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Detection Antibiotic susceptibility and some virulence factors for bacterial isolated from Pharyngitis patients

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ABSTRACT

The third most frequent primary complaint seen in outpatient medical facilities is pharyngitis. The most frequent causes of pharyngitis are usually infectious. This infection may lead to abnormally big tonsils and pharynx, which make it difficult to breathe and swallow. The purpose of this study is to evaluate the effectiveness of pharyngeal swabs for detecting some virulence in patients with pharyngitis, antibiotic susceptibility, and the most prevalent bacterial species that may be isolated from smear samples. In total, 115 throat swab samples were taken from selected patients in the pharyngitis department at Balad hospital. The results show 50 samples show positive growth on the media used, the number of Gram-positive samples are 42(84.0%) while number of Gram-negative samples are 8(16.0%), number of females is 15(30%), and the number of males is 35(70%), with ages ranging 1-69years. After that, samples were sent for culture, sensitivity, and detection of some virulence factors. The results showed that *S.aureus* 28(56%) , *S.pyogenes* 12(24%), *S.epidermidis* 2(4%), *K.Pneumonia* 2(4%), *E.coli* 3(6%), *P.aeruginosa* 3(6%). The sensitivity of the bacterial isolates under study was tested to 11 antibiotics. This study demonstrated that all isolates of *S.aureus* were sensitive to Imipenem, Azithromycin, Chloramphenicol, Gentamycin, and Amikacin, however resistance to Penicillin, Amoxicillin/Clavulanic acid, Ampicillin, Cefotaxime, Amoxicillin, and Tetracycline. *S.pyogenes* were sensitive to Imipenem, Tetracycline, Amoxicillin, Amoxicillin/clavulanic acid, Azithromycin, Chloramphenicol, Gentamycin, and Ampicillin, while resistance to Amikacin, Cefotaxime, *P.aeruginosa* were resistance for all antibiotic except Azithromycin and Imipenem, *E.coli* and *K.pneumonia* were resistance to Tetracycline, Amoxicillin, Ampicillin, Amoxicillin / clavulanic acid, and cefotaxime, *S.epidermidis* were resistance to Ampicillin, Amoxicillin, Amoxicillin/Clavulanic acid, Gentamycin, and Tetracycline . The results showed that *S. aureus* produced four virulence factors: hemolysin, Protease, lecithinase, and DNase. While it was *S.pyogenes* possesses three virulence factors hemolysin, DNase, and Protease, *P.aeruginosa* possess hemolysin, protease, and lecithinase, *S.epidermidis* and *K.pneumonia* possess only lecithinase, *E.coli* only hemolysin .

Key words: Pharyngitis, *S.aureus*, *S.pyogenes*, *K.pneumonia*, *E.coli*

Introduction

Inflammation or irritation of the posterior oropharynx is represented by pharyngitis, most popularly known as a sore throat. While non-infectious or non-bacterial causes account for the majority of instances, a sizable part of pharyngeal infections are caused by bacteria. These infections appear differently, have distinct consequences, and require different treatments [1].

Any front-line or specialized physician must be proficient at recognizing bacterial pharyngitis and know when to screen for its more unusual origins to prevent both needless therapy and the morbidity brought on by missed or inappropriately treated infection [2]. Both children and adults can have acute pharyngitis, which often results from non-infectious (such as seasonal allergies and acid reflux) or non-bacterial (such as viral) reasons [1].

However, when it comes to germs, group A beta-hemolytic Streptococcus (GABHS), often known as strep throat, is the most prevalent cause of pharyngitis.[3] GABHS is spread by ingestion or airborne transmission and is linked to immediate and delayed problems, which will be discussed more in this article. Other bacteria may also cause pharyngitis, including Streptococcus group C and G, *Staphylococcus aureus*, *E. coli*, *Klebsiella ssp*, *Proteus*, *Pseudomonas aeruginosa* [4,5]. *Arcanobacterium haemolyticum* is an uncommon cause of pharyngitis, and *Neisseria gonorrhoeae* can cause acute pharyngitis in sexually active teens. Other bacteria, such as *Francisella tularensis*, *Yersinia enterocolitica*, *Corynebacterium diphtheria*, and combination infections with anaerobic bacteria, are uncommon causes. *Chlamydomphila pneumonia*

and *mycoplasma pneumonia* have been linked to pneumonia in humans on rare occasions.

