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Conventional Phenotypic Identification and Serotype Characterization of Streptococcus Pneumoniae in Respiratory Specimens from Baghdad, Iraq

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Abstract

Background: *Streptococcus pneumoniae* remains an important bacterial cause of lower respiratory tract infection. Although molecular methods are becoming more available, many hospital microbiology laboratories in Iraq still depend on conventional phenotypic identification. So, this workflow is still not old practice only; it is part of the daily diagnostic work. ***Objective:*** To assess the practical diagnostic value of conventional phenotypic methods for identifying *S. pneumoniae* from respiratory specimens in a routine clinical microbiology laboratory in Baghdad, Iraq. ***Methods:*** This prospective, single-center, laboratory-based observational study was performed at the Microbiology Laboratories of Baghdad Medical City from February to May 2026. Seventy-four adult patients with suspected lower respiratory tract infection were enrolled. Respiratory specimens were assessed by direct Gram stain, cultured on 5% sheep blood agar under CO_2-enriched incubation, and screened for alpha-hemolytic colonies. Presumptive isolates were further examined by Gram stain from culture, optochin susceptibility, bile solubility, capsule staining, biochemical identification, and slide agglutination serotyping. ***Results:*** Of 74 respiratory specimens, 52 (70.3%) showed neutrophil-predominant inflammatory smears, and 34 (45.9%) showed Gram-positive diplococci on direct microscopy. Bacterial growth was obtained from 58 specimens (78.4%). Thirty-six isolates showed presumptive alpha-hemolytic streptococcal morphology, and 30 were finally identified as *S. pneumoniae*. The pneumococcal identification rate was 40.5% among all specimens and 83.3% among presumptive alpha-hemolytic isolates. Optochin susceptibility and bile solubility provided the clearest separation between confirmed pneumococci and non-confirmed alpha-hemolytic isolates ($p < 0.001$ for both), with perfect internal agreement (kappa = 1.00). Serotype 6 was the most frequent serotype, detected in 11 isolates (36.7%), followed by serotype 3 in 4 isolates (13.3%). ***Conclusion:*** Conventional phenotypic methods can still provide useful identification of *S. pneumoniae* in routine Iraqi microbiology laboratories when applied as a combined systematic workflow, not as isolated single tests. Still, because no molecular reference method was used, the findings should be interpreted as pragmatic diagnostic utility rather than full analytical diagnostic accuracy.

Keywords: *Streptococcus pneumoniae*; conventional identification; optochin susceptibility; bile solubility; serotyping; respiratory tract infection; clinical microbiology; Iraq.

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INTRODUCTION

Streptococcus pneumoniae remains one of the most important bacterial pathogens in clinical microbiology. It causes community-acquired pneumonia, meningitis, bacteremia, sinusitis, and otitis media, and it still produces a heavy clinical burden even in the vaccine era [1, 2]. The picture is not fixed. With vaccine use, the circulating serotypes have changed, but pneumococcal disease has not disappeared. Some serotypes decreased, some persisted, and some non-vaccine serotypes became more visible [3, 4]. So, the

organism continues to move, biologically and also in a public-health sense.

Accurate identification of *S. pneumoniae* in the clinical laboratory is important because the result may affect antibiotic selection, local surveillance, and sometimes infection-control decisions. The difficulty is that pneumococcus sits very close to other members of the viridans group streptococci. Alpha-hemolytic colonies on blood agar may look familiar, but familiar is not the same as confirmed. This small difference is where many laboratory errors can quietly start. Optochin susceptibility and bile solubility are still commonly used because they are simple, inexpensive, and practical. Still, they are not perfect tests, especially when atypical isolates are present [5-7].

In high-resource settings, MALDI-TOF MS, PCR, LAMP-based assays, and sequencing platforms now provide faster and more refined identification. These methods are useful, particularly when the isolate is atypical, optochin-resistant, or closely related to *Streptococcus pseudopneumoniae* [8-12]. But this is not always the daily reality in many Iraqi laboratories. Instruments may be unavailable. Reagents may arrive late. Maintenance is not always stable. In this setting, the conventional workflow is not just an outdated procedure written in a textbook. It is the actual work done on the bench.

