE

1.1.introduction

The tonsils are fleshy pieces of lymphatic tissue that rest in the back of the throat above and below the tongue. As part of the immune system, the tonsils help fight infections. However, if bacteria or viruses contaminate the tonsils, the result is an infectious inflammation and swelling of the tonsils - or **tonsillitis**. Although tonsillitis can occur at any age, young people are much more likely to suffer from the condition. In fact, tonsillitis is more common in anyone who spends time in a school, or in an environment where people are in close contact and germs can be easily spread like classrooms or residence halls.

Tonsillitis has both viral and bacterial causes, though many cases are caused by viruses(1). When tonsillitis is caused by the Group A streptococcal bacteria, the condition is more commonly termed "strep throat". Most infections begin when a person breathes in bacteria- or virus-infected droplets that another person has breathed, coughed, or sneezed out. Other types of contact, though, can also spread the infection.

The most common symptoms of Tonsillitis are:

- Fever and enlarged inflamed tonsils with white patches (exudate)
- Tender lymph nodes in neck
- Sore throat, difficulty swallowing
- Runny, stuffy nose
- Headache

A physical examination will allow a Healthcare Practitioner to find the symptoms of tonsillitis. A strep test (throat swab) may be employed to determine if the cause of tonsillitis is the bacterial infection known as strep throat. If it is determined that the cause is viral, a test for mononucleosis be performed to help distinguish between mononucleosis

and tonsillitis. Tonsillitis is usually treated with a course of antibiotics, be sure to take these as directed and to complete the entire course. Failure to do so, may lead to antibiotic resistant bacteria and reinfection in 2-3 weeks.

Patients with Gr. A streptococcal tonsillitis were significantly older than EB-viral or adenoviral

tonsillitis .Sixty six per cent of patients with adenoviral tonsillitis were under 4 years of age, 67%

of the patients with EB-viral tonsillitis were under 6 years of age, whereas 71% of the patients with

streptococcal tonsillitis were over 6 years of age.

Group A B-hemolytic streptococci tonsillitis in person 6 years of age or more .fever associated with group A B- hemolytic streptococci tonsillitis responded to pencillin therapy significantly more rapidly than fever associated with viral infection.

1.2.The study aim

1- make survey comprise spp that causes tonsillitis.

2.Isolated and Diagnosis the bacteria that causes tonsillitis.

3-known the effect of the some factor on the bacteria that causes the tonsillitis like nutrition & economic state and age and sex of patient.

4.Ways for prevention from tonsillitis.

CHAPTER TWO

2.Literture reivew

2.1.Define of Tonsillitis

Tonsillitis refers to inflammation of the pharyngeal tonsils. The inflammation may involve other areas of the back of the throat including the adenoids and the lingual tonsils (areas of tonsil tissue at the back of the tongue). There are several variations of tonsillitis: acute, recurrent, and chronic tonsillitis and peritonsillar abscess. Viral or bacterial infections and immunologic factors lead to tonsillitis and its complications.

2.2.Etiology;

The most common cause of acute suppurative tonsillitis include group Group A β -hemolytic streptococci, Epstein-Barr (EB) virus and adenovirus. About 30% to 40% of tonsillitis cases are caused by

Group A β -hemolytic streptococci is documented (2)

2.3.Transmission

Colds easily spread from person to person from coughs, sneezes, and infected fluids from the nose and throat. People can pass on viruses when they first show signs of being sick until five days after the illness starts.

2.4.Symptom

Acute tonsillitis: Patients have a fever, sore throat, foul breath, dysphagia (difficulty swallowing), odynophagia (painful swallowing), and tender cervical lymph nodes. Airway obstruction due to swollen tonsils may cause mouth breathing, snoring, nocturnal breathing pauses, or sleep apnea. Lethargy and

malaise are common. These symptoms usually resolve in three to four days but

may last up to two weeks despite therapy.

! Recurrent tonsillitis: This diagnosis is made when an individual has multiple

episodes of acute tonsillitis in a year.

! Chronic tonsillitis: Individuals often have chronic sore throat, halitosis, tonsillitis, and persistently tender cervical nodes.

! Peritonsillar abscess: Individuals often have severe throat pain, fever, drooling, foul breath, trismus (difficulty opening the mouth), and muffled voice quality, such as the hot potato voice (as if talking with a hot potato in his or her mouth)(3).

2.5.Epidemiology

Acute tonsillitis is caused by viral and bacterial agents.

Bacterial sore throat accounts for only about 30% of cases, of which GAS is the most common etiologic agent in childhood.

