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EVALUTION OF MULTI-DRUG RESISTANCE AND B-LACTAMASE PRODUCTION IN THROAT INFECTED BY GRAM POSITIVE BACTERIA

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ABSTRACT

Tonsillitis and Pharyngitis are an infection of the throat region poses one of the most common problems in the upper respiratory tract infection and are one of the main reasons leading the persons to seek advice from a physician. This study was carried out during the period (December, 2012 to August, 2013). A total of two hundred of throat sawbs were collected from patients with tonsillitis and pharyngitis infection at the age ranged between (1-70) years, who were attending the clinic outpatient unit in Rizgary and Raparin teaching hospitals in Erbil city. Throat swabs were collected and examined for microscopically using Gram stain examination and culture technique. Isolated microorganisms were identified using microscopical, morphological, biochemical tests and Vitek2 compact system. The results showed that 134(67%) of throat swabs were culture positive the total number of isolated microorganism isolates obtained from patients were (185) isolates. These isolates were distributed between Gram-positive bacteria 143(77.3%), Gram-negative bacteria 34(18.4%) and fungi 8(4.3%). Single isolates found in 84(62.7%) and mixed isolates in 50(37.3%). The most frequent isolated microorganisms from tonsillitis/pharyngitis patients were *Staphylococcus aureus* 54(30.5%), *Streptococcus pyogenes* 16(9%), *Streptococcus parasanginis* 12(6.8%). According to antibiotic sensitivity test most of the Gram-positive bacterial isolates showed high resistance to Penicillin 137(95.8%) and Ampicillin 136(95.1%) and the most effective antibiotics and less resistance were Ciprofloxacin 6(4.2%) and Imipenem 33(23.1%). All (143) Gram-positive bacterial isolates were screened for their ability to produce β-lactamase enzyme using Nitrocefin test, of them 116(81.1%) were found to be β-lactamase positive.

KEYWORDS: *Staphylococcus aureus, Streptococcus parasanginis and Streptococcus pyogenes.*

INTRODUCTION

A cute pharyngo-tonsillitis caused by β-haemolytic group A Streptococcus is a common disease in childhood and it is exclusively a human pathogen. Epithelial cells are the initial sites of the host invasion by group A Streptococcus has been considered an extracellular pathogen, recent studies have demonstrated that strains of this bacterium can internalize into epithelial cells both in vitro and in vivo (Passali *et al*., 2007). Group A Streptococcus is a β-hemolytic, Gram-positive human group A Streptococcus is one of predominant causes of acute bacterial tonsillopharyngitis.

Tonsillopharyngitis is an acute infection of the palatine tonsils and pharynx often presenting symptomatically with sore throat, fever and cervical lymphadenopathy (Roberts *et al*., 2012).

The diagnosis of Streptococcal pharyngitis has been studied extensively, as it can have serious long-term outcomes, such as rheumatic fever, heart disease and poststreptococcal glomerulonephritis. It is also very difficult to distinguish from nonbacterial causes of tonsillitis and pharyngitis (Cirilli, 2013).

A Streptococcal pharyngitis is the only common form of the disease for which antimicrobial therapy is definitely indicated. Therefore, when a clinician evaluates a patient with acute sore throat, the most important clinical task is to decide whether or not the patient has "strep throat." This illness occurs predominantly, though not exclusively, in school-age children (Bisno, 2001).

Recent studies on the development of the early microflora indicate that the acquisition and succession of different bacterial species in the oral cavity is a selective process in terms of the age at which infants are susceptible to colonization (Könönen *et al*., 2002).

Staphylococcus aureus one of three pathogenic species of the Gram-positive cocci, is often found as part of the normal microflora of the human skin, the upper respiratory tract, especially the vestibulum nasi and the intestinal tract (Bachert *et al*., 2002). *Staphylococcus*

aureus is a significant human pathogen world-wide particularly in health-care settings. The public health burden caused by this species is severely exacerbated by the widespread dissemination of clones resistant to β-lactam antibiotics methicillin resistant *Staphylococcus aureus* (MRSA) (Fan *et al*., 2009*).*

Physicians prescribe antibiotics for acute pharyngitis as they are concerned that patients with this complaint may be suffering from GABHS infection that if left untreated might develop suppurative complications such as tonsillar abscess or non suppurative complications such as rheumatic fever Antibiotics, however; confer only minor symptomatic benefits for GABHS sore throat. They shorten the duration of symptoms by merely half a day on average. Older studies showed that treatment with penicillin reduced the incidence of rheumatic fever (Michael and Shmuel, 2012). β-lactamase producing bacteria can play an important role in polymicrobial infections. They have a direct pathogenic impact in causing the infection as well as an indirect effect through their ability to produce the enzyme β-lactamase. β-lactamase producing bacteria may not only survive penicillin therapy but can also, as was demonstrated in vitro and in vivo studies, protect other penicillin susceptible bacteria from penicillin by releasing the free enzyme into their environment (Brook, 2009).