Good respiratory microbiology begins even before identification. A sputum specimen may truly represent the lower respiratory tract, or it may be mainly oral contamination collected in a container. Therefore, macroscopic assessment and Gram-stained smear examination remain important gates before culture interpretation. Several studies have shown that sputum Gram stain and culture results are strongly affected by specimen quality and previous antibiotic exposure [8,9]. This makes a structured workflow more important than reliance on any single test.

The literature often discusses molecular diagnostics, serotype replacement, antibiotic resistance, and vaccine coverage. Yet fewer studies describe how conventional phenotypic identification performs as a complete routine workflow in resource-limited hospital laboratories. This gap is relevant for Iraq, where many laboratories still depend on Gram stain, blood agar culture, optochin susceptibility, bile solubility, capsule demonstration, biochemical testing, and serotyping when it is available.

Therefore, the present study was conducted to assess the practical diagnostic value and internal consistency of conventional phenotypic methods for identifying *S. pneumoniae* from respiratory specimens in a routine clinical microbiology laboratory in Baghdad, Iraq. The study also describes the serotype distribution of confirmed isolates, but cautiously, because the number of isolates is modest and cannot support broad epidemiological claims.

MATERIALS AND METHODS

Study Design and Setting

This prospective, single-center, laboratory-based observational study was carried out at the Microbiology Laboratories of Baghdad Medical City, Baghdad, Iraq for a period of four months from February to May 2026. The study was conducted to assess the practical diagnostic value of standard phenotypic approaches for laboratory detection of S. pneumoniae in respiratory tract specimens. The investigation was based on regular microbiological procedure, but not on molecular confirmation.

Study Population and Specimen Collection

A total of 74 consecutive adult patients with clinical suspicion of lower respiratory tract infection were included. Patients were referred from respiratory medicine, internal medicine and emergency clinical units for routine bacteriological examination. Only one respiratory specimen per patient was included to prevent duplication isolation analysis. The patients were asked to deliver deep cough specimens rather than saliva and the freshly expectorated sputum specimens were collected in sterile, wide-mouthed, leak-proof containers. Specimens were sent to the laboratory as soon as possible, and were processed as soon as possible.

Inclusion and Exclusion Criteria

Patients were included if they were 18 years or older, had clinical suspicion of lower respiratory tract infection, provided an expectorated sputum specimen, and had sufficient specimen quality on microscopic examination. Samples were eliminated if they included mostly squamous epithelial cells, had insufficient volume, were duplicates of respiratory sampling, had incomplete laboratory processing or lacked critical microbiological data.

Sputum Quality Assessment

Each specimen was examined macroscopically for color, consistency, and purulent appearance. A direct Gram-stained smear was then prepared to assess specimen quality and the inflammatory cellular response. Specimens were considered acceptable when they showed abundant polymorphonuclear leukocytes with minimal squamous epithelial cell contamination. Samples with a predominance of squamous epithelial cells were judged to represent oropharyngeal contamination and were eliminated from culture-based analysis. This quality step was maintained, as sputum culture interpretation might be misleading when substandard samples are accepted without microscopic scrutiny [8,9].

Culture Conditions

Accepted sputum specimens were inoculated onto 5% sheep blood agar plates using a standard semi-quantitative streaking technique. Plates were incubated at 35-37°C in a 5% CO2-enriched atmosphere for 18-24 hours. Colony morphology, hemolytic pattern and growth properties were studied on culture plates. Suspected colonies of S. pneumoniae were tiny, moist, alpha-hemolytic colonies, occasionally showing a central depression following incubation.

Phenotypic Identification of S. pneumoniae

Presumptive pneumococcal isolates were identified using a stepwise conventional laboratory approach. Initial criteria were alpha haemolysis on blood agar, distinctive colony morphology and Gram positive lancet shaped diplococci on microscopy. Optochin susceptibility testing was performed on freshly inoculated blood agar plates incubated in 5% CO2. The inhibition zone was measured in millimeters. Bile solubility testing was performed for suspected isolates, and a positive reaction was interpreted as supportive evidence for pneumococcal identification. Capsule staining was employed as an additional approach. Routine laboratory procedures were used for biochemical characterization. The final phenotypic identification was established on agreement between morphology, Gram stain, optochin susceptibility, bile solubility, capsule demonstration and biochemical profile [5-7].