GAS tonsillitis occurs most frequently in the late fall, winter, and spring in temperate climates. Crowding in day care centers, schools, and military installations facilitates transmission. Food- and waterborne outbreaks of GAS tonsillitis have also been documented [4]. The infection can occur at all ages but is most common among children and adolescents 5 to 15 years of age; it is rare in children younger than 3 years of age. There is no sex predilection; transmission of GAS throat infection almost always follows close person-to-person contact via droplets of saliva or nasal secretions. Communicability of patients with streptococcal tonsillitis is highest during acute infection, and in untreated individuals gradually decreases over a period of several weeks. Patients are not contagious within 24 hours of the initiation of appropriate antimicrobial therapy. The bacteria may also colonize the throats without causing infection. During the disease season, the asymptomatic carriage rate in school children has been found to be 15% to 20%, with a lower rate among adults [4].

2.6.pathogenesis

Group A b-haemolytic streptococcus

The role of group A b-haemolytic streptococcus as a bacterial pathogen in sore throat is evident and is not questioned. Reviews and guidelines considering the diagnosis of sore throat have therefore been focused mainly or exclusively on group A streptococci and related symptomatic presentation. Asymptomatic carriage of b-haemolytic streptococci is frequent, especially in children. According to Tanz and Shulman [5], over 20% of asymptomatic school children may be carriers of group A streptococcal infection during the winter and spring. Several European investigations examined the carriage rates in children and adults. The highest rate was found in subjects aged 14 years or less (10.9%), whereas rates were 2.3% in patients aged 15–44 years and 0.6% in those aged 45 years or older [6].

Similar results emerged in a Swedish study [7], reporting carriage rates of 11.3% in 4-year-old children, 5.9% in school children and 0.8% in adults. In a study from Croatia [8], carriage rate of group A streptococci was 8.3% overall, with highest rates being reported for subjects aged 6–14 years. Higher rates were found in a prospective study conducted in Turkey on 351 asymptomatic primary school children, as about 26% of them were group A streptococcal infection carriers [9].

Complications of group A b-haemolytic streptococcal tonsillitis are generally rare in both children and adults [10–11]. Potential adverse outcomes include both suppurative (i.e. quinsy, acute otitis media, cervical lymphadenitis, mastoiditis, acute sinusitis) and non-suppurative (i.e. acute rheumatic fever, acute glomerulonephritis) complications. In particular, acute rheumatic fever has been widely investigated during the last decades, but its incidence is very low in Europe. Prevention of acute rheumatic fever depends on effective control of group A streptococcal tonsillitis [10] and is important for patients at high risk (e.g. those who have had rheumatic fever before). Acute glomerulonephritis glomerulonephritis is another rare consequence of sore throat, following group A streptococcal tonsillitis after a latency period of a few weeks. Quinsy, a complication that occurs mainly in young adults, is a polymicrobial infection but group A streptococcus is the main organism associated with the disease [10–12, 13, 14–15].

Tanz and Shulman [5] conclude that pharyngeal carriers of group A streptococci show an extremely low risk of poststreptococcal complications, and their likelihood of transmitting the infection is also small.

Group C and G b-haemolytic streptococci

A number of studies are available on the symptomatic presentation of b-haemolytic streptococci other than group A streptococci. Two observational studies (one cohort study, one case–control study) supported a milder clinical presentation of group C or group G streptococcal tonsillitis than group A streptococcal tonsillitis [15–16]. On the other hand, five observational studies (three cohort, two case–control) and one case series investigation reported a similar clinical picture.

At least 12 original studies, mostly case series and case reports, described severe symptoms or complications following acute sore throat associated with group C and group G streptococci [17,16–18]. Cases of severe or recurrent tonsillitis because of group C streptococci have been reported. A case–control study of college students found that patients with group C streptococci had exudative tonsillitis and anterior cervical adenopathy more frequently than subjects negative for this infection [19]. On the other hand, there is little evidence to address the issue of whether there is an association between group G streptococci and severe or recurrent tonsillitis.

Uncommon complications of tonsillitis caused by group C or G streptococci that have been reported include reactive arthritis, subdural empyema and acute glomerulonephritis, but a causal relationship was not clearly established. In 1997, Efstratiou reported consistent results of group C and G septicaemia over a 10-year period [20]. While sore throat caused by group A streptococci is known to be rarely associated with acute rheumatic fever in developed countries, this has not been reported as a complication following group C or group G streptococcal infection [21]. There are, however, studies and expert opinions indicating that group C and group G streptococci might contribute to acute rheumatic fever pathogenesis in high-incidence settings [22,23].

Group C streptococci can cause severe or recurrent

tonsillitis, but there is insufficient evidence for a role of group C streptococci in other adverse outcomes. There is insufficient evidence for a role of group G streptococci in severe/recurrent tonsillitis and other adverse outcomes.