However, for patients hypersensitive to β-lactam antibiotics and in whom therapy with these drugs fails, macrolides are often the recommended substitute. Azithromycin is an azalide antibiotic chemically related to erythromycin and has a long half-life and excellent tissue penetration. Short-course azithromycin therapy has been reported to be effective for the eradication of oropharyngeal GAS (Bingen *et al*., 2002).

Defining the most prominent bacterial species in recurrent tonsillitis patients as compared to peritonsillar abscess patients and elucidating the preferred site at which these bacteria persist in between the infection periods could help to develop new strategies for a successful therapy besides the well established but, considering e.g. Post-surgical hemorrhages, potentially harmful tonsillectomy. Therefore, swabs and surgically removed specimens from recurrent tonsillitis and peritonsillar abscess patients and a large panel of complementing methods were used for a prospective and comprehensive study of the involved bacterial species and their precise anatomical location (Zautner *et al*., 2010).

MATERIALS AND METHODS

Sample Collection

Throat swabs obtained from two hundred patients attending outpatient clinic unit center complaining of sore throat and fever more than 38.5˚C and diagnosed as tonsillitis and pharyngitis which are collected during the period from December 2012 to April 2013 in Rizgary and Raparin teaching hospitals in Erbil city. Their age ranged between (1-70) years. Two swabs were taken from each patient by (physician). The swabs were put in transport media and sent immediately to the laboratory. One of swabs was examined directly using stain and the other was cultured immediately.

Much information was taken directly from each patients using special questionnaire sheet. The information included.

Patient name, age, location (residence) of stay, educational status, gender, symptoms, history of tonsillectomy, Drug history, past medical history, past surgical history.

Isolation of microorganisms

For isolation of microorganisms, the specimen of throat swabs was directly inoculated on culture media:- Blood agar and MacConkey agar plates were incubated aerobically at 37˚C for (24-48) hours. Chocalate agar plates were incubated microaerophically at 37˚C for (24-48) hours. Microaerophilic incubation was done using candle jar which supplied (5-10) % CO2 (Razzak *et al.*, 2011).

Identification of microorganisms

Pure colonies of isolated microorganisms were identified using morphological, biochemical tests. Species identification and antibiograms for pathogens were performed using Vitek 2 compact system (Nagaraja, 2008).

Antimicrobial susceptibility testing

Disc diffusion method, also known as the Kirby- Bauer method was carried out according to the Clinical and Laboratory Standard Institute guidelines (CLSI), (Wayne, 2005). As follows.

1- Mueller Hinton agar was prepared and sterilized then the medium was cooled to 45˚C and poured to sterilized Petri dish.
2- Mueller Hinton agar containing 5% blood is used for testing Streptococci.
3- Inoculums suspension were prepared using colony suspension standarized to match the turbidity of a McFarland 0.5 standard.
4- The plates were inoculated by a sterile cotton swab dipped in bacterial suspension, then swabbed evenly across the surface of a Mueller Hinton agar plates.
5- Sterile forceps was used to place the antibiotic disc on the surface of the inoculums.
6- The inoculated plates were incubated at 37˚C for 18-24 hours.
7- After incubation the diameters of inhibition zone produced by antimicrobial inhibition of bacterial growth were measure using a ruler.
8- Each inhibition zone interpreted chart table which is recommended by CLSI.

β-Lactamase detection

2.2.8.1. Detection of β- lactamase enzyme in Gram positive bacteria by identification sticks β- lactamase (Nitrocefin)

A total of 143 of Gram positive bacteria were screened for β- lactamase enzyme by nitrocefin test. Rapid nitrocefin test consist of sticks, one end of each stick is impregnated with a solution of nitrocefin, phosphate buffer and dimethysulphoxide. The opposite end is colored black to identify the correct for handling.

1- The container was removed from the freezer and allowed to reach room temperature.
2- Well separated representative colony from the primary isolation medium was selected.
3- One stick were removed (color coded black) from the container, and holding the colored end then touched the colony with the impregnated end of the stick, the stick was picking off a small mass of cells.
4- The reagent- impregnated tip of the stick were examined for up to 5 minutes, if negative, re- examined after 15 minutes.
5- A positive reaction were shown by the development of a pink-red color. No color change is observed with organisms that do not produce beta-lactamase [Basingstoke, Hampshire Company (England)].

RESULTS AND DISCUSSION

A total of two hundred throat swabs were collected from patients attending Rizgary and Raparin Teaching Hospitals in Erbil city suspected of having tonsillitis/pharyngitis. The results as showed in figure (1) that among 200 throat swabs only 134(67%) were culture positive, while 66(33%) were culture negative.

These results were in agreement with those dictated by Raju and Selvam, (2012) from India who found that (68%) of samples show positive cultures, Sadoh *et al*., (2008) from Nigeria reported of (53.42%). While in a study done in Bangladesh by Amin *et al*., (2009) their results showed that (26.3%) were culture positive which is lower than that reported in the present study.