Serotyping

All confirmed S. pneumoniae isolates were subjected to serotyping by slide agglutination using pneumococcal type-specific antisera according to the manufacturer instruction and the routine laboratory protocol. Serotyping was conducted only after phenotypic confirmation and was utilized for microbiological characterization, not as a substitute for species level identification. The interpretation was descriptive because several of the serotype categories contained a limited number of isolates [14].

Outcome Measures

The primary outcome was successful identification of S. pneumoniae using the conventional phenotypic workflow routinely applied in the clinical microbiology laboratory. Secondary outcomes were culture positivity, presumptive alpha-hemolytic isolate rate, concordance of major phenotypic findings, internal agreement between chosen conventional tests and serotype distribution in confirmed pneumococcal isolates.

Data Collection

Laboratory data were collected using a structured data sheet. Recorded variables included specimen quality category, direct Gram stain findings, culture result, colony morphology, optochin susceptibility, bile solubility result, capsule staining result, biochemical identification result, and serotype. Patient identifiers were removed prior to analysis. Age and sex were not kept in the anonymized laboratory dataset utilized for this manuscript and hence age- and sex-stratified analyses were not performed.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 27.0. Categorical variables are defined as frequencies and percentages. We estimated Wilson method 95% confidence intervals for the key descriptive proportions. Because of the low number of non-confirmed isolates, the Fisher exact test was utilized to compare selected phenotypic findings between verified S. pneumoniae isolates and non-confirmed alpha-hemolytic isolates. Internal agreement between chosen traditional phenotypic tests was assessed using Cohen kappa coefficient. A p-value <0.05 was considered statistically significant. Because no molecular reference method was used, sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were not calculated.

Ethical Considerations

The study protocol was reviewed and approved by the Quality Evaluation Committee, Baghdad Medical City, Baghdad, Iraq (Approval No. 32-114, dated 12 May 2026). The study was conducted using respiratory specimens submitted as part of routine clinical microbiological investigation. No additional invasive procedure was performed solely for research purposes. All laboratory data were anonymized before analysis to protect patient confidentiality. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

RESULTS

Study Population and Available Baseline Data

A total of 74 respiratory specimens were taken from clinically suspected adult patients of lower respiratory tract infection. All specimens were processed using the standard microbiology methodology. As patient age and sex were not retained in the anonymized laboratory dataset, the demographic description was confined to available study-level descriptors. It's not perfect, but it's more honest than creating demographic data after anonymization.

Table 1: Available baseline and study descriptors

Variable	Value
Study design	Prospective single-center laboratory-based observational study
Study site	Microbiology Laboratories, Baghdad Medical City, Baghdad, Iraq
Study period	February-May 2026

Variable	Value
Patients/specimens included	74 adult patients / 74 respiratory specimens
Age eligibility	Adults, >=18 years
Age and sex distribution	Not retained in the anonymized laboratory dataset
Specimen type	Expectorated respiratory specimens submitted for routine bacteriological investigation

Specimen Processing and Microscopic Assessment

Initial microscopic assessment showed that 52 specimens had a neutrophil-predominant inflammatory pattern, 22 specimens had a mixed inflammatory pattern, but nevertheless met the minimum acceptance criteria for culture. Gram-positive diplococci compatible with pneumococcal shape were found in 34 specimens. As expected, the direct microscopy was useful for early screening, but not for final identification alone. That would be too easy and microbiology is rarely so cooperative.

Table 2: Initial microscopic assessment of respiratory specimens

Microscopic finding	Number of specimens	Percentage (%)
Neutrophil-predominant inflammatory smear	52	70.3
Mixed inflammatory smear	22	29.7
Gram-positive diplococci observed on direct smear	34	45.9
Total specimens examined	74	100.0

Culture Findings and Presumptive Isolates

In 58 of 74 specimens' growth of bacteria was found. Of the culture-positive specimens 36 isolates had alpha-hemolytic morphology consistent with growth of presumptive pneumococcal or viridans group streptococcal isolates. These isolates were selected for the full conventional identification workflow. Of the 36 presumptive alpha-hemolytic isolates, 30 fulfilled the combined phenotypic criteria for S. pneumoniae, while 6 isolates showed incomplete or discordant profiles and were not classified as pneumococci.