The causative agent of *Lemierre syndrome*, *Fusobacterium necrophorum*, can also cause uncomplicated pharyngitis [6]. A virus that several diseases may produce causes about 70% of pharyngitis or sore throat, among which Rhinoviruses, Coronaviruses, Adenoviruses, Influenza, and Parainfluenza viruses are more common, Respiratory syntactical viruses, Herpes Simplex viruses (type1 and type2), enteroviruses, Cocksackie viruses, Epstein-Barr viruses, Cytomegaloviruses, Human immunodeficiency virus (HIV) rarely occur [3,7]

Materials and Methods

A total 115 throat swab samples were collected from symptomatic cases of both sexes (male &female) and ages 1-69. These patients were admitted to Balad hospital during the period 1/11/2021 until 25/3/2022, and the samples are transferred directly to the microbiology laboratory, specimens are planted on Blood agar, MacConkey agar, Nutrient agar and Mannitol salt agar for growth bacteria species, and incubated at 37C° for 18-24hours, to explore bacterial species linked with pharyngitis, the bacteria were diagnosis through phenotypic and microscopic examination and through biochemical tests that included (xidase, Coagulase, Catalase, IMVIC test)[8,9]. The diagnosis is confirmed by using VITKE2 system. Use this device to confirm the diagnosis most types after diagnosing them using the usual methods, and investigation the production some virulence factors by using suitable medium. The ability of the isolates to analyze red blood cells, analyze proteins, lecithin and DNase was tested using assay [11,12].

antibiotic sensitivity test was carried out using 11 types of antibiotics for Gram-positive and Gram-negative bacterial isolates under study on Muller Hinton-agar medium according to the Kirby-bauer method described by the World Health Organization [10].

Results & Discussions

115 throat swab samples were collected from patients confirmed to be infected with pharyngitis. It found that 50 samples showed positive growth, with the number of males 35(70%) and 15(30%), the number of Gram-positive samples are 42(84.0%) while number of Gram-negative samples are 8(16.0%). The age group (less than10 years) recorded the highest infection rates with pharyngitis (50%). At the same time, the lowest infection rate for pharyngitis was noticed in the age group (40-69). This may be explained may be due to the immaturity of the immune system and evolution of young people compared with adults, or that adults acquired immunity due to their exposure to infections in various stages of their lives. In addition to these changes, children in this age group are subjected to various diseases such as influenza, measles, and other

diseases that could lead to a weak immune system. The result of the present study was consistent with the results of [13,14] which found that the age group (1-10 years) recorded the highest infection rates.

The isolates are distributed into 42(84.0%) belonging to Gram-positive bacteria and 8 (16.0%) belonging to Gram-negative bacteria. Table (1) show bacteria isolates from pharyngitis patients. Of which 28(56%) isolates were *S.aureus*, 12(24%) isolates were *S.pyogenes*, *P.aeruginosa* 3(6%) isolates, *S.epidermidis* 2(4%) isolates, *E.coli* 3(6%), *K.pneumonia* 2(4%) isolates.