The high rate of positive cultures of bacterial pharyngitis in our study may be due to that bacterial tonsillitis/pharyngitis may be common in Erbil city and others might due to sample size and our target populations were selected by physician only patients with symptoms of tonsillitis and pharyngitis.

The number and percentage of microorganisms isolated from 134 patients with tonsillitis/pharyngitis are showen in table (1) the most common isolated organisms was Gram positive bacteria including 143(77.3%) isolates that was including *Stapylococcus aureus* 54(30.5%) followed by *Streptococcus pyogenes* 16(9%), *Streptococcus parasanginis* 12(6.8%), *Pseudomonus aeruginosa* 11(6.2%) and *Proteus mirabilis* 10(5.6%).The identification of microorganisms causing tonsillitis/pharyngitis was based on cultural morphology, biochemical characteristics and Vitek2 compact system.

The highest percentage of tonsillitis/pharyngitis was caused by *Staphylococcus aureus* (30.5%). Relatively similar observation was also reported by many investigators in our country by Almalki, (2011) from Thi Qar (Iraq) reported (29.4%) and AD'hlah *et al*., (2006) from Baghdad (Iraq) reported (23.8%) and also agree with other investigators in other countries, Raju and Selvam (2012) they showed that *Staphylococcus aureus* was the most common bacteria isolated from tonsillitis (83%) which is higher than our results. Lee *et al*., (2011) from New York isolated (42.7%) of *Staphylococcus aureus* from throat swabs in prison population, Zautner *et al*., (2010) from Germany also demonstrated that intracellular residing *Staphylococcus aureus* is the most common cause of recurrent tonsillitis and Islam *et al*., (2011) from Pakistan showed that *Staphylococcus aureus* was (21.13%) from infected throat swabs that taken from patients with respiratory disease and Sadoh *et al*., (2008) reported that (12.83%) of isolates from pharyngitis and tonsillitis were *Staphylococcus aureus* which were lower than our results.

The second most common isolated microorganisms from pharyngitis was *Streptococcus pyogenes* (9%) other results consistent with our results since Raju and Selvam (2012) reported (5%). Our results disagree with Zautner *et al*., (2010) was (20.2%) and AD' hlah *et al*., (2006) from found (39.3%) that were higher than our results and Treebupachatsakul *et al*., (2006) from Thailand was isolated (16%) which was higher than our results.

The presence of this bacteria in large percent in pharyngitis and tonsillitis might be attributed to the fact that these bacteria are often part of the resident flora and different virulence factors contributing to their pathogenicity and the difference in the results might be attributed to the number of taken sample size and the difference in the time (season) of the study.

In this study the prevalence of *Streptococcus agalactiae* was (3.4%) (Lancefield group B) are frequently inhabitants of the human pharynx and tonsils where they can adopt either a commonsal or a pathogenic role (Savini *et al*., 2008).

The frequency of other groups of Streptococci such as viridians Streptococci and non- haemolyticus Streptococci in our study were (21.5%). Our results agree with the finding of Harrison *et al*., (1999) from UK who found were (27.5%) of alpha (viridians) and non haemolyticus Streptococci from nasopharyngeal swabs and agree with Bista *et al*., (2006) from Nepal in which isolated the same species from tonsil and concluded that the most common organisms affecting the tonsil in infected state were Streptococcus viridians and β-haemolytic Streptococci. Therefore, laboratories which report β-hemolytic Streptococcal isolates from the

pharynx only as group A or non-group A should be encouraged to perform group identification of all β-hemolytic isolates to further evaluate the role of non-group A hemolytic Streptococci in tonsillitis, pharyngitis and infections at other bodily sites (Savini *et al*., 2008).

Isolation of *Streptococcus thoraltensis* (2.3%) the new species, cultures of this species were recovered from the intestinal tract of swin, identification of this species from human has not been documented (Facklam, 2002). That mean this is the first first report of this microorganism in hman in the present study in our country.

Isolation of some species in our study like *Leuconostoc pseudomesenteroides* (0.6) in which it was members of the family Streptococcaceae have recently been recognized as potential pathogens and it describe in case of bacteremia (Handwerger *et al*., 1990) and also isolatd from fecal samples by Walter *et al*., (2001) from Germany. Furthermore it isolated from patient with Cholesteatome (chronic middle ear) by Frickman and Zautner, (2012) from Germany. This concluded that the first time that isolated of this microorganism in hman in the present study in our country.

Isolation of *Arecococcus viridian* in our results was (0.6%) this species had been isolated from infected third-molar pericoronal pockets by Rajasuo *et al*., (1996) from Finland the isolation of *Alloiococcus otitis* from pharyngitis in our study was (1.12%) in which it isolated from ear discharge by Marsh *et al*., (2012) from Australia that was documented that this bacteria from previous studies from other populations have reported in nasopharyngeal swabs.

The percentage of Staphylococci Coagulase negative was (7.9%) our results consistent with Yousef *et al*., (2010) from Al-Diwanya (Iraq) was (12.33%). While Islam *et al*., (2011) from Pakistan about (4.23%) in which is lower than our results.