Table 3: Culture yield and recovery of confirmed S. pneumoniae

Culture category	Number	Percentage (%)
Total respiratory specimens processed	74	100.0
Specimens with bacterial growth	58	78.4
Presumptive alpha-hemolytic streptococcal isolates	36	48.6
Confirmed S. pneumoniae isolates	30	40.5
Non-confirmed alpha-hemolytic isolates	6	8.1

Phenotypic Identification Profile

Among the 36 presumptive alpha-hemolytic isolates, 30 pneumococcal isolates confirmed were Gram-positive diplococci on repeat smear from culture, optochin susceptibility and bile solubility. Capsule staining was detected in 28/30 confirmed isolates. The remaining two isolates were retained as confirmed pneumococci because optochin susceptibility, bile solubility, and biochemical compatibility were concordant. The 6 non-confirmed alpha-hemolytic isolates lacked the confirmatory pattern required for final classification.

Table 4: Phenotypic identification profile of presumptive alpha-hemolytic isolates

Identification parameter	Confirmed S. pneumoniae (n=30)	Non-confirmed alpha-hemolytic isolates (n=6)
Gram-positive diplococci on culture smear	30 (100.0%)	4 (66.7%)
Alpha-hemolytic colonies on blood agar	30 (100.0%)	6 (100.0%)
Optochin susceptibility	30 (100.0%)	0 (0.0%)
Bile solubility positive	30 (100.0%)	0 (0.0%)
Capsule staining positive	28 (93.3%)	0 (0.0%)
Compatible biochemical profile	30 (100.0%)	0 (0.0%)
Final classification as S. pneumoniae	30 (100.0%)	0 (0.0%)

Statistical Comparison and Internal Agreement

Optochin molecule susceptibility and bile solubility gave the clearest differentiation between proven pneumococci and non-confirmed alpha-hemolytic isolates. All confirmed isolates were positive in both tests, while non-confirmed isolates were negative in both assays. Helpful but less specific Gram-positive diplococcal morphology, and it was also seen in four non-confirmed alpha-hemolytic isolates. Internal

agreement was strongest between optochin susceptibility and bile solubility.

Table 5: Statistical comparison of selected phenotypic findings

Phenotypic parameter	Fisher exact test	Interpretation
Gram-positive diplococci	p=0.024	Useful screening feature, but not fully discriminatory
Alpha-hemolysis	Not tested	Present by selection in both groups
Optochin susceptibility	p<0.001	Strong discriminatory feature
Bile solubility	p<0.001	Strong discriminatory feature
Capsule staining	p<0.001	Supportive adjunctive feature
Biochemical compatibility	p<0.001	Strong supportive confirmation

Table 6: Internal agreement between selected conventional tests

Comparison	Cohen kappa	Interpretation
Optochin susceptibility vs bile solubility	1.00	Perfect agreement
Optochin susceptibility vs biochemical compatibility	1.00	Perfect agreement
Optochin susceptibility vs capsule staining	0.82	Almost perfect agreement
Gram morphology vs optochin susceptibility	0.45	Moderate agreement

Descriptive Proportions with Confidence Intervals

The main proportions were summarized with Wilson 95% confidence intervals. This helped to frame the precision of the observed laboratory yield and did not mean that the study was measuring molecular diagnostic accuracy.

Table 7: Key descriptive proportions used in interpretation

Indicator	n/N	Percentage (%)	95% CI (Wilson)
Bacterial growth	58/74	78.4	67.7-86.2
Presumptive alpha-hemolytic isolates	36/74	48.6	37.6-59.8
Confirmed S. pneumoniae among all specimens	30/74	40.5	30.1-51.9
Confirmed S. pneumoniae among presumptive isolates	30/36	83.3	68.1-92.1
Capsule staining positive among confirmed isolates	28/30	93.3	78.7-98.2
Serotype 6 among confirmed isolates	11/30	36.7	21.9-54.5

Stepwise Laboratory Flow

Table 8: Stepwise laboratory flow from specimen receipt to final pneumococcal identification

Laboratory step	Number	Percentage of total specimens (%)
Total respiratory specimens received	74	100.0
Specimens showing bacterial growth	58	78.4
Presumptive alpha-hemolytic isolates selected for workup	36	48.6
Confirmed S. pneumoniae isolates	30	40.5
Serotyped confirmed isolates	30	40.5

Serotype Distribution

All 30 verified S. pneumoniae isolates were serotyped. Eight serotypes were found. Serotype 6 was the most frequent single serotype, next to serotype 3. The other isolates were found among serotypes 1, 2, 4, 5, 7 and 8. Some serotype groups had just two or three isolates and serotype data were thus interpreted solely descriptively.