Table 1: Bacterial species isolated from pharyngitis patients

Bacterial isolates	Number	Percentage %
<i>Staphylococcus aureus</i>	28	56%
<i>Streptococcus pyogenes</i>	12	24%
<i>Staphylococcus epidermidis</i>	2	4%
<i>Klebsiella pneumonia</i>	2	4%
<i>Escherichia coli</i>	3	6%
<i>Pseudomonas aeruginosa</i>	3	6%
Total	50	%100

The results show that 28 isolates of *S.aureus* with a percentage (56%) were the most frequent bacteria among isolated bacteria. *S.aureus* is one of the most common and virulent causes of pharyngitis. It may exist on the skin, nose, and throat as a commensal organism. *S.aureus*, which persistently colonizes the anterior nares in 10%–20% of the population and sporadically colonizes 30%–50% of the population, infects around 30% of healthy individuals without any symptoms. The remaining population is never colonized. Notably, this colonization is a recognized infection risk factor [15,16,17]. The present study results agreed with [18], who demonstrated that *S.aureus* is the principal causative agent of pharyngitis with a percentage (50.9%), [19,20] who founded *S.aureus* were is one causative agent of pharyngitis with percentage 29% and 60%, respectively.

As for *S.pyogenes* showed that 12(24%) isolates from these bacteria. The only agent that needs an etiologic diagnosis and a particular course of therapy is *S.pyogenes*, (commonly known as Lancefield group A -hemolytic streptococci) Because they can induce post-infection systemic problems, post-Streptococcal glomerulonephritis, and severe rheumatic fever, which happen 1-3 weeks after the pharyngeal infection, these bacteria have major clinical value[21]. These results are close to what was found [22,5] who demonstrated that *S.pyogenes* is the principle causative agent of pharyngitis with a percentage of 61.76% and 25%, respectively.

To determine the antibiotic susceptibility of bacterial isolates in this study, 11 antibiotics were tested due to [23], and the results of this study found that most isolates of bacteria from pharyngitis infection (Table 2). All 28 *S.aureus* isolates were subjected susceptibility test against different antibiotic that

were used in the present study. *S.aureus* the results showed that these bacteria were resistance to Cephalosporins. *S.aureus* were resistance to Cefotaxime. Bacteria develop resistance to cephalosporins by altering the target protein of cephalosporins, which reduces the affinity of the protein to bind to cephalosporins or by changing the permeability of the bacteria to antibiotics or by producing the enzyme beta-lactamase that destroys the drug structure [24]. These results were compatible with [25] reported that these bacteria were resistance to Cefotaxime 88.9%. Penicillin group *S.aureus* were resistance to Amoxicillin. These result was agreed with [26,27] demonstrated that *S.aureus* were resistance to Amoxicillin and Amoxicillin/clavulanic acid.

Effectively of Augmentin explained by the structure of this antibiotic which is a mixture of amoxicillin and clavulanic acid, useful for the treatment of a number of bacterial infections. This combination results in an antibiotic with an increased spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β -lactamase amoxicillin to affect the synthesis of the cell wall of the bacteria either clavulanic acid, it destroys the enzyme penicillin which found that nullifies the effect of the antibiotic used to therapy of infections [28]. Many bacteria can develop resistance to penicillin, the most important type of resistance is the production of penicillinase, a narrow-acting beta-lactamase enzyme that break the beta-lactam ring and inhibits penicillin, and most staphylococci can produce this enzyme [24]. Aminoglycoside *S.aureus* were sensitive to Amikacin and Gentamycin. These results were consistent with [29,30] who recorded that (100%) of isolates were more sensitive to Amikacin. The higher sensitivity rates of *S.aureus* isolates to Amikacin can be explained by the structure and work mechanism of this antibiotic, which is an aminoglycoside antibiotic used to treat different types of bacterial infections such as hospital-acquired infections. Its works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth, [31] reported that these bacteria were sensitive to Gentamycin 100%. Macrolide group *S.aureus* were sensitive to Azithromycin. This result was agreed with [18] observed that these bacteria were sensitive 94.4%.

Tetracycline group *S.aureus* were resistance to this antibiotic. Tetracyclines attach to the 30S subunit and prevent it from synthesizing proteins. Tetracycline resistance occurs via two distinct mechanisms: first, active drug efflux encoded by plasmid-borne genes tet(K) and tet(L), and second, ribosome protection, the genes tetO/M that encode the tetracycline target site [32]. [26] show that isolates of *S.aureus* were resistance for tetracycline 62%. Chloramphenicol group *S.aureus* were sensitive to Chloramphenicol. These outcomes closely match what was found [33]

founded that these bacteria were sensitive to Chloramphenicol 92.9%. Carbapenem *S.aureus* were sensitive to Imipenem. This result was compatible with [34] observed that these bacteria were sensitive to imipenem 98%.

