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# Isolation and identification of *Streptococcus pneumoniae* that causes Respiratory Diseases

Shaimaa Habbaib Hassan Al-Jowrani *[1a] and Hanaa Daaj Khalaf Al-Mozan[1b]

*Department of Biology, College of Science, University of Thi-Qar , Thi-Qar,Iraq.*

[b]E-mail: hanaa.d@sci.utq.edu.iq

[a*]Corresponding author: shaymaa.hasan@utq.edu.iq

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***Abstract—*** ***Streptococcus pneumoniae*** **is one of the bacterial species that colonize the respiratory system, and it causes many respiratory diseases, including pneumonia. Sputum specimens were collected from 270 patients of all ages suffering from respiratory diseases at the Consultants Clinic for Respiratory and Chest Diseases, Al-Hussein Teaching Hospital, and Public Health Laboratory in Thi-Qar Governorate during the period from October 2024 to March 2025.The aim in this study is the isolation and identification of** ***Streptococcus pneumoniae*****, and morphological and (conventional and advanced) biochemical tests were performed. Results showed that** ***Streptococcus pneumoniae*** **isolates that cause respiratory diseases were (3.72%).** ***Streptococcus pneumonia*****e had high rates of resistance to some antibiotics.This study showed that vancomycin is the best to treat** ***Streptococcus pneumoniae*****, but** ***S. pneumoniae*** **is characterized by high resistance to tetracycline.**

***Keywords—*** ***Streptococcus pneumoniae*****, Antibiotic resistance , VITEK 2 System , API 20 strep.System.**

## I .INTRODUCTION

Respiratory infections are among the most significant causes of illness worldwide. With the presence of the COVID-19 pandemic, there has been a high interest to identify the causes of respiratory infections [1]. Respiratory tract infections may be increased by viral co-infection or environmental stressors [2].

The main causes of acute respiratory diseases are bacteria such as *Streptococcus pneumoniae* [3]. *Streptococcus pneumoniae* (pneumococcus) is a pathogen of human respiratory diseases and a leading cause of illness and death worldwide. *Streptococcus pneumoniae* is commensal bacteria that colonize the nasopharynx , however it can also cause fatal diseases such as meningitis, sepsis, and pneumonia, particularly in immunocompromised patients, the elderly, and young children [4].

Morphological and (routine and advanced) biochemical tests were used to bacterial diagnosis.Gram staining and sputum culture are the first diagnostic steps for identifying *Streptococcus Pneumoniae* [5-7].Routine tests are the basic tests used to diagnose bacteria, which include Gram staining, the optochin test, and the bile salts test. However, these tests require a lot of effort and time compared to advanced tests, including API and VITK 2 , which are faster and more accurate.

*Streptococcus pneumoniae* is becoming increasingly resistant to commonly used antibiotics.Antibiotic resistance is a widespread problem that significantly contributes to the spread of disease.Multidrug-resistant (MDR) bacteria is bacterial resistance to more than three of antibiotics [8-10].

## II. METHODS

### *A .Collection of Specimens*

Two hundred and seventy sputum specimens were collected from patients with respiratory diseases from Consultants Clinic for Respiratory and Chest Diseases, Al-Hussein Teaching Hospital, and the Public Health Laboratory in Thi-Qar Governorate during the period from October 2024 to March 2025. The specimens were collected directly using Transport Swab media and transferred to the laboratory at Al-Hussein Teaching Hospital.

### *B .Culturing the Specimens*

Sputum specimens were incubated in brain heart infusion broth (BHI) at 37°C for 24 hours to activate bacteria, and they were cultured directly on enrichment medium using the planning method such as blood agar, chocolate agar with nystatin, an antifungal, added to the


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medium to prevent fungal growth and facilitate bacterial isolation. Plates were incubated in (5-10)% $CO_2$. Subculturing was repeated several times to purify bacterial isolates. The diagnosis was made by tracing bacterial morphology and using routine and advanced biochemical tests.

*C .Microscopic Examination*

Under sterile conditions, one or two colonies were transferred from the agar surface and stain by Gram stain pursuant to scientific procedures.

*D .Biochemical Tests*

Biochemical methods are used to identify bacteria. The most important of these tests are:

1. *Catalase Test*

This test was performed by adding drops of catalase reagent to a single colony on a glass slide. The appearance of gas bubbles indicates a positive result.

2 . *Optochin Sensitivity Test*

*Streptococcus pneumoniae* is the only known streptococci sensitive to optochin. Based on this feature, *S. pneumoniae* can be easily differentiated from other streptococci.

