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Article

Identification and Phenotypic Characterization of Macrolide-Lincosamide-Streptogramin B Resistance in *Streptococcus* Spp. Isolates from Pharyngeal Swabs in Basra, Iraq

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Abstract: Acute pharyngotonsillitis remains a widespread health concern affecting adults and children alike. Effectively treating this condition is increasingly challenged by the emergence of antibiotic-resistant bacteria, particularly among Streptococcus species. This study investigates the prevalence of these pathogens in Basra, Iraq, focusing on their resistance patterns to standard treatments, including the macrolide-lincosamide-streptogramin B (MLSB) class of antibiotics. For this purpose, throat swabs samples were collected from 270 adult patients presenting with symptoms of pharyngitis. The samples were cultured and analyzed using the Vitek system to accurately identify bacterial species and determine their antibiotic susceptibility profiles. Special attention was given to detecting MLSB phenotypes, which indicate resistance to erythromycin and clindamycin. Our findings revealed that bacterial growth was confirmed in 127 cases (47%), with a noticeably higher infection rate observed in women (59%) compared to men. The most frequently isolated pathogen was *Streptococcus parasanguinis* (20.5%), followed closely by Group A *Streptococcus* (S. *pyogenes*, 16.5%). Resistance testing revealed concerning trends: *Streptococcus pyogenes* isolates demonstrated a remarkably high resistance rate to penicillin (90.5%), deviating from global susceptibility norms. Furthermore, a constitutive MLSB resistance pattern where bacteria are resistant to both erythromycin and clindamycin was dominant across several species, including *Streptococcus sanguinis* and *Streptococcus. orails.* In conclusion, this study reveals a notable change in the region's throat infection antibiotic resistance landscape. The prevalence of MLSB phenotypes and the unexpectedly high penicillin resistance indicate that conventional first-line treatments may not be effective, especially for those who are allergic to penicillin. In order to stop the spread of strains that are resistant to multiple drugs, it is imperative that local treatment guidelines be updated and that antibiotic use be closely monitored.

Keywords: Acute Pharyngotonsillitis, *Streptococcus Parasanguinis*, *Streptococcus Pyogenes*, Vitek System

Introduction

Acute pharyngotonsillitis is the most common disease that occurs in both males and females, for adults as well as children, in a high occurrence ratio and this illness contributes to viral infection and bacterial infection in many cases [1]. *Streptococcus* bacteria associated with this illness, especially *Streptococcus pyogenes,* also named Group A *Streptococcus* (GAS) and this *Streptococcus* bacteria had many virulence factors, such as M protein [2], which is considered the primary surface-anchored virulence factor of Group A *Streptococcus* (GAS) and also plays an important role in resisting phagocytosis by human leukocytes [3] and provides a major adhesion able the bacteria to attach to host cells. Production of Streptolysin O (SLO) toxin by Group Group A, C, and G Streptococcus bacteria, notably *S. pyogenes* had effect of cell lysis and damage of several tissues [4].

Another issue contributing to these types of bacterial infections is how to choose the optimal treatment (antibiotics) for the patient that could eliminate the infection and bacterial growth in the body. Many physicians recommend erythromycin and clindamycin for the patients [5]. Erythromycin is prescribed for patients that have a penicillin allergy especially Streptococcus pyogenes infection. Also, clindamycin for the same purpose had a positive effect for the patient by inhibiting bacterial growth and halting the production of toxin formation because its mode of action is attributed to stopping the 50S ribosome subunit from mating with the small 30S subunit and preventing protein production [6]. MLSB resistance is a mechanism of streptococcus and staphylococcus bacteria resistance [7] to three class of antibiotics classes (macrolide, lincosamide and streptogramin B-type antibiotics) due to possessing the erm gene that is responsible for altering the site of these antibiotics binding with ribosomes, which made these antibiotics inactive as a treatment [8]. Using erythromycin and clindamycin together is considered the standard and very important method in medical laboratories for detecting known resistance MLSB phenotype patterns.

MSLB can be classified as cMLSB, which is constitutive macrolide-lincosamide-streptogramin B resistance, while iMLSB can be referred to as susceptibility to clindamycin and resistance to erythromycin [9]. In this study we aimed to identify the causative agent of pharyngotonsillitis in throat swab samples and determine the MLSB bacteria in the streptococcus isolates by using the Vitek system.