In our study *Staphylococcus aureus* were resistance to Ciprofloxacin 1(1.8%), similar to Islam *et al*., (2011) from Pakistan who reported the resistance of *Staphylococcus aureus* to Ciprofloxacin was (0%) and Žemlićkovâ *et al*., (2006) from Czech also found it was (0%). In our study *Staphylococcus aureus* showed highly resistance to Penicillin 52(96.2%) and Ampicillin 50(92.5%) our results similar to Wang *et al*., (2009) from China who reported that the resistance of *Staphylococcus aureus* to Penicillin was (93.5%) and Ampicillin (85.2%).

Antimicrobial susceptibility test against of *Streptococcus pyogenes* isolates in present study showed that the most effective antibiotics was Imipenem (0%) and Ciprofloxacin 3(18.8%). *Streptococcus pyogenes* showed 16(100%) resistance to Penicillin other results reported by Hameed and Al-Saidi, (2010) from Wasit (Iraq) reported that Ciprofloxacin resistance among GABHS in patients with tonsillitis was (16%) also they mentioned that the resistance to penicillin was 94% which is similar to our finding.

In contrast to our study results Ndiaye *et al*., (2009) from Senegal reported that the *Streptococcus pyogenes* isolated from tonsilitis/pharyngitis patients were highly Sensitive to Penicillin (100%) showing no resistance completely.

One explanation for the failure of Penicillins to eradicate GABHS tonsillitis is that repeated administration of these agents may select BLPB that can protect not only themselves from Penicillins but also penicillin-susceptible pathogens. The recovery of aerobic and anaerobic BLPB in more than three fourths of the patients with recurrent GABHS tonsilitis the ability to measure β-lactamase activity in the core of the tonsils and the response of patient with recurrent GABHS pharyngo tonsillitis to antimicrobial agents effective against BLPB support this explanation (Brook and Gober, 2009).

As shown in table (4) 143 isolates of Gram-positive bacteria were screened for β-lactamase production by nitrocefin test figure (2) out of them 116(81.1%) isolates were β-lactamase positive, where as 27(18.9%) were β-lactamase negative. All isolates (100%) of *Staphylococcus lentus, Staphylococcus intermedius, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus agalactiae, Streptococcus gordonii, Streptococcus thoraltensis, Streptococcus mitis, Streptococcus mutans* produced β-lactamase and the lowest percentage found with *Staphylococcus epidermidis* (33.3%). However no β-lactamase production was detected by *Staphylococcus warneri, Kocuria kirstinae, Arecoccus viridians, Alloiococcus otitis.*

β-lactamases are enzymes that hydrolyze the β-lactam ring of β-lactam antibiotics like Cefotaxime, Ceftriaxone, Ceftazidime, Cefipime and Aztreonam (Samatha and Parveen, 2011).

β-lactams are a group of antibiotics acting on the cell wall of bacterial cell (Rawat and Nair, 2010). An interested finding in this part of the study there was an association of throat infection with β-lactamase. Therefore in present study Nitrocefin-based stick were used for detection of β-lactamase production among Gram-positive out of (143) Gram-positive bacterial isolates 116(81.1%) produce β-lactamase. The present results were similar to Ahmad, (2013) who reported that out of (118) Gram-positive bacterial isolates 86(72.9%) produce β-lactamase while in another study by Sanaa *et al*., (2006) from Eygept who reported the incidence of β-lactamase production by the Gram-positive Cocci was (96%) were higher than our results.

Also among (70) isolates of *Staphylococcus spp*. were 60(85.7%) isolates produced β-lactamase. This results is agreement with Ahmed (2013) who reported that among of (77) isolates of *Staphylococcus* spp. isolated from viaginal swab 59(76.6%) were produced β-lactamase also our results was agreement with Hussein (1998) from Baghdad (Iraq) reported that (76.5%) of *Staphylococcus* spp. were β-lactamase producer.

In the present study among (65) isolates of *Streptococcus spp*. were 53(81.5%) produce β-lactamase. While, *Streptococcus pyogenes* produce (93.8%) of β-lactamase. This finding was similar Brook and Gober (1995) from USA who was reported that *Streptococcus pyogenes* produce (93.8%) of β-lactamase.

Avilès and Zaragoza (2000) from Spain found lower percentage than our finding among *Streptococcus pyogenes* isolated from pharyngiotonsillitis in which was (69%).

In the present study 51(94.4%) of *Staphylococcus aureus* were β-lactamase producer this results similar to Al-Kasi (2001) from Baghdad (Iraq) who reported that (94.8%) of *Staphylococcus aureus* produced β-lactamase which was similar to our finding. Also Kilic and Cirak, (2006) from Turkey found that the percentage of β-lactamase producer of *Staphylococcus aureus* was (83.9%) in which is consistence with our results.

β-lactams includes Penicillin and Cephalosporin are broad spectrum antibiotics, which are highly effective against the Gram-positive genera Sreptococcus and Staphylococcus (woodhead and Blasi, 2005).

