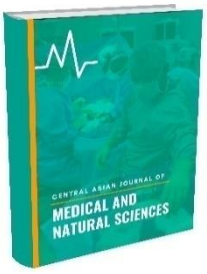
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 03 | May-Jun 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Antimicrobial Resistance of *Streptococci* Sp. Isolated From Different Clinical Sources in Al-Muthanna Province

1. **Zahraa Latif Abd AL-Ameer**
2. **Ghaidaa Jihadi Mohammed**

Received 5th Apr 2023,
Accepted 6th May 2023,
Online 8th June 2023

[1, 2] Department of Biology, College of Science, University of Al-Qadisiyah, Al-Diwaniayh, Iraq

Key words: Streptococci sp., Antibiotic resistance, Pharyngitis, Dental caries, Pneumonia.

Abstract:

Background: The genus Streptococcus includes Gram-positive organisms cocci shape and organized in chains. Streptococcus has spacious importance in medicine, different streptococci are significant as part of the commensals of humans; some can as well cause illnesses that may be subacute, acute or chronic. From the considerable human illnesses belong to streptococci are rheumatic heart disease, scarlet fever, pneumococcal pneumonia and glomerulonephritis. Antibiotic Resistance is a growing concern for public health and the main cause for the failure of streptococcal infections treatment is the increase resistance to antibiotics.

Aim: Determine the antimicrobial resistance of Streptococci sp .isolated from different clinical sources in Al- Muthanna Province.

Method: The study included the collection of (270) samples from different clinical source(Throat swabs,burns, wounds, abscess, pus, vagina swabs,teeth,ear,mouth and sputum) from patients in different hospitals and clinics in Al-Muthanna Province, during the period from first of September to the mid December of 2022 .The patients were with different ages ranging from 1 day-80 year, and from both sexes. The identification of bacterial isolates were achieved by morphological characteristics of bacterial colonies on selective media, result of Bacitracin sensitivity test, Optochin sensitivity test, CAMP test, Gram stain, and biochemical tests and then confirmed the diagnosis by using Remel RapID™ STR System. The antibiotics sensitivity test was achieved for all isolates by disk diffusion method.

Results: A total of 50 (18.5 %) isolates were identified as Streptococcus spp. from (270) clinical samples that collected from different sources, that distributed by 16 (20.77%)from Throat Swab,

12 (33.33%) from sputum, 10(29.41%) from Tooth swab, 7 (13.20%) from Vaginal swab, 3 (10%) from Mouth swab, 2 (10%) from Ear swab, and No isolate detected from burns, wounds, abscess and pus samples. The Fifty isolates included 16(32%) of them were identified as S. pyogenes, 11(22%) were *S.sanguins*, 10(20%) were *S.aglactiae*, 9(18%) were S. *mutans* and 4(8%) were identified as S. *pneumoniae*

The antibiotic susceptibility of the bacterial isolates was achieved against 10 types of antibiotics; most *S.pyogenes* isolates have resistance to three or more antibiotics. The highest percentage of *S. pyogenes* resistance was (93.7%) to Ceftriaxone, 87% to Penicillin and Ampicillin, (62.5%) to Tetracycline, 50% to Chloramphenicol and Clindamycin. Whereas (81.25%) of the isolates were sensitive to Levofloxacin, Vancomycin (75%), Azithromycin (62.5%), erythromycin (56.25%), and Clindamycin (50%). All *S. agalactiae* isolates were resistance (100%) for ceftriaxone, tetracycline, Azithromycin, Chloramphenicol, clindamycin and erythromycin. On the other hand,the isolates were resistance to vancomycin and Levofloxacin with rate(90%) and(60%) respectively. While they were sensitive for Ampicillin and Penicillin with percentage (80%, 60%) respectively. Antibiotic susceptibility profile for *Streptococcus mutans* isolates revealed that all the isolates were resistant to Ampicillin and clindamycin, 77.77 % of them were resistant to penicillin, 66.66% isolates were resistant to Ceftriaxone. Whereas all isolates were sensitive to Azithromycin, erythromycin and Levofloxacin. While, the sensitivity to Chloramphenicol, vancomycin, ceftriaxone and Tetracyclin were (88.88 %, 77.77%, 33.33%,33.33%) respectively. *Streptococcus pneumoniae* isolates were resistant to penicillin, Ampicillin, Ceftriaxone, tetracycline, clindamycin and vancomycin (100,100.100,75,75,and 50) respectively. While, all isolates were sensitive to Azithromycin, Erythromycin, Levofloxacin, Chloramphenicol. *S.san*guinis isolates were sensitive to clindamycin, Levofloxacin, Azithromycin and erythromycin (90.90%,81.81%,81.81%,63.63%)) respectively. and all the isolates were resistant to Ampicillin and Ceftriaxone, nine isolates were resistant to vancomycin and penicillin (81.81%) and six isolates were resistant to tetracycline (54.54%).