Materials and Methods

Sample collection

The 270 throat swabs were taken from adults at three different medical laboratories between February 2024 and December 2024 (Table 1), and the individuals were suffering from symptoms of pharyngitis. To ensure accurate results, the physician carefully swabbed the tonsils and the back of the throat. Each swab was then secured in a sterile transport container and rushed to the laboratory within two hours of collection.

Table 1. Sample types, numbers and percentage.

Type of sample	No.	Percentage (%)
Male	131	48.5
Female	139	51.5
Total	270	100

Bacterial cultivation

The throat swabs that were taken were put on a 5% sheep blood agar plate by streaking them across the surface. The optimal incubation time is 24 hours at 37°C and 5% CO_2 concentration. If bacterial cultures are negative, re-examine after another 24 hours of incubation. Then, all positive growth was identified using the vitek system and AST.

Bacterial identification by vitek system

Identification for positive growth sample was subjected to Gram stain methods for detecting if the bacteria were gram-positive or gram-negative. The positive growth isolates are subjected to the Vitek system in order to identify the bacteria. These procedures were done according to the

manufacturer's instructions, which took isolated colonies from the plate and prepared a 3 ml suspension by adding selected colonies with Vitek sterile saline and checking the turbidity by using the Vitek DensiCheck instrument. Then selected the types of card for identification: the GN card was used for gram-negative bacteria, while the GP card was used to identify the gram-positive bacteria.

AST analysis by vitek system

After performing Gram staining for positive growth isolates on the plate and preparing 3 ml suspension for Vitek identification by using the Densicheck instrument, 280µl volume was transferred to a 3 ml sterile saline tube for AST gram-positive card (AST- ST03) analysis by the Vitek system. detects the antibiotics resistances [10] through MIC assays validate isolates' resistance or susceptibility and determine if bacteria in isolates were MLSB or otherwise.

Statistical analysis

GraphPad Prism software was utilized to identify significant differences at $p<0.05$ (Gharban et al).

Results

Out of 270 throat swab samples taken from adults with symptoms of tonsillitis, only 127 isolates showed growth on the culture plates after being cultured in bacterial growth plates Growth rates were 57.04% for isolates obtained from women and 47.96% for men (Table 2) and The P value results less than 0.05

Table 2. Showed the percentage of positive growth in blood agar.

Type of sample	No.	Percentage (%)
Male	52	40.94
Female	75	59.05
Total	127	100

Bacterial Identification

Pure colonies were taken from culture plates and passed through the Vitek system for identification and antibiotics susceptibility testing. The Vitek reports, after identifying the bacterial species present in each isolate, (Table 3) showed that the highest percentage was for Streptococcus parasanguinis 19.26%, followed by Streptococcus pyogenes 15.56% (Figure1).

Table 3. The percentage of bacterial identification in samples.

Organism	No.	Percentage (%)
Streptococcus parasanguinis	26	20.47
Streptococcus pyogenes	21	16.54
Streptococcus orails	13	10.24
Granulicatella adiacens	10	7.87
Streptococcus thoraltensis	9	7.09
Streptococcus sanguinis	8	6.30
Streptococcus sal.salivarius	6	4.72
Klebsiella pneumoniae	4	3.15
Rothia dentocariosa	3	2.36
Streptococcus viridans .	4	3.15
Streptococcus pneumoniae	9	7.09
Enterococcus spp	3	2.36
Streptococcus mitis	2	1.57
Staphylococcus aureus	2	1.57
Streptococcus mutans	2	1.57
Gardnerella vaginalis	1	0.79
Gemella morbillorum	1	0.79
Entero.casseliflavus	1	0.79

Streptococcus pasteurianus	1	0.79
Streptococcus gallolyticus	1	0.79
Total	**127**	**100**