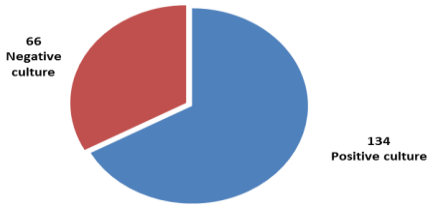

Figure (1): Relation between culture results and tonsillitis/pharyngitis infection.

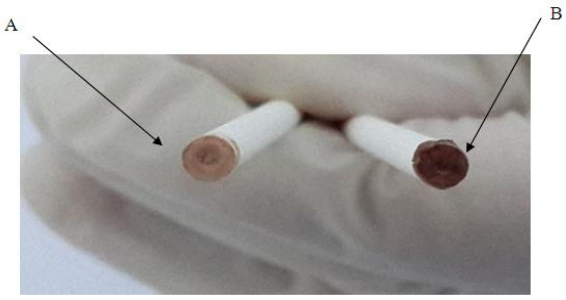

Figure (2): Detection of β- lactamase by sticks Nitrocefin test for Gram positive bacteria (A) Control (Negative result), (B) Positive result.

Table (1): Frequency of microorganism's positive culture from Throat infection.

Isolated pathogens	Total No. and % of isolates	
	No.	%
Staphylococcus aureus	54	37.7
Staphylococcus haemolyticus	2	1.4
Staphylococcus warneri	2	1.4
Staphylococcus intermedius	2	1.4
Staphylococcus auricularis	6	4.2
Staphylococcus epidermidis	3	2.1
Staphylococcus lentus	1	0.7
Streptococcus pyogenes	16	11.2
Streptococcus sanginus	6	4.2
Streptococcus parasanginus	12	8.4
Streptococcus agalactiae	6	4.2
Streptococcus pneumoniae	7	4.9
Streptococcus gordonii	5	3.5
Streptococcus thoraltensis	5	3.5
Streptococcus mitis	5	3.5
Streptococcus salivaris	2	1.4
Streptococcus mutans	1	0.7
Leuconostoc pseudomesenteroides	1	0.7
Alloiococcus otitis	2	1.4
Arecoccus viridans	1	0.7
Kocuria kristinae	2	1.4
Dermacoccus nishinomiyaensis	2	1.4
Total	143	100

Table (2): The number and percentage of antibiotics resistance in *Staphylococcus spp*.

Total No. of isolated bacteria	1 AMC	2 E	3 AM	4 DA	5 AK	6 IMP	7 CIP	8 CRO	9 VA	10 P	11 CFM	12 CTX	13 CN	14 CAZ
Staphylococcus aureus (54)	25 46.3%	28 51.8%	50 92.5%	23 42.5%	16 29.6%	15 27.7%	1 1.9%	51 94.4%	8 14.8%	52 96.2%	53 98.1%	52 96.2%	24 44.4%	53 98.1%
Staphylococcus auricularis (6)	0 0%	2 33.3%	6 100%	0	1 16.6%	1 16.6%	0	6 100%	0	6 100%	6 100%	6 100%	2 33.3%	6 100%
Staphylococcus epidermis(3)	2 66.7%	1 33.3%	2 66.7%	1 33.3%	0	0	0	3 100%	0	3 100%	3 100%	3 100%	1 33.3%	3 100%
Staphylococcus intermedius (2)	0 0%	1 50%	2 100%	0	2 100%	1 50%	0	2 100%	0	2 100%	2 100%	2 100%	0	2 100%
Staphylococcus haemolyticus (2)	0 0%	2 100%	2 100%	0	2 100%	0	0	2 100%	0	2 100%	2 100%	2 100%	0	2 100%
Staphylococcus warneri (2)	0 0%	2 100%	2 100%	2 100%	0	1 50%	0	2 100%	2 100%	2 100%	2 100%	2 100%	1 50%	2 100%
Staphylococcus lentus (1)	0 0%	0	1 100%	1 100%	0	0	0	1 100%	0	1 100%	1 100%	1 100%	0	1 100%

*Amoxicillin and Clavunic acid (AMC), Erythromycin (E), Ampicillin (AM), Clindamycin (DA), Amikacin (AM), Impimenem (IMP), Ciprofloxacin (CIP), Ceftriaxone (CRO), Vancomycin (AV), Penicillin (P), Cefixime (CFM), Cefotaxime (CTX), Gentamycin (CN), Ceftazidime (CAZ).

Table (3): The number and percentage of antibiotics resistance in *Streptococcus spp*. and other Gram-positive bacteria.