Table 9: Serotype distribution among confirmed S. pneumoniae isolates

Serotype	Number of isolates	Percentage (%)
Serotype 6	11	36.7
Serotype 3	4	13.3
Serotype 1	3	10.0
Serotype 2	3	10.0
Serotype 8	3	10.0
Serotype 4	2	6.7
Serotype 5	2	6.7
Serotype 7	2	6.7
Total	30	100.0

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Table 10: Grouped serotype frequency pattern

Serotype group	Included serotypes	Number of isolates	Percentage (%)
Dominant individual serotype	6	11	36.7
Intermediate-frequency serotypes	1, 2, 3, 8	13	43.3
Low-frequency serotypes	4, 5, 7	6	20.0
Total	-	30	100.0

Grouped categories were used only for descriptive visualization, not for inferential epidemiological comparison.

Figures

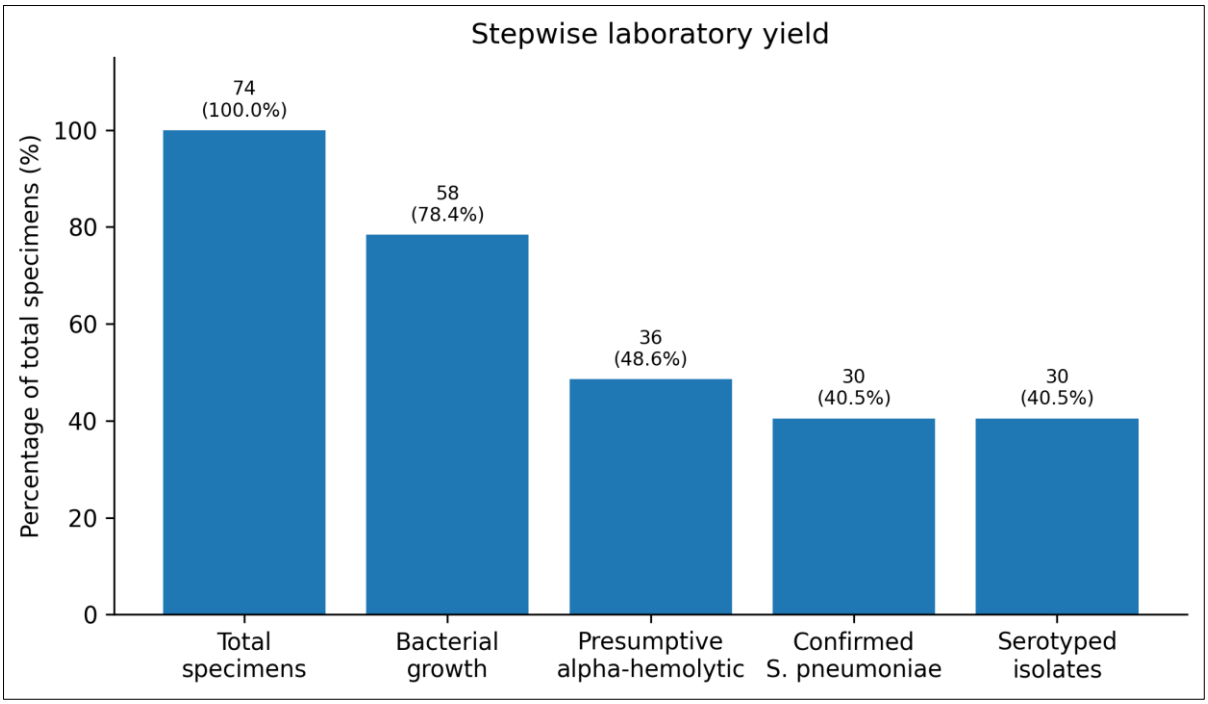

Figure 1: Stepwise laboratory yield from specimen receipt to final phenotypic identification