Introduction

Streptococcus strains are normally spherical or ovoid, less than 2 µm in diameter, occurring in chains or in pairs when grown in liquid media. Cells are nonmotile, endospores are not formed, and Gram-positive. Virtually all species are facultatively anaerobic, some requiring additional CO2 for growth. Chemo-organotrophic with fermentative metabolism. Carbohydrates are fermented to produce mainly lactic acid but no gas. Catalase-negative. Nutritional requirements are complex and variable(1). The genus Streptococcus belongs to phylum Firmicutes, class Bacilli, order Lactobacillales, and family Streptococcaceae (1,2).Streptococci are found in almost every location in the human body and are the dominant species in the human oral cavity and upper respiratory tract. Species within the genus Streptococcus were initially differentiated based on hemolysis patterns on blood agar plates with β-hemolysis (complete lysis), α-hemolysis (partial lysis), and γ-hemolysis (nonhemolytic) (3,4).The heterogeneous genus of Streptococci plays an important role in human diseases. Streptococci are estimated to cause 700 million human infections each year worldwide, with an estimated total of 500,000 deaths (5,6).Streptococci sometimes use mechanisms similar to those of other intracellular bacterial species and viruses to invade host cells. Due to their variable repertoire of adhesins and invasins, streptococci have evolved numerous strategies to be internalised and survive in host cells for their own benefit, namely escaping antibiotic treatment and the host immune system (7,8,9,10,11,12,13,14). Most streptococcal infections are of a mild nature. However, these bacterial species also cause highly invasive diseases. These include but are not limited to necrotizing skin and soft tissue infections (NSTIs; *S. pyogenes*) (15), pneumonia, sepsis, and meningitis (*S. pneumoniae*, *S.*

suis) (16,17,18), neonatal sepsis (*S. agalactiae*) (19), and endocarditis (*S. anginosus*) (20,21). Antibiotic resistance is a growing concern for public health and global economy, with the cost of over 50,000 lives per year only in Europe and US. If the problem is not adequately addressed worldwide, it is assumed that by 2050 the presence of resistance would lead to 10 million people dying every year and it would cost trillions of USD to healthcare system (22). The main cause for the failure of streptococcal infections treatment is the increase resistance to antibiotics. So, this study was carried out to determine the antimicrobial resistance of Streptococci sp. isolated from different clinical sources in Al- Muthanna Province.

Methodology

Collection of Samples

The study included the collection of (270) samples from different clinical cases in different hospitals and clinics in Al-Muthanna Province during the period from first of September to the mid December of 2022 .These samples included (77) samples were collected from throat swabs of patients suffering from pharyngitis; (20) Ear swabs from patients with the middle ear infections;(53) samples from high vaginal swabs were taken from the pregnant woman in the period (36-40)weeks by physician; 20 samples from burns, wounds , abscess and pus ;36 samples from Sputum of patients suffering from respiratory tract infections;34 samples from tooth swabs of individuals with dental caries ; 34 samples from mouth swabs were collected from newborn; Cotton swabs containing the transport medium were used during collection to ensure the vitality of the isolates and its survival for the longest period . Samples were taken from patients after get their consent and recording their data of age and gender, the age of participant patients were (1day-80years) ,and from both sexes. The collected samples were planted directly on blood agar medium, chocolate agar, HiCrome Strep B Selective Agar Base, Mutans-Sanguis Agar, and Mitis Salivarius Agar Base and incubated under 5- 10% CO2 for 24 hours at 37˚C .

Identification of bacterial isolates

The identification of bacterial isolates were achieved by bacteriological methods including morphological characteristics of bacterial colonies on agar (Blood agar medium, chocolate agar, HiCrome Strep B Selective Agar Base, Mutans-Sanguis Agar, and Mitis Salivarius Agar Base), result of Bacitracin sensitivity test , sensitivity to Optochin disk ,Gram stain, and traditional biochemical tests and then confirmed by Moleculer by 16Sr RNA,VITEK-2 system and Remel RapID™ STR System.

Antibiotic susceptibility test

The isolates were tested for antimicrobial susceptibility to 10 antibiotics using the agar disc diffusion method on Mueller–Hinton agar(Bioanalyse /Turkey) following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI , 2022). The 10 antibiotics tested were as follows: pencillin (10 µg), Ampicillin (10 µg),Erythromycin (15 µg),Azithromycin (15 µg), Tetracycline (10 µg), Clindamycin (10 µg),Levofloxacin (5 µg), (10 µg), Vancomycin (30 µg), Chloramphenicol (10 µg) , ceftriaxone (10 µg), .These antibiotics are representative of the major classes of antimicrobial drugs important to both veterinary and human medicine. Isolates were classified as susceptible, moderately resistant, and resistant using breakpoints specified by the CLSI.

Results

Bacterial Isolation and Biochemical Identification

The culture of different clinical samples(270) on blood agar and selective media showed that the bacterial agent were detected in 50 (18.5%) samples as shown in figure (1).

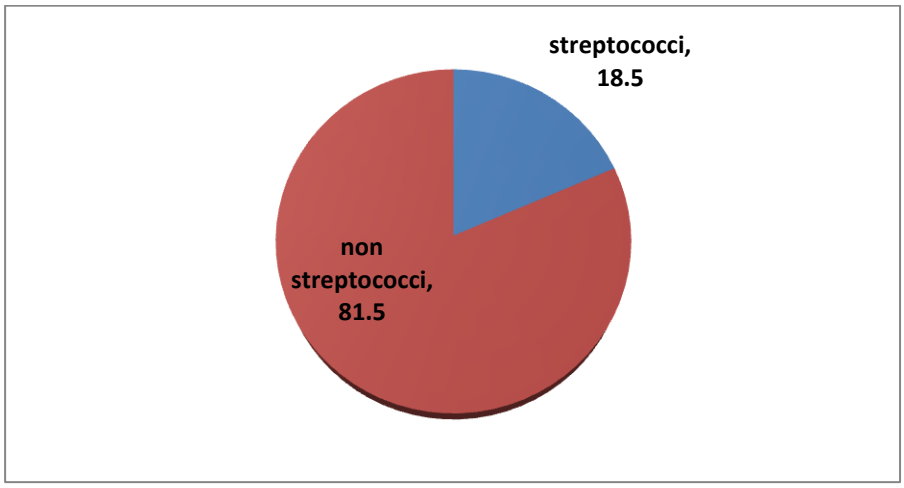


Figure (1) : Percentage of Bacterial culture from clinical samples.