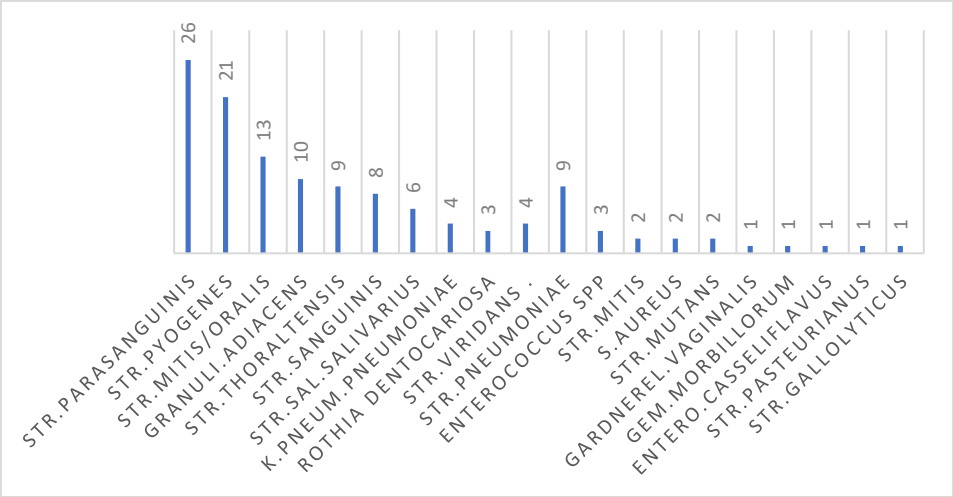

Figure 1. The bacterial Identification Percentage in the Urine samples.

The results of the bacterial diagnosis showed that the percentage of Streptococcus pyogenes diagnosed was 59.20% (Figure 2) of the remaining Streptococcus isolates (Table 4).

Table 4. Showed the Number and Percentage of Streptococcus isolates.

Type of *Streptococcus*	No.	Percentage (%)
Streptococcus parasanguinis	26	25.49
Streptococcus pyogenes	21	20.59
Streptococcus orails	13	12.75
Streptococcus pneumoniae	9	8.82
Streptococcus thoraltensis	9	8.82
Other types of *Streptococcus*	24	23.53
Total	**102**	**100**

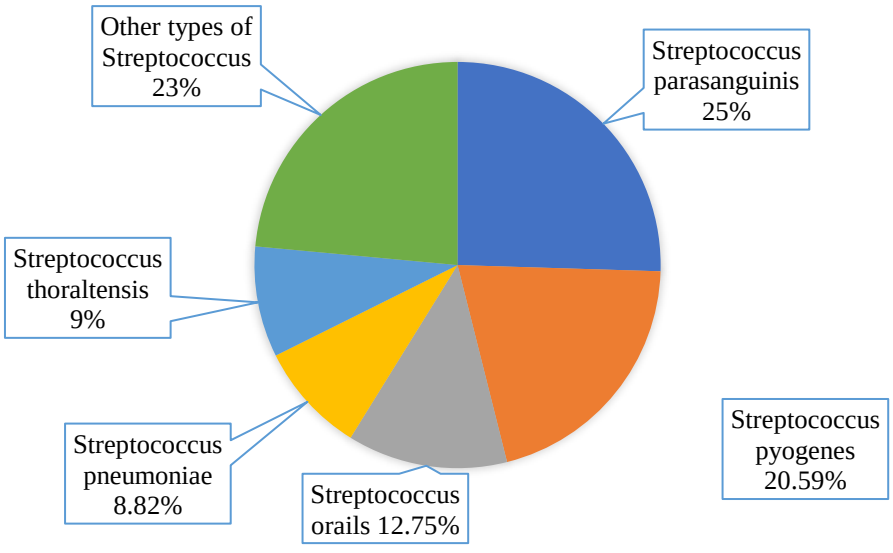

Figure 2. Showed the percentage of *Streptococcus* isolates in this study.

The results of Antibiotics susceptibility testing of the growth-positive isolates showed that a number of isolates exhibited resistance rates of up to 100% to penicillin in Streptococcus mitis and Streptococcus mutans isolates, while Streptococcus pyogenes isolates showed resistance rates of up to 90.50% (Table 5). The P value results less than 0.002.

Table 5. Showed the number and percentage of penicillin-resistant isolates.

Organism name	Number of isolates	Number of penicillin-resistant isolates	Penicillin resistance Percentage (%)
S. orails	13	13	100.00
S. sanguinis	8	7	87.50
S. pyogenes	21	19	90.50
S. oralis	13	13	100
S. mitis	2	2	100
S. smutans	2	2	100
S.viridans	4	3	75
S. salivarius	6	6	100
S. parasanguinis	26	15	60
S. thoraltensis	9	0	0

MSLB Results

The results revealed 100% concordance between resistance to erythromycin and clindamycin in several bacterial species, namely: Streptococcus sanguinis, Streptococcus orails, and Streptococcus salivarius. This complete concordance indicates the dominance of the Continuous MLSB resistance pattern (Table 6). Streptococcus pyogenes showed Majority MSLB phenotype mean Dominant MSLB resistance across the species while Streptococcus viridans showed partial MLSB phenotype it might their resistance due to mixed mechanisms (some MLSB, some Efflux/M-phenotype). The P value results less than 0.005.