Streptococcus pyogenes the results showed that these bacteria were sensitive to Penicillin and Cephalosporin group included, Penicillin 83.3%, Amoxicillin/Clavulanic acid 75%, Amoxicillin 75%, Ampicillin 58.3% and were resistance to cefotaxime 66.7%, these results were compatible with [33,35,31] who recorded that these bacteria were sensitive for penicillin 98%, Amoxicillin/calavulanic acid 100%, and Ampicillin 100%, respectively, [36] mentioned that these bacteria were resistance to cefotaxime with percentage 79%. As for Aminoglycoside *S.pyogenes* were sensitive to gentamycin 83.3%, these result was agreed with [37] reported that these bacteria were sensitive to Gentamycin 100%, while Amikacin were *S.pyogenes* resistance.

Aminoglycoside-modifying enzymes (AMEs) are frequently responsible for the enzymatic inactivation of aminoglycosides that completely abolishes synergistic bactericidal action, whereas ribosomal changes are a less frequent mechanism for high-level resistance to aminoglycosides (MICs >2,000 g/ml) [38]. Be note that plasmids are frequently where the genes that encode for AMEs are found. The three kinds of AMEs—aminoglycoside acetyltransferases (AACs), aminoglycoside phosphotransferases (APHs), and aminoglycoside nucleotide transferases—depend on the process they catalyze (ANTs) [39]. This result was consistent with [40] showed that 60% of isolates were resistance to Amikacin.

Macrolides *S.pyogenes* were sensitive to Azithromycin. This result was consistent with the [35,31] demonstrated that these bacteria were sensitive to Azithromycin 63.3% and 100%, respectively. Chloramphenicol group *S.pyogenes* were sensitive to Chloramphenicol. These result was compatible with [33,36] observed that *S.pyogenes* were sensitive to Chloramphenicol 95% and 93%, respectively. Tetracycline group *S.pyogenes* were sensitive to Tetracycline. This result was agreed with [35] Who founded that these bacteria were sensitive to tetracycline 40%. Carbapenems group *S.pyogenes* were sensitive to imipenem.

P.aeruginosa results showed that these bacteria were sensitive to Imipenem and Azithromycin. These results were agreed with [4], who found *P.aeruginosa* was sensitive to imipenem 100%. While [41] found that *P.aeruginosa* was sensitive to Azithromycin 72%. In contrast, these bacteria were resistant to Tetracycline 100%, Amoxicillin 100%, Cefotaxime 100%, Ampicillin 100%, Amoxicillin / Clavulanic acid 100%, Amikacin 100%, Penicillin 100%, Gentamycin 100%, and Chloramphenicol 66.7%. The present study results agreed with (Sadoh *et al.*, 2009), who found that all

P.aeruginosa isolates were resistant to Amoxicillin 100% and Ampicillin 100%. Also, [4] found that the resistance of isolates to Gentamycin was 100%.

Klebsiella Pneumonia the present study results showed these bacteria were sensitive to Imipenem, Gentamycin, Penicillin, Amikacin, and Azithromycin. These results were consistent with [18] reported that these were sensitive to Gentamycin 83.3%, Azithromycin 100%, and Chloramphenicol 100%. *Klebsiella Pneumonia* was ultimately resistant to Amoxicillin/Clavulanic acid 100%, Ampicillin 100%, Cefotaxime 100%, Amoxicillin 100%, and Tetracycline 100%, while Amikacin and Chloramphenicol were 50%. The present study's results [42] found that these bacteria are resistant to Amoxicillin 81.8%, Ampicillin 93.9%, and Cefotaxime 57.6%.