A total of 50 (18.5 %) bacterial isolates were identified as *Streptococcus spp.* from (270) clinical samples that collected from different sources, that distributed by 16 (20.77%)from Throat Swab , 12 (33.33%) from sputum , 10(29.41%) from Tooth swab , 7 (13.20%) from Vaginal swab , 3 (10%) from Mouth swab , 2 (10%) from Ear swab ,and No isolate detected from burns , wounds , abscess and pus samples ,as shown in table (1).

Table (1): Prevalence of *Streptococci* sp. isolated from different clinical specimens

Source	Total No.	Positive specimens No. for *Streptococci* sp.	%
Throat swab	77	16	20.77
Ear swab	20	2	10
Vaginal swab	53	7	13.20
Sputum	36	12	33.33
Burns ,Wounds, Abscess and Pus	20	0	0
Tooth swab	34	10	29.41
Mouth swab	30	3	10
Total	270	50	18.51
X^2	16.11		
P value	0.013*		

* Significant difference at P<0.05

The 50 isolates that were identified as *Streptococcus spp.* by Gram stain, traditional biochemical tests and by Remel RapID™ STR System (Table 3), included 16(32%) of them were identified as *S. pyogenes* , 11(22%) were *S.sanguins*, 10(20%) were *S.aglactiae* ,9(18%) were *S. mutans* and 4(8%) were identified as *S. pneumonia*, and these results are more clarified in (Figure 2) (Table 2).

Table (2). Percentage of *Streptococccci* species from different clinical samples.

Bacterial isolation	No. of isolation	Percentage
S.pyogenes	16	32%
S.sanguinis	11	22%
S.agalactiae	10	20%

S.mutans	9	18%
S. pneumoniae	4	8%
Total	50	100%
X^2	9.25	
P value	0.055*	

* No significant difference at P<0.05

Table (3) : Results of Gram stain and Biochemical tests of S*treptococci spp* isolates

No	Tests	*Streptococcus pyogenes* Group A	*Streptococcus agalactiae* Group B	*Streptococcus pneumoniae*	*Streptococcus mutans*	*Streptococcus sanguinis*
1.	Gram stain	+	+	+	+	+
2.	CAMP test	-	+	-	-	-
3.	Hemolysis	β-Hemolysis	β-Hemolysis	α-hemolysis	α-hemolysis	α-hemolysis
4.	Catalase	-	-	-	-	-
5.	Oxidase	-	-	-	-	-
6.	Shape	cocci	cocci	Diplococc	cocci	cocci
7.	Bacitracin test	+	-	-	-	-
8.	Optochin test	-	+	-	-	-
9.	Growth in the presence of CO2	+	+	+	+	+

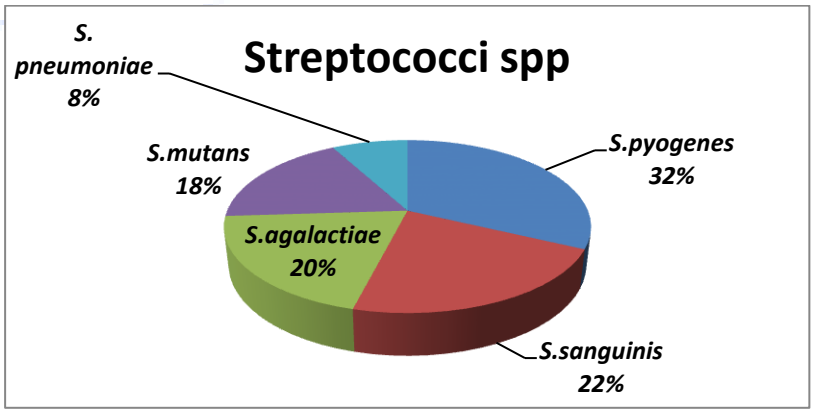


Figure (2) Percentage of *Streptococccci* species from different clinical samples.

Bacitracin sensitivity test

The positive bacterial culture of *S. pyogenes* tested for bacitracin sensitivity and the results was shown in figure (3 A&B). *Streptococcus pyogenes* (GAS) is inhibited by a small amount of bacitracin (0.04 U) in the disk. After an overnight incubation at 35°C in 5% CO_2, a zone of inhibition surrounds the disc, indicating the strain's susceptibility . Whereas *Streptococcus agalactiae* were resistant as shown in Figure 3C.