Table 6. Showed the MLSB phenotype in study isolates.

Organism name	Total count	Erythromycin resistance	Clindamycin resistance	MLSB phenotype
S. sanguinis	8	8	8	Yes (Complete)
S. orails	13	13	13	Yes (Complete)
S. salivarius	6	6	6	Yes (Complete)
S. pyogenes	21	20	20	Yes (Majority)
S. anginosus	8	7	7	Yes (Majority)
S. parasanguinis	26	0	9	No (CC only)
S. mitis	2	0	2	No (CC only)
S. thoraltensis	9	0	0	No
S. mutans	2	2	2	Yes
S. viridans	4	4	2	Yes (Partial)

CC: Clindamycin resistance only

Discussion

The study results showed a higher number of bacterial isolates in women compared to men. This is attributed to several factors, including those related to female hormones, such as Estrogen , which plays a different role in the immune response compared to men [11]. Another factor is related to the characteristics of the oral and pharyngeal mucosa and the difference in pH between the sexes, which may provide a favorable environment for the growth of this type of bacteria [1]. The use of cosmetics and beauty tools can be contaminated and contribute to the introduction of this type of bacteria into

the oral cavity of women. Another factor is women's close contact with children, as children are reservoirs for this type of bacteria [1].

The diagnostic results, obtained through the Vitek system, showed that Streptococcus parasanguinis was identified at a higher rate than other bacteria in throat samples. This is attributed to several factors, including the bacteria's ability to adhere due to the presence of fimbriae, thus preventing their removal during swallowing [12]. Additionally, biological competition exists, as this bacterium has the capacity to produce H2O2, which negatively impacts the growth of other bacteria, leading to its dominance [13].

The incidence and diagnosis of Streptococcus pyogene bacteria is 16.54%, compared to 20.59% for other Streptococcus species. This bacterium, also known as GAS, is the most important microbe and the primary cause of tonsillitis in adults and children [13]. This is due to its virulence marker, which includes the presence of m protein that protects the bacteria from the immune system. The bacteria also secrete the toxin Streptolysin O (SLO), which causes hemolysis that breakdown of red blood cells [13]. The importance of this bacterium extends beyond the initial tonsillitis; if left untreated, it can lead to various complications, including rheumatic fever and glomerulonephritis [14].

The results of the Antibiotics susceptibility testing for penicillin-resistant Streptococcus pyogenes, showing a resistance rate of 90.50% in the bacterial isolates, are very significant, as global indicators suggest that the bacteria are still susceptible to penicillin [15]. This antibiotic resistance is attributed to significant mutations in (PBPs) and the overuse of antibiotics, which can lead to the emergence of such high-risk resistant Streptococcus stains [16].

The results of the Antibiotic sensitivity Testing of Streptococcus bacteria showed variations in drug resistance levels to both erythromycin and clindamycin, and thus the phenotype of MSLB resistance can be determined. Susceptibility testing revealed a Streptococcus bacteria correlation between resistance to both erythromycin and clindamycin in isolates of Streptococcus sanguinis, Streptococcus orails, and Streptococcus salivarius. These results indicate that these bacteria exhibit a complete concordance indicates the dominance of the Continuous MLSB resistance pattern, and this resistance is attributed to the presence of an erm gene, rendering these bacteria resistant to both erythromycin and clindamycin [7].

On the other hand, Streptococcus pyogenes bacteria have shown high levels of resistance to erythromycin and clindamycin, reaching 90.5%. This poses a public health problem, as erythromycin is the treatment alternative for patients with penicillin allergies, and this pattern of resistance (MLSB resistance) renders other options, such as azithromycin and clindamycin, ineffective [15]. This forces physicians to resort to other antibiotics that require close medical monitoring of patients due to their side effects, such as vancomycin [17], or expensive antibiotics like linezolid, whose overuse can cause thrombocytopenia [18].

Conclusion

The study highlights a significant shift in the antibiotic resistance landscape for throat infections in this region. The unexpected high resistance to penicillin and the prevalence of MLSB phenotypes suggest that traditional first-line treatments may fail, particularly for penicillin-allergic individuals. These findings underscore the urgent need for updated local treatment guidelines and careful monitoring of antibiotic use to prevent the spread of multi-drug resistant strains.

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