Total No. of isolated bacteria	1 CIP	2 AV	3 AMC	4 AM	5 E	6 DA	7 P	8 CTX	9 CRO	10 CN	11 IPM	12 CFM
Streotococcus pyogenes (16)	3 18.75%	14 87.5%	15 93.75%	16 100%	16 100%	15 93.75%	16 100%	10 62.5%	14 87.5%	13 81.25%	0	10 62.5%
Streptococcus parasanginus(12)	1 8.3%	6 50%	8 66.6%	12 100%	8 66.6%	6 50%	10 83.3%	7 58.3%	3 25%	11 91.6%	3 25%	9 75%
Streptococcus pneumoniae(7)	0	7 100%	7 100%	7 100%	7 100%	7 100%	7 100%	7 100%	7 100%	7 100%	2 28.5%	7 100%
Streptococcus sanginus(6)	0	2 33.3%	3 50%	5 83.3%	2 33.3%	3 50%	5 83.3%	3 50%	2 33.3%	3 50%	0	4 66.6
Streptococcus agalactiae (6)	0	6 100%	6 100%	6 100%	6 100%	6 100%	6 100%	4 66.6%	0	5 83.3%	5 83.3%	2 33.3%
Streptocccus gordonii (5)	0	5 100%	5 100%	5 100%	5 100%	5 100%	5 100%	1 20%	2 40%	5 100%	1 20%	1 20%
Streptococcus thoraltensis (5)	0	5 100%	5 100%	5 100%	5 100%	5 100%	5 100%	0	3 60%	5 100%	4 80%	0
Streptococcus mitis (5)	1 20%	4 80%	5 100%	5 100%	4 80%	5 100%	5 100%	4 80%	5 100%	4 80%	0	5 100%
Streptococcus salivaris (2)	0	2 100%	0	2 100%	2 100%	2 100%	2 100%	0	0	2 100%	0	2 100%
Streptococcus mutans (1)	0	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	0	0	1 100%	0	0
Alloiococcus otitis (2)	0	0	1 100%	1 100%	0	0	1 100%	0	0	1 100%	0	1 100%
Leuconostoc Pseudomesenteroides (1)	0	0	1 100%	1 100%	0	0	1 100%	0	0	1 100%	0	1 100%
Arecococcus viridians (1)	0	0	0	1 100%	1 100%	0	1 100%	1 100%	1 100%	0	0	1 100%
Kocuria kristinae (2)	0	0	0	2 100%	0	0	2 100%	0	0	0	0	0
Dermacoccus nishinomiyaensis(2)	0	0	0	2 100%	0	0	2 100%	0	0	0	0	0

Table (4): Frequency of β-lactamases producing Gram-positive bacteria isolates.

Gram- positive bacteria isolates	Total No. of isolated	No. & % of isolated with β- lactamase positive		No. & % of isolated with β- lactamase negative	
		No.	%	No.	%
Staphylococcus aureus	54	51	94.4%	3	5.6%
Staphylococcus auricularis	6	3	50%	3	50%
Staphylococcus epidermidis	3	1	33.3%	2	66.7%
Staphylococcus intermedius	2	2	100%	0	0%
Staphylococcus warneri	2	0	0%	2	100%
Staphylococcus haemolyticus	2	2	100%	0	0%
Staphylococcus lentus	1	1	100%	0	0%
Streptococcus pyogenes	16	15	93.8%	1	6.2%
Streptococcus parasanginis	12	7	58.3%	5	41.7%
Streptococcus pneumoniae	7	7	100%	0	0%
Streptococcus sanginus	6	5	83.3%	1	16.7%
Streptococcus agalactiae	6	6	100%	0	0%
Streptococcus gordonii	5	1	100%	0	0%
Streptococcus thoraltensis	5	5	100%	0	0%
Streptococcus mitis	5	5	100%	0	0 %
Streptococcus salivaris	2	1	50%	1	50%
Streptococcus mutans	1	1	100%	0	0%
Kocuria kristinae	2	0	0%	2	100%
Dermacoccus nishinomiyaensi	2	2	100%	0	0%
Alloiococcus otitis	2	0	0%	2	100%
Leuconostoc pseudomesenteroides	1	1	100%	0	0%
Arecoccus viridans	1	0	0%	1	100%
Total	143	116	81.1%	27	18.9%