E. coli the current study demonstrated that these bacteria were sensitive to Imipenem, Gentamycin, Chloramphenicol, Azithromycin, Amikacin, and Penicillin. These outcomes closely match what was found [43] reported that these bacteria were sensitive to Gentamycin 100%. *E. coli* were resistant to Tetracycline, Amoxicillin, Cefotaxime, Ampicillin, and Amoxicillin/Clavulanic acid. These results, compatible with [44], demonstrated that these bacteria were resistant to Tetracycline, Amoxicillin, Gentamycin, and Amoxicillin/Clavulanic acid.

S.epidermidis, the present study showed these bacteria were sensitive to Azithromycin 100%, Penicillin 100%, Amikacin 100%, Chloramphenicol 100%, Imipenem 100%, and Cefotaxime 50%. The result was consistent with those [45] mentioned that these bacteria were sensitive to Amikacin 83.87%, Imipenem 96.77%, Cefotaxime 65%, and Azithromycin 50%. *S.epidermidis* were resistant to Gentamycin 100%, Amoxicillin/Clavulanic acid 100%, Ampicillin 100%, Amoxicillin 100%, and Tetracycline 100%. These results are compatible with those [46] who found that these bacteria were resistant to Penicillin 95.65% and Tetracycline 91.30%.

Hemolysin is a virulence factor possessed by bacteria and can destroy the cell membrane of red blood cells [47]. The source of red blood cells employed in the medium used to test the bacteria's capacity to make hemolysin is the most significant of numerous variables that affect the ability of bacteria to produce hemolysin. It is also impacted by the test technique and the blood's serum and cholesterol levels, both of which cause hemolysis to be inhibited [48]. The current study results showed *S.aureus* 92.9%, *S.pyogenes* 100%, *P.aeruginosa* 100%, *E.coli* 66.7% (Table 2). These results were agreed with [49,40,50] reported that these bacteria were produce for hemolysin.

An enzyme known as a protease, also known as a peptidase or proteinase, catalyzes (increases reaction rate or "speeds up") proteolysis, which causes proteins to be broken down into smaller polypeptides or just single amino acids, resulting in the formation of new protein products [51]. They do this by cleaving the peptide bonds contained in proteins by hydrolysis, a process in which water destroys bonds. Proteases play a role in a variety of biological activities, including cell signaling, protein catabolism (the breakdown of aging proteins), and post-ingestion protein digestion [52,53]. The present study results showed *S.aureus* 71.4%, *S.pyogenes* 83.3% Table (2). These results were compatible with [54,55] mentioned that those bacteria were produce for protease. Extracellular DNases / nucleases, produced by a variety of bacteria, are necessary for virulence, biofilm formation, extracellular DNA use as a nutrient, and (NETs) [56]. The current study results showed *S.aureus* 96%, *S.pyogenes* 91.7% and *P.aeruginosa* 100%, Table (2). these results were consistent with [57,58, 59].

The cell membrane comprises phospholipid lecithin, which is affected by this enzyme. This enzyme breaks down lecithin into two components (diglyceride and phosphorylcholine), which causes the membrane to perforate. Any membrane flaw causes the lysis of cells, including red blood cells, myocytes, fibroblasts, Platelets, and leukocytes [60]. The present study showed *S. aureus* 96.4%, *P.aeruginosa* 100%, and *S.epidermidis* 50% and *K.pneumonia* 60% Table (2). These results agreed with [61,62,63].