Mycoplasma pneumoniae and *Chlamydia pneumoniae*
Mycoplasma pneumoniae and *C. pneumoniae* infection has been associated with non-streptococcal acute tonsillitis in selected studies [24]. It is not clear whether tonsillitis due to these infections may have an unwanted outcome, including longer duration or recurrence of symptoms and occurrence of other complications. The available evidence is scanty and limited to paediatrics [24–25]. Two observational studies (one prospective cohort, one case–control) reported increased risk of recurrence of symptoms after *M. pneumoniae* infection. One prospective cohort study reported an increased risk of recurrence of respiratory illness after *C. pneumoniae* infection. Case reports and case series found a possible association between *M. pneumoniae* infection and Bell’s palsy or Stevens–Johnson syndrome.

2.7.Prevention

There is no immunisation to prevent tonsillitis.

You can try to avoid the infection spreading by:

- keeping yourself or your child away from others while unwell (if bacteria caused the tonsillitis, it will be safe to be around others after about 24 hours on antibiotics)
- washing your hands often (help children to do the same)
- keeping the sick person’s eating and drinking utensils separate from others
- not sharing toothbrushes
- not kissing a person with tonsillitis, or kissing someone if you have tonsillitis yourself.

2.8.Treatment

Are analgesics effective in sore throat?

A systematic review [26] and six randomized-controlled trials (RCTs) [27,26–28] found that non-steroidal antiinflammatory drugs and paracetamol are more effective than placebo for reducing acute sore throat symptoms in adults. Ibuprofen and diclofenac are slightly more effective than paracetamol for pain relief [29,27 ,26–30].

Paracetamol and ibuprofen were the safest. In a large RCT, ibuprofen, when used in accordance with the usual contraindications, was as well tolerated as paracetamol for the short-term treatment of the pain of cold and flu symptoms and of sore throat in adults [31,32]. No trials were found comparing ibuprofen and diclofenac. A

systematic review showed that ibuprofen and paracetamol are more effective than placebo for reducing acute sore throat symptoms in children [26]. Another systematic review assessed the efficacy and safety of single doses of ibuprofen and paracetamol for short-term treatment of children's pain or fever [33]. The results did not indicate any difference

between the drugs in analgesic efficacy or safety.

CHAPTR THREE

3.Material and methods

3.1.collection of specimens

In the present study a total of 44 tonsil swab were collected from students of the College of Veterinary Medicine-Diyala University.the study was extended from 1st.february/2014 to 30th.April /2014.the students were 34 male and 10 female from different classes. The age range of students was [18-24].the mean age of female students was 20.76 1.67SD,and the mean age of male students was 21.13 1.88SD.students information were collected directly by short interview

3.2.preparation of culture media

3.2.1.blood agar media;

It was prepared by dissolving 40.0 gram of blood agar powder in 1000 milliliters of distilled water in pyrex glass flask and heated on gas burner till completely dissolved.the of the flask was tightly plugged by tampon and warped by aluminum foil.there after,the medium was sterilized by autoclaving at 121c and 15lb for 30 minutes .after that the medium was left to cool for about 45-50c,and 5 whole blood was added to the medium and mixed well by gentle shaking of the flask.the blood was collected from sheep by vein puncture in

E.D.T.A.[anticoagulant]tubes.the medium was distributed in petri dishes [about 10 milliliters each]and left to solidify,then kept in refrigerator 4c till used.

3.3.culturing of specimens;

After the collection and labeling of the tonsil swab ,it was transferred to microbiology laboratory as soon as possible.culturing of specimens was done by streaking on blood agar plates which were previously labeled as the same on the swabs.the plates were incubated at 37c for over night.