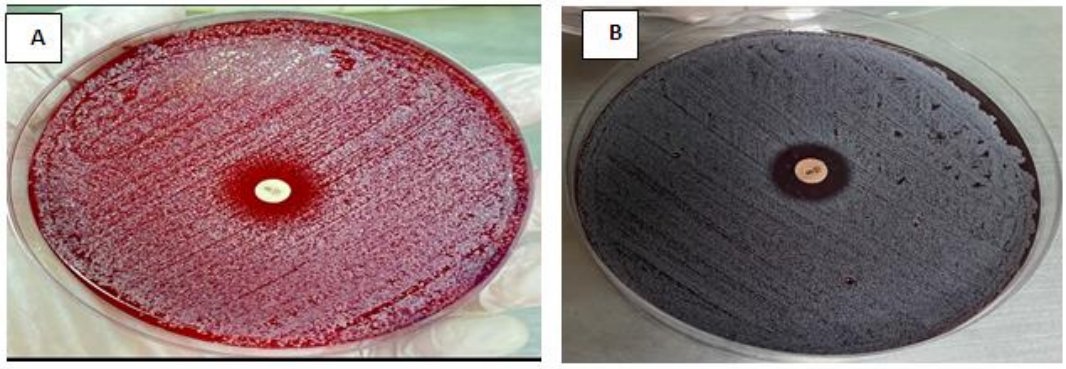
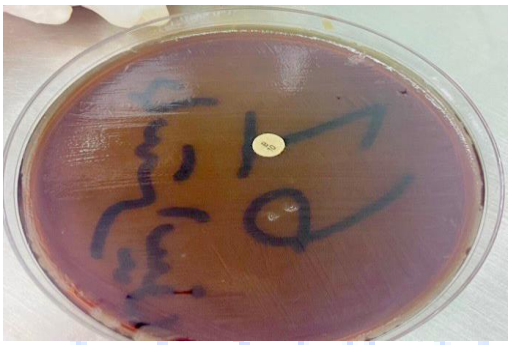


Figure (3): Bacitracin sensitivity test (A) On Blood Agar;(B) On Chocolate agar



(C) Bacitracin resistant*Streptococcus agalactae* (GBS) on chocolate agar

• Positive: Any zone of inhibition greater than 10 mm; susceptible.

• Negative: No zone of inhibition; resistant

Antibiotic susceptibility of *Streptococci* sp. isolates

The Antibiogram testing was performed with selected antibiotics that is commonly recommended by (CLSI, 2022). The rates of sensitivity and resistance to antibiotics by *Streptococci sp.* isolates are shown in table(3) to table (7) . The results showed most of *S.pyogenes* isolates have resistance to three or more antibiotics. The highest percentage of resistance was (93.7%) to Ceftriaxone , 87% to Penicillin and Ampicillin , (62.5%) to Tetracycline, 50% to Chloramphenicol and Clindamycin. Whereas sensitivity results showed (81.25%) of isolates were sensitive to Levofloxacin, Vancomycin (75%), Azithromycin (62.5%), erythromycin (56.25%), and Clindamycin (50%), as shown in table(4).

Table (4) Results of Antibiotic susceptibility test of *S. Pyogenes* isolates

Pattern	PEN	AMP	CRO	VA	E	AZT	TET	LEV	C	DA
	No. %									
Sensitive	2 12.5%	2 12.5%	1 6.25%	12 75%	9 56.25%	10 62.5%	1 6.25%	13 81.25%	6 37.5%	8 50%
Intermediate	0 0%	0 0%	0 0%	0 0%	1 6.25%	1 6.25%	5 31.25%	2 12.5%	2 12.5%	0 0%
Resistance	14 87%	14 87%	15 93.75%	4 25%	6 37.5%	5 31.25%	10 62.5%	1 6.25%	8 50%	8 50%
X2	74.78									
P value	0*									

* Significant difference at P<0.05

In consider to *S. agalactiae* isolates, the results observed all the isolates were resistance (100%) for ceftriaxone , tetracycline , Azithromycin , Chloramphenicol ,clindamycin and erythromycin. On the other hand, the isolates were resistance to vancomycin and Levofloxacin with rate(90%) and(60%) respectively. While the isolates were sensitive for Ampicillin and Penicillin with percentage(80% and 60%)respectively, table (5).

Table (5) Results of Antibiotic susceptibility test of *S. Agalactiae* isolates

Pattern	PEN	AMP	CRO	VA	E	AZT	TET	LEV	C	DA
	No. %									
Sensitive	6 60%	8 80%	0 0%	1 10%	0 0%	0 0%	0 0%	4 40%	0 0%	0 0%
Intermediate	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Resistance	4 40%	2 20%	10 100%	9 90%	10 100%	10 100%	10 100%	6 60%	10 100%	10 100%
X2	52.56									

* Significant difference at P<0.05

Antibiotic susceptibility profile for *Streptococcus mutans* isolates against ten antibiotics was tested. The results revealed that all the isolates were resistant to Ampicillin and clindamycin , 77.77 % of them were resistant to penicillin , 66.66% isolates were resistant to Ceftriaxone. Whereas all isolates (100%) were sensitive to Azithromycin, erythromycin and Levofloxacin. While, the sensitivity to Chloramphenicol and vancomycin were 88.88 %,and 77.77% respectively, and 33.33% for ceftriaxone and Tetracyclin, as clarified in table(6) .