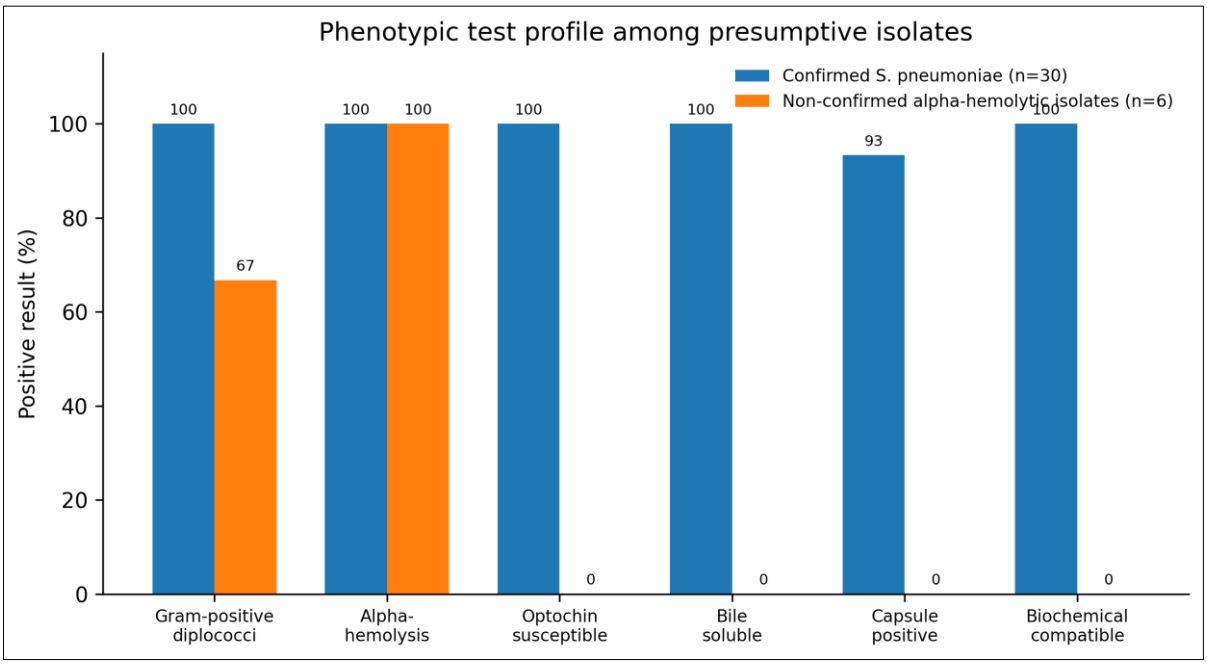

Figure 2: Phenotypic test profile among presumptive alpha-hemolytic isolates

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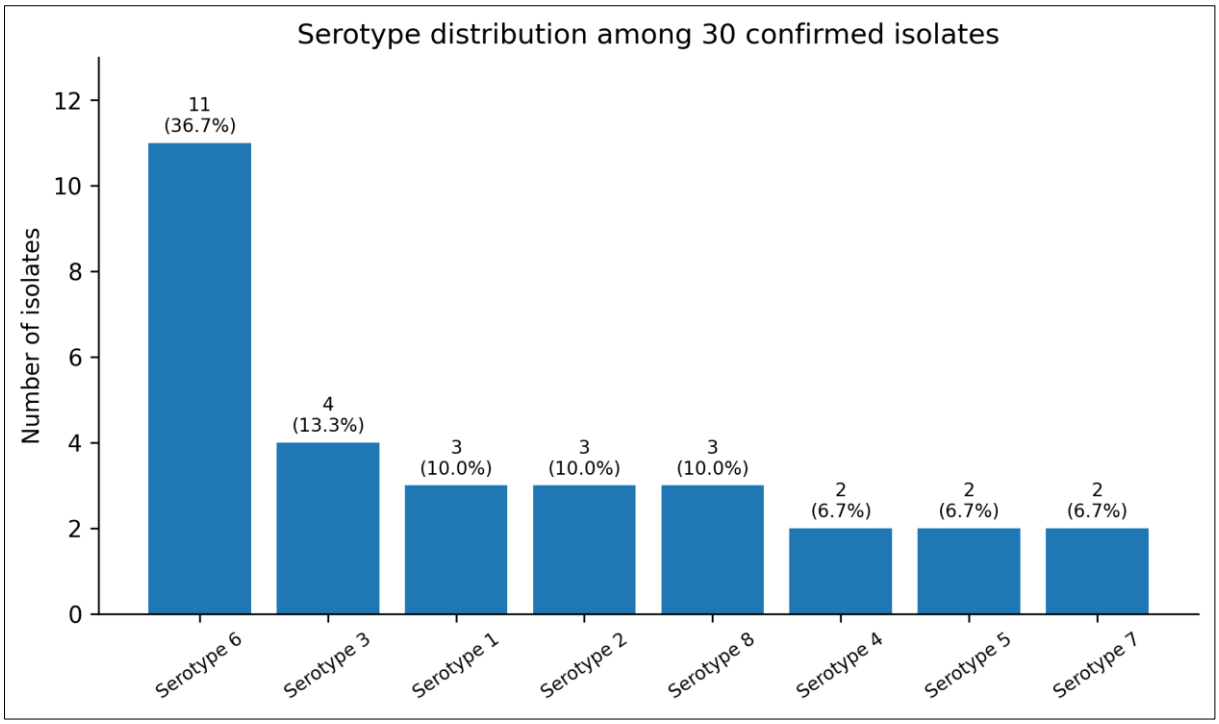