3. *Bile Solubility Test*

The bile salt test is the test that differentiates between *Streptococcus pneumoniae* and other streptococci. *Streptococcus pneumoniae* is soluble in bile while all other streptococci are insoluble.

*E.API 20 strep. System*

This test was performed according to Leboffe and Pierce [11] and it is used for the rapid identification of bacterial isolates. Each of the test's 25 plastic strips contains 20 small tubes with top and bottom openings that contain dry material representing the biochemical test. Where changes occur in the colors present in the tubes after incubation or after adding reagents, these colors indicate the presence or absence of chemical activity, thus indicating a positive or negative result.

*F. Detection of S. pneumoniae by VITEK ® 2 System*

For detection of the isolates, isolates were cultured on chocolate agar and it is analyzed using VITEk® 2 system according to the company's instructions [12].

*G. Antibiotics Susceptibility Test*

Antibiotic susceptibility test was performed using the disc diffusion method according to standards of the Clinical Laboratory Standards Institute (CLSI).The antibiotic susceptibility of *Streptococcus pneumoniae* isolates was done to some antibiotic discs. Antibiotic discs were used in the study shown in Table 1.

TABLE 1. Antibiotic discs used in this study.

| **No.** | **Antibiotics** | **Concentration µg** | **Zone diameter** | | |
|---|---|---|---|---|---|
| | | | **S** | **I** | **R** |
| 1 | Tetracycline (TE) | 10 | ≥ 28 | 25-27 | ≤ 24 |
| 2 | Erythromycin (E) | 15 | ≥ 21 | 16-20 | ≤ 15 |
| 3 | Penicillin (P) | 10 | ≥ 20 | - | - |
| 4 | Ceftriaxone (CTR) | 30 | ≥ 27 | 25-26 | ≤ 24 |
| 5 | Vancomycin (VA) | 30 | ≥ 17 | - | - |
| 6 | Azithromycin (AT) | 15 | ≥ 18 | 14-17 | ≤ 13 |

## III. RESULTS AND DISCUSSION

*A. Isolation and Identification of Streptococcus Pneumoniae*

A total of 270 sputum sample , only 10 samples (3.71%) were isolated , the proportion of *Streptococcus Pneumoniae* bacterium in this study consistent to [13] , who isolated 37 (6.7%) of the 558 sputum samples, and this disagree with [14], where the percentage was (40%) of *Streptococcus Pneumoniae.* This may be because the outbreak of the COVID-19 pandemic, has led to an increase in the spread of *Streptococcus pneumoniae* bacteria during coinfection with the virus.

*B. .Morphological Properties*

*Streptococcus pneumoniae* colonies appeared on chocolate agar with a yellow color and surrounded by a green color Fig.1

Fig.1. *Streptococcus pneumoniae* on chocolate agar.

*B . Microscopic Examination*

This test has shown *that S. pneumoniae* was Gram-positive, ovoid or lancet-shaped, typically in pairs or short chains Fig. 2.

Fig.2. Staining of *S. pneumoniae* with Gram stain.

Gram staining can differentiate between Gram-positive and Gram-negative bacteria based on differential crystal violet staining. The cell walls of Gram-positive organisms retain this compound after treatment with alcohol and appear purple, while Gram-negative organisms lose their color after this treatment and appear pink [15].

*C . Biochemical Tests*

There is a difference between routine and advanced tests. Table 1 shows the results of routine and advanced biochemical tests.

TABLE 2. Comparison between routine and advanced biochemical tests

| **Routine biochemical tests** | **Advanced biochemical tests** | |
|---|---|---|
| | **Api 20 Strep** | ***VITEK*® *2 system*** |
| **110** | **20** | **10** |

The results are consistent with [5],[6], where they were found the same difference. This is due to routine tests taking a long time and they are performed at different times, therefore they may be exposed to different conditions where they are susceptible to contamination that gives false positive results, while the API and VITEK ® system are rapid and advanced tests that are performed simultaneously.

*Streptococcus pneumoniae* has the ability to dissolve in bile salts due to the presence of an autolysin, which breaks the cross-linking of the cell wall and peptidoglycan, leading to bacterial lysis [17].

1. Catalase Test

The result of this test has shown that *S. pneumoniae* was catalase negative.

2 .Optochin Sensitivity Test

This test has shown that *Streptococcus pneumoniae* is sensitive to optochin Fig. 3.