Table (6) Results of Antibiotic susceptibility test of *S. Mutans* Isolates

Pattern	PEN	AMP	CRO	VA	E	AZT	TET	LEV	C	DA
	No. %									
Sensitive	0 0%	0 0%	3 33.33%	7 77.77%	9 100%	9 100%	3 33.33%	9 100%	8 88.88%	0 0%
Intermediate	2 22.22%	0 0%	0 0%	0 0%	0 0%	0 0%	5 55.55%	0 0%	1 11.11%	0 0%
Resistance	7 77.77%	9 100%	6 66.66%	2 22.22%	0 0%	0 0%	1 11.11%	0 0%	0 0%	9 100%
X2	99.53									
P value	0*									

* Significant difference at P<0.05

The results inducted that *Streptococcus pneumoniae* isolates were resistant to penicillin, Ampicillin, Ceftriaxone, tetracycline , clindamycin and vancomycin with rate (100%,100%,100%,75%,75%,and

50%) respectively. While, all isolates were sensitive to Azithromycin , Erythromycin, Levofloxacin, Chloramphenicol, Table(7).

Table (7) Results of Antibiotic susceptibility test of *S. Pneumoniae* isolates

Pattern	PEN	AMP	CRO	VA	E	AZT	TET	LEV	C	DA
	No. %									
Sensitive	0 0%	0 0%	0 0%	2 50%	4 100%	4 100%	1 25%	4 100%	4 100%	1 25%
Intermediate	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Resistance	4 100%	4 100%	4 100%	2 50%	0 0%	0 0%	3 75%	0 0%	0 0%	3 75%
X2	30									
P value	0*									

* Significant difference at P<0.05

The results of antibiotic sensitivity for *S.sanguinis* isolates showed that they were sensitive to clindamycin , Levofloxacin, Azithromycin and erythromycin (90.90%, 81.81%,81.81%,63.63%)) respectively .and all the isolates were resistant to Ampicillin and Ceftriaxone, nine isolates were resistant to vancomycin and penicillin (81.81%) and six isolates were resistant to tetracycline (54.54%) ,as shown in table(8).

Table (8) Results of Antibiotic susceptibility test of *S. Sanguinis* isolates

Pattern	PEN	AMP	CRO	VA	E	AZT	TET	LEV	C	CD
	No. %									
Sensitive	2 18,18%	0 0%	0 0%	2 18.18%	7 63.63%	9 81.81%	5 25%	9 81.81%	1 9.09%	10 90.90%
Intermediate	0 0%	0 0%	0 0%	0 0%	3 27.27%	1 9.09%	0 0%	1 9.09%	6 54.54%	0 0%
Resistance	9 81.81%	11 100%	11 100%	9 81.81%	1 9.09%	1 9.09%	6 54.54%	1 9.09%	4 36.36%	1 9.09%
X^2	94.57									
P value	0*									

* Significant difference at P<0.05

Discussion

The results of isolation indicate that the rate of *Streptococcus pyogenes* was 16(32%) included,1(5%) isolate from patient with the middle ear infections; 15(19.48%) isolates from throat swabs from patient with pharyngitis .Regarding , the percentage of isolates from throat swab is approximately similar to

the study in Babylon who was found that the percentage of GAS was (16.95%) (23) and other study found that the percentage of GAS was (17%)(24) . In addition , the results of this study was disagree with that carried by (25,26,27,28) were they found the rate of *Streptococcus pyogenes* isolated from throat swab reach to(7.4%) (25%) ,(37%) ,and (41%) respectively, as well as the ratio of isolation of *S.pyogenes* from patients with tonsillitis in the city of Muqdadiya was 11% in a study by(29).

The isolates of *S.aglactiae* were 10 (20%) , included ,3(10%) obtained from mouth swab from newborn, 7 (13.20%) obtained from high vaginal swabs from pregnant women in (36-40) weeks. This result was correlated with study of (30) who found that the prevalence of GBS by culture sample was (13%) .The result was in agreement with results of a study done in Babylon city by(31) who found that ratio of isolation *S.aglactiae* (10%) .

The results of this study was in agreement with results obtained by (32) who he found that (9.3%) were identified as carriers of group B streptococci depending on the cultures of specimens. The study done by (33) showed that the highest percentage(24.3%) of GBS infected Iraqi pregnant women. Other study reported that the prevalence of *S. agalactiae* was(23%) by (34).This result was also correlated with a study by (35) who found that the prevalence of GBS by culture sample were (7.39%). Other study indicate high prevalence of GBS in Al-Muthanna province the percentage (49.51%) of the pregnant women aged under 25 years of age, the percentage (29.61%) the pregnant women more than 25 years (36). Four (8%) isolates were identified as *S. pneumonia*. This percentage agrees with (25)who he found the rate of isolation was(8.8%) in Kirkuk city. This percentage is slightly different with the percentage obtained by(37)where the rate of isolation was 4.8%. Also, it disagrees with what found by (38) where the rate of isolation was (22%). About 9(18%) were identified as *S. mutans* and 11(22%) isolates were *S.sanguins* . This percentage for *S. mutans* corresponds with a percentage of a study in Diyala by (39) which was (13.30 %). The current results were slightly different from a study done in Diyala by (38) who found that (14%) were *S. mutans*.

This variation in percentage of infection could be belong to several reason, including the different techniques used to isolate the bacteria , differing objectives of the study and age groups , random intake to antibiotics in some times as a result of negative culture wrong , season on which the sample also effect the isolated bacteria proportion of the collection as some seasons are the infection rate more than others because of the climatic condition and cases of overcrowding, case economic and social for patients under study (40).