Figure 3: Serotype distribution among 30 confirmed S. pneumoniae isolates

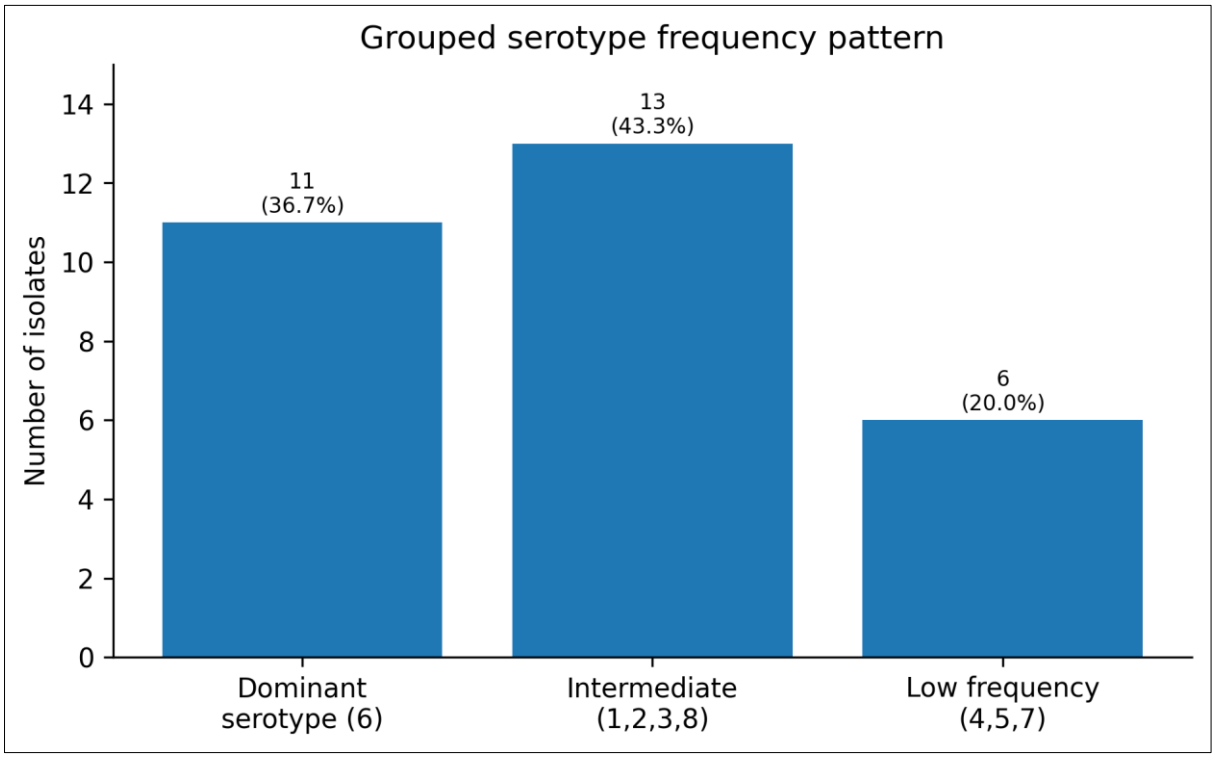

Figure 4: Grouped serotype frequency pattern. This grouping is descriptive and should not be over-read