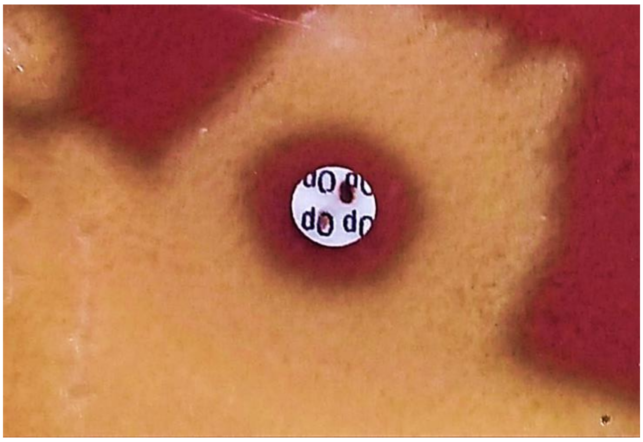

Fig.3. Susceptibility of *Streptococcus pneumoniae* to optochin.

*Streptococcus pneumoniae* can be distinguished from other alpha-hemolytic streptococci based on their sensitivity to optochin. This agent activates an autolytic enzyme in *S. pneumoniae* that degrades peptidoglycan [16].

3 . Bile Solubility Test

This test has shown *Streptococcus pneumoniae* is positive for bile solubility Fig. 4.

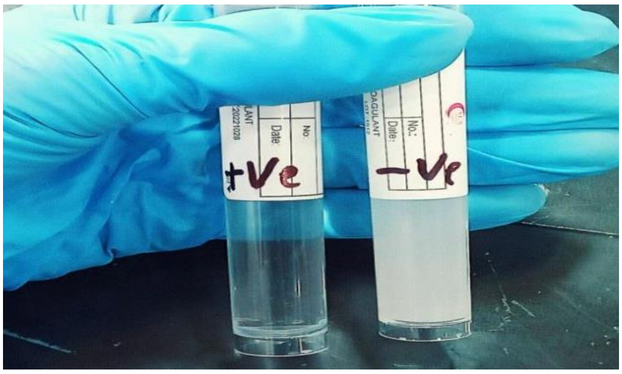


Fig. 4. Bile solubility test.

*D .Api 20 Strep. System*

The analytical profile index for *Streptococcus* was used to identify *S. pneumoniae* isolates Fig. 5.