The results of antibiotic sensitivity test of *S. agalactiae* showed that the sensitivity of isolates to Ampicillin was (80%) . Ampicillin is a ß-lactam antibiotic that has been used extensively to treat bacterial infections. It inhibits the third and final stage of bacterial cell wall synthesis, which ultimately leads to cell lysis (41).While the sensitivity to penicillin was (60%) , and the results of this study were similar to a study of (42) which showed high sensitivity rate to penicillin . Other study found that group B streptococci (GBS) isolates were completely sensitive to penicillin(43). Also, a study by (36) found that group B streptococci (GBS) isolates were sensitive to penicillin with rate of (76%). All the isolates of *S. agalactiae* were resistance (100%) for clindamycin and erythromycin. This result was similar to the results of a study by(42) which showed *S. agalactiae* isolates were resistance to erythromycin and clindamycin with rate of above 90 %. The isolates of this bacteria were resistance to levofloxacin (60%) and this result was slightly different from the study carried by(42),where the resistance rate of *S. agalactiae* to levofloxacin was 71.88%, as well as different from the study done by (43) in Al –Diwaniyah city which reported the resistance rate of (50%) . Vancomycin had a resistance of 90% and this finding was somewhat differs with a study done by(44) in Al –Diwaniyah city where the resistance rate was (62.5%%) .all the of *S. agalactiae* isolates were resistance (100%) for tetracycline Chloramphenicol. The streptococcal genetic elements carriers of macrolide resistance

determinants., also carry a number of other genes conferring resistance to a variety of other antibiotics, including tetracyclines, aminoglycosides, spectinomycin, and chloramphenicol (45).

The results showed most of *S.pyogenes* isolates have resistance to three or more antibiotics. The highest percentage of resistance was (93.7%) to Ceftriaxone , 87% to Ampicillin , this result was somewhat similar with results of a study by(25) in Kirkuk city in which he found the resistance rate was (100%). *S.pyogenes* isolates have resistance (62.5%) to Tetracycline , and this disagree with a study of (25) where the resistance rate was (100%). *Streptococcus pyogenes* isolates have resistance rate 50% to Chloramphenicol and this finding was slightly different with study of (25) that reported the resistance rate of (60%). The sensitivity results of *S.pyogenes* isolates showed (81.25%) of them were sensitive to Levofloxacin ,this result agrees with(41) which showed *Streptococcus pyogenes* were not resistant to levofloxacin, the present result results disagree with what found by (25) where the resistance rate was (100%). The study has shown the sensitivity rate to Vancomycin was about (75%) ; this result was similar to the results of a study by(42) where *Streptococcus pyogenes,* were highly sensitive to vancomycin. The sensitivity results of *S.pyogenes* isolates to erythromycin was (56.25%) and Clindamycin was (50%). The results disagree with (25) who he found the resistance rate was (100%). The sensitivity results of this bacteria showed that the sensitivity to Azithromycin was(62.5%). This result was slightly different with a study of (25) which showed the sensitivity rate to Azithromycin was(40%). The macrolides are considered bacteriostatic against most susceptible organisms; however, azithromycin is bactericidal against *Streptococcus pyogenes* (45).

The streptococcal geneticelements carriers of macrolide resistance determinants., also carry a number of other genes conferring resistance to a variety of other antibiotics, including tetracyclines, aminoglycosides, spectinomycin, and chloramphenicol (46).

The results inducted that *S. pneumoniae* isolates were resistant to penicillin, tetracycline and vancomycin with rate (100%,75% and 50%) respectively .This result corresponds with a study which showed that *S. pneumoniae* had a resistance rate of more than 50% to tetracycline, penicillin, and vancomycin(42). While, all isolates were sensitive to Levofloxacin, and this agrees with study of (42), in which *S. pneumoniae,* were not resistant to levofloxacin, this result came in line with a study done in Kirkuk city which found the sensitivity rate was (41%)(25).

S. pneumoniae isolates were resistant to tetracycline with rate (75%) and this result came in line with what found by a study of (38) which reported resistance rate 63.3% ,as well as was agrees with study of (47) where the resistance of bacteria to this antibiotic was 77%, but this result disagree with a study (25) which found the resistance rate (100%).

S. pneumonia and *S.mutans* isolates in this study showed that all isolates were resistant to Bacitracin and by 100% this result agrees with the study of (39). All isolates of *Streptococcus mutans* (100%) were sensitive to erythromycin . While, the sensitivity to Chloramphenicol was (88.88 %), the results were slightly different with a study of (39) where the resistance rate to Erythromycin was(65%), Chloramphenicol was (45%).

While they showed resistance to antibiotics , Ampicillin and Optochin (100%).The present results in agreement with results of a study done in Diyala (39). All the isolates of *Streptococcus mutans* were resistant to clindamycin, the results was disagreement with results of a study done in Diyala(38) where *S. viridans* isolate had a resistance rate of 71.4% for tetracycline , 28% for clindamycin and 57% for erythromycin.

Internationally, the acquisition of resistance in streptococci is somewhat low, may be due to the restricted horizontal transfer of resistance genes. In addition to that, conjugative elements; Plasmids , integrative elements or transposons can publicize resistance factors among streptococci (48).

Conclusion

The widespread and irrational use of antimicrobial drugs in the past few years has altered pathogenic bacteria and enhanced drug-resistant strains . Which is a major threat to global health .So, the preforming drug sensitivity analyses before treatment, select efficient and sensitive antibacterial drugs, and standardize their use to effectively control the infection at the earliest, and reduce the creation of drug-resistant strains.