Table 2: shows bacteria that produce virulence factors

Bacteria	Hemolysin	Protease	DNase	Lecithinase
<i>S.aureus</i>	92.9%	71.4%	96%	96.4%
<i>S.Pyogenes</i>	100%	83.3%	91.7%	-
<i>S.epidermidis</i>	-	-	-	%50
<i>K.pneumonia</i>	-	-	-	%60
<i>P.aeruginosa</i>	100%	%100	-	100%
<i>E. coli</i>	%66	-	-	-

Recommendations and Conclusions

This present study showed that *Staphylococcus aureus* was the commonest microorganism followed *Streptococcus pyogenes* in patients. Where it was Imipenem, Chloramphenicol, Azithromycin, Gentamycin more effectiveness for these bacteria. However, *S.aureus* and *S.pyogenes* can produce multiple virulence factors such as hemolysin, protease, DNase, and lecithinase. It is recommended to conduct extensive survey research to find other pathogenic species in Pharyngitis, additional research on various virulence factors may be accountable for the pathogenicity of bacteria, and study the relationship of multiple antibiotic resistance to forming virulence factors.

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الكشف عن الحساسية للمضادات الحيوية وبعض عوامل الضراوة للبكتيريا المعزولة من المرضى المصابين بالتهاب البلعوم

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الملخص

الشكوى الأولية الثالثة الأكثر شيوعاً في العيادات الخارجية هي التهاب البلعوم. عادة ما تكون الأسباب الأكثر شيوعاً لالتهاب البلعوم معدية. قد تؤدي هذه العدوى إلى تضخم اللوزتين والبلعوم بشكل غير طبيعي، مما يجعل التنفس والبلع صعباً. الغرض من هذه الدراسة هو تقييم فعالية مسحات البلعوم في الكشف عن بعض الفوعة في مرضى التهاب البلعوم، وقابلية المضادات الحيوية، وأكثر أنواع البكتيريا انتشاراً التي يمكن عزلها من عينات المسحات. في المجموع، تم أخذ 115 عينة مسحة من الحلق من مرضى مختارين في قسم التهاب البلعوم في مستشفى بلد. أظهرت النتائج أن 50 عينة أظهرت نمواً إيجابياً في الوسط المستخدم، وبلغ عدد العينات الموجبة للجرام 42 (84.0%) بينما عدد العينات سالبة الجرام 8 (16.0%)، وعدد الإناث 15 (30%)، وبلغ عدد الذكور 35 (70%) وتتراوح أعمارهم بين 1-69 سنة. بعد ذلك، تم إرسال عينات للزرع والحساسية والكشف عن بعض عوامل الضراوة. أظهرت النتائج أن بكتيريا المكورات العنقودية الذهبية كانت 56 (28%)، البكتيرية العقدية المقيحة 24 (12%)، البكتيرية العنقودية البشرية 4 (2%)، الكلبسيلا الرئوية 4 (2%)، الإشريكية القولونية 6 (3%)، الزائفة الزنجارية 3 (6%). اختبرت حساسية العزلات البكتيرية قيد الدراسة لـ 11 مضاد حيوي. أظهرت هذه الدراسة أن جميع عزلات بكتيريا المكورة العنقودية الذهبية كانت حساسة لكل من Imipenem، Azithromycin، Chloramphenicol، Gentamycin، Amikacin، ولكن مقاومة Penicillin، كانت البكتيرية العقدية المقيحة حساسة لكل من Imipenem وTetracycline وAmoxicillin / clavulanic acid وAmoxicillin وChloramphenicol وAzithromycin وGentamycin وAmpicillin، بينما كانت مقاومة Amikacin وCefotaxime والزائفة الزنجارية مقاومة لجميع المضادات الحيوية باستثناء Imipenem وAzithromycin. كانت الكلبسيلا الرئوية والإشريكية القولونية مقاومة Ampicillin، Amoxicillin، Tetracycline، Amoxicillin/clavulanic acid، Cefotaxime والبكتيرية العنقودية البشرية كانت مقاومة Amoxicillin/clavulanic acid، Hemolysin، Protease، Lecithinase، وDNase. في حين أن المكورات العقدية المقيحة تمتلك ثلاثة عوامل ضراوة هي: Hemolysin، Protease، وDNase، تمتلك الزائفة الزنجارية Hemolysin وProtease وLecithinase، تمتلك البكتيرية العنقودية البشرية والكلبسيلا الرئوية فقط Lecithinase والإشريكية القولونية فقط Hemolysin