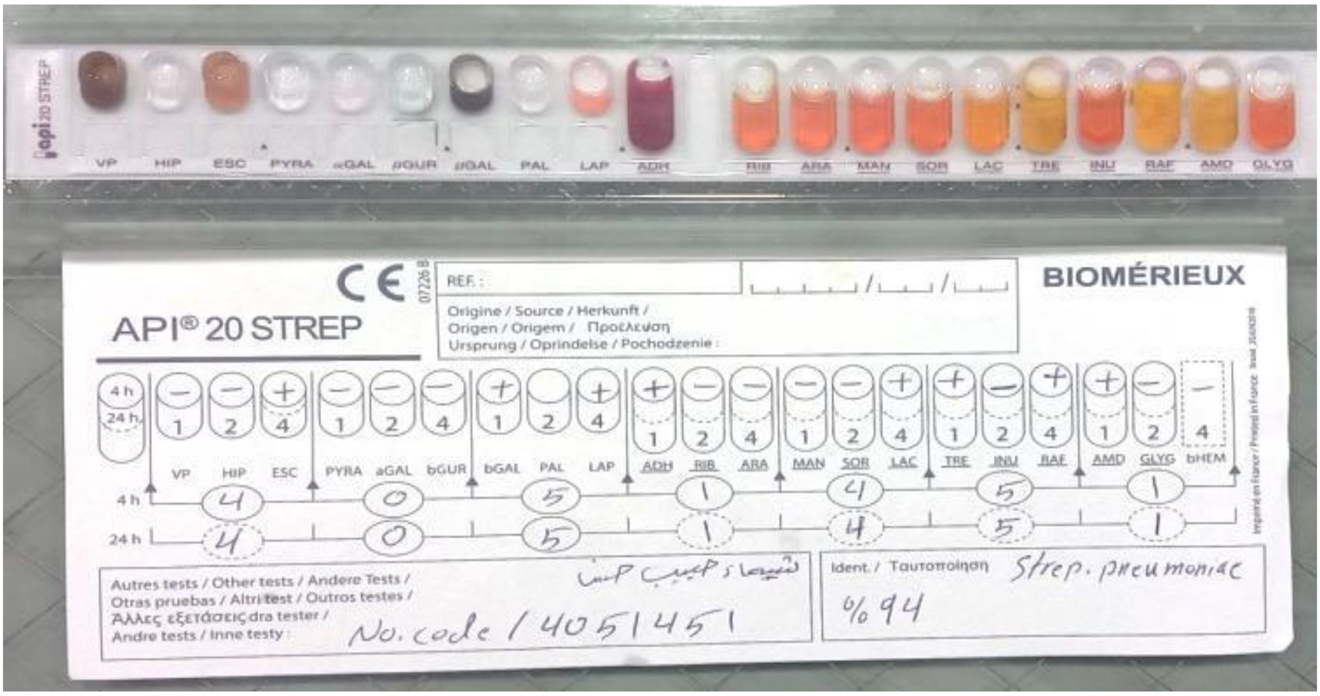


Fig. 5. API-20 Strep. Test of *Streptococcus pneumoniae*.

The first half of the strip, which starts from VP and ends with LAP, showed that only ESC, bGAL, and LAP gave a positive result. The second half of the strip, which starts from ADH and ends with GLYG, which are sugars, and oil is added to provide anaerobic conditions for fermentation, showed positive results for ADH, LAC, TRE, RAF, and AMD.

*E. Detection of S. pneumoniae by VITEK ® 2 System*

Tests of the Vitek® 2 system were used to identify these bacteria, and the test revealed that all isolates were *Streptococcus pneumoniae*.

*2)Multi-drug Resistance Pattern of Streptococcus pneumoniae*

This study showed that all *Streptococcus pneumoniae* were sensitive to vancomycin, which is consistent with [18]. This may be due to its antibacterial efficacy and ability to treat severe infections caused by bacteria [19]. High rates of resistance were found to tetracycline (100%), erythromycin (80%), and Penicillin (70%), which disagrees with [20], [18], where Tetracycline (78.7%), Erythromycin (96.6%), and Penicillin (98.5%). The overuse of antibiotics is considered an important cause and a major factor in the emergence of resistance [21]. *Streptococcus pneumonia*e isolates resistance to Ceftriaxone (50 %)is less than the percentage (67.6%) reported by [22]. Resistance of *Streptococcus pneumonia*e to azithromycin is 20%, which is inconsistent with the results of [23]. which found azithromycin resistance at 87.5%. This resistance may be attributed to the indiscriminate use of antibiotics during the COVID-19 pandemic, which has led to an increase in antibiotic. The results of this study may be indicated that overuse of unspecified drugs increases bacterial resistance to antibiotics Table 2 and Fig .7.

TABLE .3. Percentage of *Streptococcus pneumoniae* resistant to some antibiotics.

| Antibiotics | R | | I | | S | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| **Tetracycline** | 10 | 100% | 0 | 0.0 | 0 | 0.0 |
| **Erythromycin** | 8 | 80% | 0 | 0.0 | 2 | 20% |
| **Ceftriaxone** | 5 | 50% | 0 | 0.0 | 5 | 50% |
| **Penicillin** | 7 | 70% | 0 | 0.0 | 3 | 30% |
| **Vancomycin** | 1 | 10% | 0 | 0.0 | 9 | 90% |
| **Azithromycin** | 2 | 20% | 0 | 0.0 | 8 | 80% |

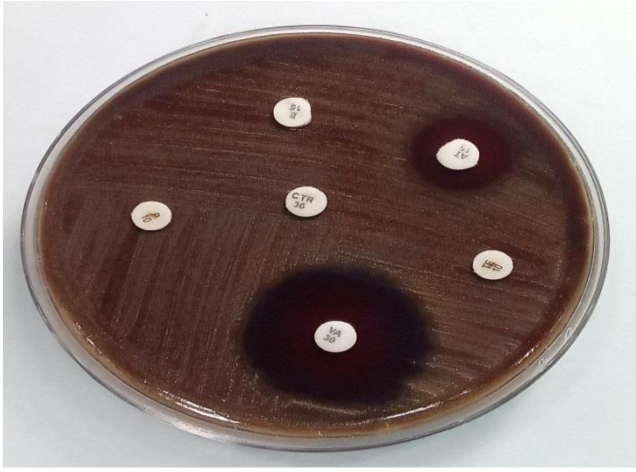

Fig.7. Susceptibility of *S. pneumoniae* to some antibiotics

## IV. CONCLUSION

*Streptococcus pneumoniae* is a significant cause of respiratory diseases. This study showed that vancomycin is best for treating respiratory diseases; however, S. pneumoniae is characterized by high resistance to tetracycline.

## CONFLICT OF INTEREST

Author declares that he has no conflict of interest.

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