References

1. Whiley R, Hardie J. (2015). Streptococcus. Bergey's Manual of Systematics of Archaea and Bacteria. Chichester, UK: John Wiley & Sons, Ltd, 1-86.
2. Kumar, S., Bansal, K., & Sethi, S. K. (2022). Phylo-taxonogenomic reconciliations of genus Streptococcus reveals the existence of sixteen novel genomospecies.
3. Sherman JM. (1937). The Streptococci. Bacteriol Rev 1:3–97.
4. Abranches, J., Zeng, L., Kajfasz, J. K., Palmer, S. R., Chakraborty, B., Wen, Z. T., ... & Lemos, J. A. (2018). Biology of oral streptococci. Microbiology spectrum, 6(5), 6-5.
5. Carapetis J. R., McDonald M., Wilson N. J. Acute rheumatic fever. Lancet. 2005;366(9480):155–168. .
6. Rohde, M., & Cleary, P. P. (2022). Adhesion and invasion of Streptococcus pyogenes into host cells and clinical relevance of intracellular streptococci.
7. Cunningham M. W. (2000)/ Pathogenesis of group A streptococcal infections. Clinical Microbiology Reviews;13(3):470–511.
8. Courtney H. S., Hasty D. L., Dale J. B.(2002). Molecular mechanisms of adhesion, colonization, and invasion of group A streptococci. Annals of Medicine;34(2):77–87.
9. Talay S. R. (2005). Gram-positive adhesins. Contributions to Microbiology;12:90–113. .
10. Nitsche-Schmitz D. P., Rohde M., Chhatwal G. S. (2007).Invasion mechanisms of gram-positive pathogenic cocci. Thrombosis and haemostasis;98(3):488–496.
11. Nobbs A. H., Lamont R. J., Jenkinson H. F.(2009). Streptococcus adherence and colonization. Microbiology and Molecular Biology Reviews;73(3):407–450. Table of Contents.
12. Rohde M., Chhatwal G. S.(2013). Adherence and invasion of streptococci to eukaryotic cells and their role in disease pathogenesis. Current Topics in Microbiology and Immunology;368:83–110.
13. Brouwer, S., Barnett, T. C., Rivera-Hernandez, T., Rohde, M., & Walker, M. J. (2016). Streptococcus pyogenes adhesion and colonization. FEBS letters, 590(21), 3739-3757.
14. Rohde, M., & Cleary, P. P. (2022). Adhesion and invasion of Streptococcus pyogenes into host cells and clinical relevance of intracellular streptococci.
15. Siemens, N., Snäll, J., Svensson, M., & Norrby-Teglund, A. (2020). Pathogenic mechanisms of streptococcal necrotizing soft tissue infections. Necrotizing Soft Tissue Infections: Clinical and Pathogenic Aspects, 127-150.
16. Votsch, D., Willenborg, M., Weldearegay, Y. B., and Valentin-Weigand, P. (2018). Streptococcus suis The "Two Faces" of a pathobiont in the porcine respiratory tract. Front. Microbiol. 9, 480. doi: 10.3389/fmicb.2018.00480.

17. Steinert, M., Ramming, I., and Bergmann, S. (2020). Impact of von willebrand factor on bacterial pathogenesis. Front. Med. 7, 543. doi: 10.3389/fmed.2020.00543.

18. Palmer, C. S., and Kimmey, J. M. (2022). Neutrophil recruitment in Pneumococcal Pneumonia. Front. Cell. Infect. Microbiol. 12, 894644. doi: 10.3389/fcimb.2022.894644.

19. Armistead, B., Oler, E., Adams Waldorf, K., and Rajagopal, L. (2019). The double life of group b Streptococcus: asymptomatic colonizer and potent pathogen. J. Mol. Biol. 431, 2914-2931. doi: 10.1016/j.jmb.2019.01.03.

20. Reissmann, S., Friedrichs, C., Rajkumari, R., Itzek, A., Fulde, M., Rodloff, A. C., et al. (2010). Contribution of Streptococcus anginosus to infections caused by groups C and G streptococci, southern India. Emerg. Infect. Dis. 16, 656-663. doi: 10.3201/eid1604.090448.

21. Bergmann, S., Fulde, M., & Siemens, N. (2022). Streptococci in infectious diseases–pathogenic mechanisms and host immune responses. Frontiers in Microbiology, 13, 2863.

22. Ojha, A.K., Shah, N.P., Mishra, V.(2021). Conjugal transfer of antibiotic resistances in Lactobacillus spp. Curr. Microbiol. 1-11 https:// doi.org/10.1007/s00284-021- 02554-1 .

23. Sahib Russell Issam Ali,2019; Molecular study of some virulence factors among streptococcus pyogenes isolated from patients with pharyngitis; department of Microbiology-College of Medicine/ Babylon University.

24. Jose ,J.J.M.; Brahmadathan ,K. N.; Abraham, V.J.; Huang ,C.Y.; Morens , D.; Hoe,N.P.; Follmann ,D.A. and Krause, R.M. (2018).Streptococcal group A, C and G pharyngitis inschool children: a prospective cohort study in Southern India. Epidemiology and Infection146,848–853.

25. Ameen Hawzheen Mohammed. (2022). Evaluation of Some Immunological Parameters In Secondary Bacterial Infections among COVID-19 Patients In Kirkuk City . College of Education for Pure Sciences University of Kirkuk.