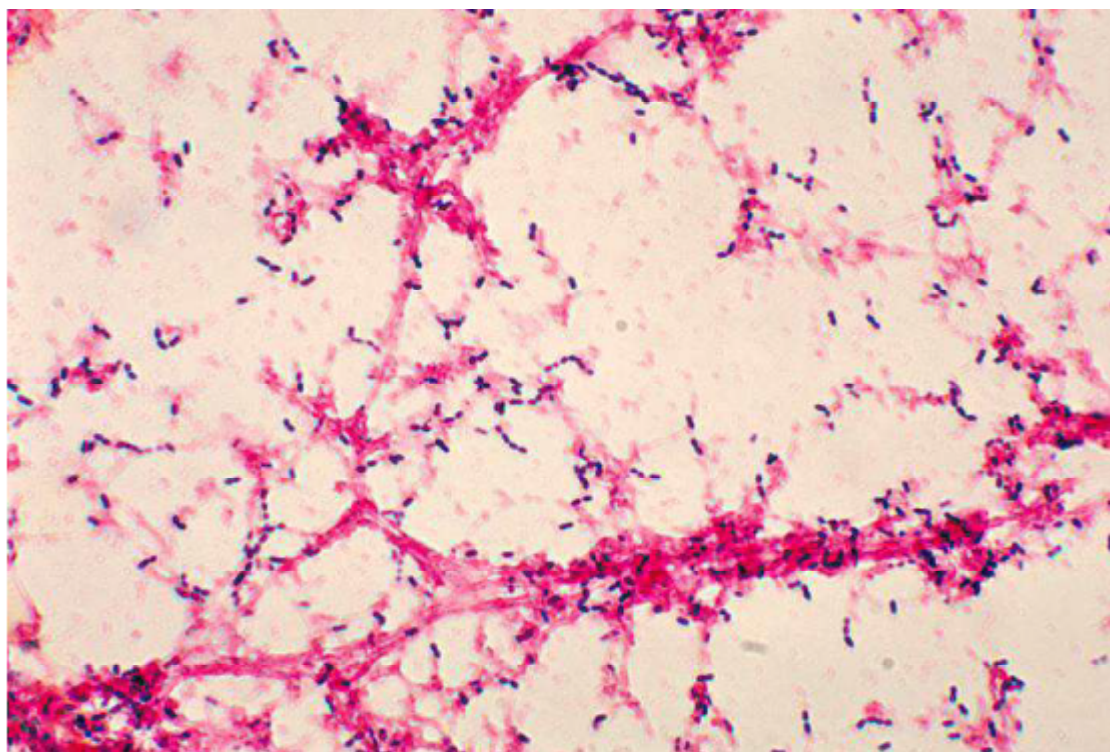
3.4. inspection and diagnosis of bacterial growth;

In the second day morning the plates were inspected for the growth of strepto coccous on the bases colonial morphology.after that make Gram stain procedure.

3.5.Gram stained film

One or two suspected colonies were picked up by bacteriological loop and mixed with adrop of normal saline solution[0.9 % sodium chloride] on a clean glass slide ,air dried and fixed by passing over gas burner flame several times to prevent sloughing during staining .the Grames stain procedure was applied as follows;

- a.crystal violet stain was added as basic stain on the smear for 1 minute.
- b.the slide was washed under gentle streaming tap water.
- c.Grames Iodine was applied on the slide for 30 second,and the slide was washed again under tap water.
- d.after that,the slide was decolorizer[70% ethanol -30% acetone]for 30 second.
- e.the safranin stain was added as a counter stain for 1 minute.
- f.the slide was air dried and examined under the oil immersion lens.streptococccous were appeared as Gram positive cocci arranged in chains as in picture below:



CHAPTER FOUR

Result

1-After taken (44), sample of students from the Collage of Veterinary Medicine/ University of Diyala, which is between the ages of 18-24 years for both sexes were isolated and diagnose the main cause of a bacterial tonsillitis is streptococcous spp.group A(B-haemolytic steptococcous)

2-The percentage of affected females is higher than the percentage of affected males with streptococcous spp

3-The percentage of male non- smokers injury is higher than the .. proportion of male smokers infecting with bacteria streptococcous spp

4-Table (1) shows the incidence precentage between males and females.

Gender	No . tested	No . positive	%
Female	25	20	80
Male	19	12	63.16
Total	44	32	72.72

Table (1) frequency of tonsillitis isolated rate by gender

5-Table (2) shows the ratio of affection between male smokers and non-smokers

Type male	No . tested	No . positive	%
Smoke	7	2	28.57
No smoke	12	10	83.33
Total	19	12	63.15

Table (2) frequency of tonsillitis isolated rate by type of male (smoke ornot)

Discussion

- The bacteria streptococcus spp. group A (B-haemolytic streptococcus) is the main cause of tonsillitis. This is consistent with both (34;35). The study also confirmed that the cause may be Virus as confirmed by (1).

And so that some isolates taken from patients with inflammation of the tonsils with the appearance of clinical signs nevertheless appear result (negative) due to the fact that the probability of the reason Virus.

Appear study that the percentage of affected females more than males proportion of bacteria streptococcus spp. This is due to the decrease immunity of females during the menstrual period and therefore we were affected various infections such as tonsillitis.

The study also showed a semi-injured male smoker's bacteria streptococcus spp. Less than non-smoker is attributed to be the cause is no bacterial (viral).

Recommendations

- 1- Conduct a research laboratory for the study of all the etiology of the disease, tonsillitis , whether bacterial , Virus, fungal
- 2- follow the health means for the prevention of disease tonsillitis
- 3- taking early treatment in an ongoing infection of the tonsils , and in order to avoid complications of the disease
- 4- follow healthy ways when antibiotic treatment
- 5- make culture of bacteria that cause tonsillitis before taking antibiotics.

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