REFERENCES

1. AD᾽ hlah A H, AL-Abdulwahed L S A and AL kashaly S S A. (2006). A Bacterial Evaluation of Tonsillitis In Baghdad. Ibn AL-Hattham Journal for Pure and APPL SCI., 19(1): 6-11.
2. Ahmed S S. (2013). Microbiological Study of Multi-Drug Resistant PathogensIsolated From Women With Genital Tract Infection in Erbil. MSc thesis. College of Midicine. Medical University of Hawler. Erbil (Iraq).
3. Avilès G P and Zaragoza O. (2000). Streptococcus pyogenes: in vitro susceptibility and role of beta-lactamase producing bacteria in the persistence of Streptococcal pharyngotonsillitis. Journal of Aten Primaria, 25(8): 542-5.
4. Al-kasi G M K. (2001). A study of enzymatic activity of Staphylococcus aureus bacteria isolated from different infections and it's susceptibility antibiotics. M.Sc. thesis. Baghdad: University of Al-Mustansiriya. College of Science. Iraq.
5. Almalki M N. (2011). Bacteriology and Antibacterial Susceptibility of Tonsillopharyngitis and Chronic Suppurative Otitis Media Cross Sectional Study in Al. Habobi Hospital-Thi-Qar. Thi-Qar Medical Journal, 5(1): 118-125.
6. Amin R, Hoque A M W, Khan R F and Rahman M. (2009). Considering respiratory tract infections and antimicrobial sensitivity: An exploratory analysis. Malaysian Journal of Microbiology, 5(2): 109-112.
7. Bachert C, Gevaert P and Cauwenberge V. (2002). Staphylococcus aureus enterotoxins: a key in air way disease?. Journal of Allergy, 57: 480-487.
8. Bingen E, Leclercq R, Fitoussi F, Brahimi N, Malbruny B, Deforche D and Cohen R. (2002). Emergence of Group A Streptococcus Strains with Different Mechanisms of Macrolide Resistance. Journal of Antimicrobial Agants and Chemotherapy, 46(5): 1199-1203.
9. Bisno A L. (2001). Acute Pharyngitis. Journal N Engl Med Primary care, 344(3).
10. Bista M, Amatya R C M and Basnet P. (2006). Tonsillar microbial flora: A comparison of Infected and non-infected tonsils. Kathmandu University Medical Journal, 4(Issue13): 18-21.
11. Brook I and Gober A E. (2009). Rate of eradication of group A beta-hemolytic Streptococci in chidren with pharyngo- tonsillitis by amoxicillin and cefdinir. International Journal of Pediatric Otorhinolaryngology, 73: 757-759.
12. Brook I and Gober A E. (1995). Role of Bacterial Interference and β-Lactamas- Producing Bacteria in the Failure of Penicillin to Eradicate Group A Streptococcal Pharyngotonsillitis. Arch. Journal of Otolaryngol Head Neck Surg, 121(2): 1405-1409.
13. Brook I. (2009). The role of beta-lactamase-producing-bacteria in mixed infections. BMC Journal of Infectious Diseases, 9: 202.
14. Cirilli A R. (2013). Emergency Evaluation and Management of the sore throat. Journal Emerg Med Clin N Am, 31: 501-515.