26. Linder, J.A.; Bates ,D.W. and Lee ,G.M. (2005).Antibiotic treatment of children with sore throat. JAMA;294:2354-6.

27. Cohen J, Bertille N, Cohen R, Chalumeau M.(2016). Rapid antigen detection test for group A streptococcus in children with pharyngitis. Cochrane Database of Systematic Reviews.

28. Aljadoa, H.H.(2019). Phenotypic and Genotypic Characterization of some bacterial causing pharyngeal Infections in Babylon Province.

29. Tamimi Zainab Amer Hatem .(2013). Bacteriological and hereditary study of *Streptococcus pyogenes* isolated from patients with tonsillitis in the city of Muqdadiya, *Master Thesis*, College of Education for Pure Sciences \ University of Diyala.

30. Alp, F., Findik, D., Dagi, H.T., Arslan, U., Pekin, A.T. and Yilmaz, S.A. (2016). Screening and genotyping of group B streptococcus in pregnant and non-pregnant women in Turkey.The *Journal of Infection in Developing Countries*, 10(03), pp.222-226.

31. Rasheed Taiseer Safaa Jwad. (2018). Molecular Study of Streptococcus agalactiae isolated from pregnant women in Babylon province, *Doctor Thesis* College of Medicine\ University of Babylon.

32. Bakhtiari, R., Dallal, M.S., Mehrabadi, J.F., Heidarzadeh, S. and Pourmand, M.R.,(2012). Evaluation of culture and PCR methods for diagnosis of group B streptococcus carriage in Iranian pregnant women. *Iranian journal of public health*, 41(3), p.65.

33. Hamid, Z.O., Zaki, N.H. and Ali, M.R., (2015). Prevalence of macrolide resistance genes among Group B Streptococci in pregnant women. *Int. J. Curr. Microbiol. App. Sci*, 4(1), pp.419-436.

34. Abdul-Razzaq, M.S., Al-taee, S.S. and al-khalidi Saleem, A., (2013). Detection of Sortase Enzymes and Cyl operon A and B of Streptococcus Agalactiae by PCR. *Medical Journal of Babylon*, 10(4), pp.1013-1020.

35. Mashouf, R.Y., Mousavi, S.M., Rabiee, S., Alikhani, M.Y. and Arabestani, M.R. (2014). Direct identification of *Streptococcus agalactiae* in vaginal colonization in pregnant women using polymerase chain reaction. Journal of Comprehensive Pediatrics, 5(4).

36. Mohammed, H. H. (2022). Assist. Prof. Maitham Abas Makki (Doctoral dissertation, Ministry of Higher Education.

37. Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S and Lynch S V .(2015). Use of16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PloS One* **10**(2), e0117617.

38. Ibrahim, Hadeel Jalil.(2018). Diagnostic and molecular study of *Streptococcus* spp isolated from oral and upper respiratory diseases in Baquba city , Master Thesis, Diyala University, College of Education for Pure Sciences Biology Department.

39. Torki Sumyah hasan .(2018).A Study on Streptococcus mutans bacteria isolated from Dental caries infection and comparing the effect of some plant extracts and antibiotics on this bacteria, Master Thesis, Diyala University College of Science.

40. Almasohode M.A.(2016) . Diagnostic study for *Streptococcus pyogenes* and molecular characterization for some virulence factor in tonsillitis patients .

41. Bose,S.C.; Kumar,V. and Raghavan.S. (2012).Qualitative Analysis of Amoxicillin, Ampicillin, Cephalexinby Quadrupole–Time of Flight (LCMS) Using Electrospray Ionization International .Journal of ChemTech Research CODEN(USA): 1151-1157.

42. Zhang, M., Liu, J., Pan, Y. C., Liu, H. L., Shu, J., Wu, X. M., & Yang, Y. (2023). Analysis of Pathogenic Bacteria and Drug Resistance Among Girls with Bacterial Vaginitis. Infection and Drug Resistance, 1849-1863 .

43. Brandon, M., & Dowzicky, M. J. (2013). Antimicrobial susceptibility among Gram-positive organisms collected from pediatric patients globally between 2004 and 2011: results from the Tigecycline Evaluation and Surveillance Trial. Journal of Clinical Microbiology, 51(7), 2371-2378.

44. ALNaayli Haneen Kadhum Abdul-Hamza.(2019).Assessment of Antagonistic effect of some nanoparticles against Biofilm, and Quorum Sensing *Streptococcus agalactiae* isolated from pregnant women in Al –Diwaniyah city, AL -Qadisiyah University ,College of Science.

45. Davidson, R.J.(2019). In vitro activity and pharmacodynamic/ pharmacokinetic parameters of clarithromycinand azithromycin: why they matter in thetreatment of respiratory tract infections. Infection and Drug Resistance .12 :585–596.

46. Varaldo, P.E., Montanari, M.P. and Giovanetti, E., (2009). Genetic elements responsible for erythromycin resistance in streptococci. Antimicrobial agents and chemotherapy, 53(2), pp.343-353.

47. Johnson A P .(2015). Surveillance of antibiotic resistance. Phil. Trans. R. Soc. B 370 (1670), 20140080.

48. Haenni , M.; Lupo ,A.; Madec,J-Y.(2018). Antimicrobial Resistance in Streptococcus spp. ASM Journals/Microbiology Spectrum; Vol. 6, No. DOI: https://doi.org/10.1128/microbiolspec.ARBA-0008-2017.