15. Facklam R. (2002). What Happened to the Streptococci: Overview of Taxonomic and Nomenculture Change. Journal of Clin Microbiol Rev, 15(4): 613.
16. Fan J, Shu M, Zhang G, Zhou w, Jiang y, Zhu y, et al. (2009). Biogeography and virulence of Staphylococcus aureus. Journal PLOS/ONE, 4(Issue7): e 6216.
17. Frickmann H and Zautner A E. (2012). Cholesteatoma-A Potential Consequence of Chronic Middle Ear Inflammation. Journal of Otolaryngology, S: 5.
18. Hameed H M and Al-Saidi M A. (2010). Is the Abuse of Antibiotics Change the Sensitivity Of Follicular Tonsillitis Bacteria in Wasit Province? College of Education Journal Thi-Qar, 1(Issue1): 14-20.
19. Handwerger S, Horowitz H, Coburn K, Kolokathis A and Wormser G P. (1990). Infection Due to Leuconostoc Species: Six Cases and Review. Journal of Clinical Infectious Diseases, 12(4): 602-610.
20. Harrison L M, Morris J A, Telford D R, Brown S M and Jones K. (1999). The nasopharyngeal bacterial flora in infancy: effects of age, geneder, season, viral upper respiratory tract infection and sleeping position. FEMS Journal of Immunology and Medical Microbiology, 25(Issue1-2): 19-28.
21. Hussein R M. (1998). Study of plasmid content of Staphylococci and its relation to the antibiotics resistance. M.Sc. thesis. Baghdad: University of Al-Mustansiriya. College of Science. Iraq.
22. Islam M, Hussin S and Rahman M. (2011). Respiratory bacterial flora from healthy as well as respiratory symptoms subjects. Pakistan Journal of biological science, 14(7): 456-460.
23. Kilic E and Cirak M Y. (2006). Comparison of Staphylococcal Beta- lactamase detection methods. Fabad Journal Pharmacceutical Sciences, 31: 79-84.
24. Könönen E, Jousimies S H, Bryk A, Kilpi T and Kilian M. (2002). Establishment of Streptococci in the upper respiratory tract: longitudinal changes in the mouth and nasopharynx up to 2 years of age. Journal of Med Microbiol, 51: 723-730.
25. Lee C J, Sankaran S, Mukherjee DV, Apa Z L, Hafer C A, Wright L, et al. (2011). Staphylococcus aureus Oropharyngeal Carriage in a Prison Population. Journal of Clinical Infectious Diseases, 52(6): 775-778.
26. Marsh R L, Binks M J, Beissbarth J, Christensen P, Morris P S, Leach A J, et al. (2012). Quantitative PCR of ear discharge from Indigenous Australian children with acute otitis media with perforation supports a role for Alloiococcus otitidis as a secondary pathogen. BMC Journal of Ear, Nose and Throat Disorders, 12(11): 1472-6815.
27. Michael S and Shmuel M G. (2012). Streptococcal pharyngitis: A prospective study of compliance and complications. Journal International Scholarly Research Article. Doi: 10. 5402/2012/ 796389.
28. Nagaraja P. (2008). Antibiotic resistance of Gardnerella vaginalis in recurrent bacterial vaginosis. Indian Journal of Medical Microbiology, 26(2): 155-157.
29. Ndiaye A G, Sarr N C, Thiam K and Boye C S. (2009). In Vitro Activity of Antimicrobial Agents Against Streptococcus Pyogenes Isolates from Patients with Acute Tonsillopharyngitis in Dakar, Senegal. Journal of Microbiology Insights, 2: 25-29.
30. Passali D, Lauriello M, Passali G G, Passali F M and Bellussi L. (2007). Group A Streptococcus and its antibiotic resistance. Journal Acta Otorhinolaryngol Ital, 27: 27-32.
31. Rajasuo A, Somer H J, Savolainen S, Leppänen J, Murtomaa H and Meurman J H. (1996). Bacteriological Finding in tonsillitis and Pericoronitis. Journal of Clinical Infectious Diseases, 23: 51-60.
32. Raju G and Selvam E M. (2012). Evalution of microbial flora in chronic tonsillitis and the role of tonsillectomy. Bangladesh Journal of Otorhinolaryngol, 18(2): 109-113.
33. Rawat D and Nair D. (2010). Extended-spectrum B-lactamases in Gram-negative bacteria. Journal of Glob Infect Dis, 2(3): 263-274.
34. Razzak M S A, Al-Charrakh A H and Al-Greitty B H. (2011). Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginittis. North American Journal of Medical Sciences, 3(4): 185-192.
35. Roberts A L, Connolly K L, Kirse D J, Evans A K, Poehling K A, Peters T R and Reid S D. (2012). Detection of group A Streptococcus in tonsils from pediatric patients reveals high rates of a symptomatic Streptococcal carriage. Journal BMC Pediatrics, 12(3): 12-3.
36. Sadoh E W, Sadoh A E, Oladipo A O and Okunola O. (2008). Bacterial isolates of Tonsillitis and Pharyngitis in a Paediatric casualty setting. Journal of Bio Medical, 7(1-2): 5-11.
37. Samatha P and Praveen K V. (2011). Prevalance of ESBL Ampc β-Lactamase in Gram negative clinical isolates. Journal of Bioscience and Technology, 2(4): 353-357.
38. Sanaa M I, Zaki A A, El-Sayed M A and Abdullah D. (2006). Bacterial infections complicating skin diseases. Egypt Journal Medical Laboratory Science, Etramural Support Information Centers (ESIC), 15(1).
39. Savini V, Favaro M, Fontana C, Consilvio N P, Manna A, Talia M, et al. (2008). Acase of pharyngitis caused by Streptococcus pneumoniae. Journal of Medical Microbiology, 57: 674-675.
40. Treebupachatsakul P, Tiengrim S and Thamlikitul V. (2006). Upper Respiratory Tract Infection in Thai Adults: Prevalence and Prediction of Bacterial Causes and Effectiveness of Using Clinical Practice Guidelines. Journal of Med Assoc Thai, 89(8): 1178-86.

41. Walter J, Hertel C, Tannock G W, Lis C M, Munro K and Hammes W P. (2001). Detection of Lactobacillus, Pediococcus, Leuconostoc and Weissella Species in Human Feces by Using Group-Specific PCR Primers and Denaturing Gradient Gel Electrophoresis. Journal of Appl. Environ Microbiol, 67(6): 2578.
42. Wang Y, Zhang R, Li W, Feng Y and Leng T. (2009). Serious Antimicrobial Resistance Status of Pathogens Causing Hospital-acquired Lower Respiratory Tract Infections in North China. International Journal of Medical Research, 37: 899.
43. Wayne P A. (2005). Clinical and laboratory standards institute. Performance standard for antimicrobial susceptibility testing. 15th ed. Informational Supplement. CLSI / NCCLS M 100- S 15. The institute.
44. Woodhead M and Blasi F. (2005). Guidelines for the management of adult lower respiratory tract infections. European Respiratory Journal, 26: 1138–1180.
45. Yousef R Y, Abid faza'a S and Yousef R Y. (2010). Comparison of The Bacteriology of Tonsills Surface and Core in Bacterial Profile Isolated from Children with Chronic Tonsillitis. Journal of Medical Journal of Babylon, 7(1-2).
46. Zautner A E, Krause M, Stropahl G, Holtfreter S, Frickmann H, Maletzki C, et al. (2010). Intracellular Persisting Staphylococcus aureus is the Major Pathogen in Recurrent Tonsillitis. Journal of PLOS/ONE, 5(Issue 3): e 9452.
47. ŽemličkovA H, Urbášková P, Adámková V, Motlová J, Lebedová V and Procházká B. (2006). Characteristics of Streptococcus pneumoniae, Haemophilus influnenza, Moraxella catarrhalis and Staphylococcus aureus isolated from the nasopharynx of healthy children attending day-care centres in the Czech Republic. Journal of Epidemiol Infect, 134: 1179-1187.