DISCUSSION

Principal interpretation of the laboratory yield

This study evaluated a conventional phenotypic workflow for identifying S. pneumoniae among respiratory specimens processed in a routine clinical microbiology setting in Baghdad. Of the 74 isolates, 58 yielded bacterial growth and 36 isolates had alpha-hemolytic morphology requiring additional pneumococcal workup. Finally, 30 isolates were identified as S. pneumoniae by the combined phenotypic approach. The key lesson of the study is the progressive narrowing. Clinical suspicion, bacterial growth, alpha-hemolytic morphology and final pneumococcal confirmation are sequential stages, although they are not

the same stage. Specimen quality and the value of early microscopic filtering

The first practical point is that conventional diagnosis begins with specimen quality. Respiratory samples are easily contaminated with oral flora and a culture result may appear relevant despite the reported specimen being suboptimal. Popova *et al.,* showed that quality control of sputum allows differentiating between probable lower respiratory tract infections and colonization flora [9]. Similarly, Cartularies *et al.,* have showed that Gram stain and sputum culture may detect only a limited number of bacteria in community-acquired lower respiratory tract infections, especially when drug exposure comes into play. [8]. In the present study, direct microscopy helped select and interpret samples, but it was not used as final evidence. That is the right place for it: useful, early, and limited.

Culture morphology as a necessary but insufficient filter

Blood agar culture was the bridge from direct microscopy to definitive testing. Alpha-hemolysis guided the workup but did not close the case. In this investigation, 36 isolates had alpha hemolytic growth, although only 30 isolates were confirmed as S. pneumoniae. The pneumococcal output would have been overcalled if colony morphology alone had been considered. This is consistent with the larger diagnostic dilemma: viridians group streptococci can be close mimics of pneumococci and a familiar colony is not always the correct species. Optochin and bile solubility as the strongest conventional pair.

The strongest separation came from optochin susceptibility and bile solubility. Both were positive in all confirmed isolates and negative in all non-confirmed alpha-hemolytic isolates, with perfect agreement between them. Ercibengoa *et al.,* confirmed that optochin testing remains useful for differentiating S. pneumoniae from viridans group streptococci, although the test is not flawless [5]. Ktari *et al.,* also described optochin-resistant S. pneumoniae and S. pseudopneumoniae strains, reminding us that optochin alone should not become a laboratory religion [6]. Thus, the present results support the classical pair, but as a pair, not as a lonely shortcut. Why absence of molecular confirmation matters

The study did not include PCR, MALDI-TOF MS, or whole-genome sequencing. Because of that, no sensitivity, specificity, PPV, NPV, or diagnostic accuracy was calculated. This limitation should stay visible. Pan *et al.,* showed that MALDI-TOF MS can discriminate many viridans group streptococci, although platform and database differences matter [10]. Basheer *et al.,* developed a LAMP-based method for identifying S. pneumoniae and S. pseudopneumoniae in clinical isolates [11]. Molecular and proteomic tools are clearly the direction of modern microbiology. Still, in many Iraqi hospital laboratories, these tools are not consistently available. Under this real condition, a disciplined phenotypic workflow remains useful when its limits are not hidden.

Phenotypic workflow in the Iraqi laboratory context

The Iraqi laboratory environment is not a trivial background note. Baghdad Medical City handles clinically heterogeneous patients, and the microbiology laboratory operates under pressure: inconsistent specimen quality, workload, limited sophisticated platforms and the necessity to report rapidly. In this context, typical tests are not museum items. These are routine diagnostic tests. Pneumococcal diagnosis currently stands between the older culture-based methods and the increasing molecular approaches, and was recently reviewed by Chen *et al.,*[1]. This is the middle zone where the present study belongs, where conventional methodologies still accomplish valuable work if employed consecutively and read carefully.

Serotype distribution and local microbiological meaning

Serotyping added a second layer beyond species-level identification. Serotype 6 was the most frequent individual serotype, accounting for 36.7% of confirmed isolates. Serotype 3 followed at 13.3%. This is interesting but should be read with caution. Non-serotype 6 isolates collectively represented 63.3%, which means the isolate population was diverse, not one-serotype dominant. In recent adult CAP monitoring in Japan, serotype 3 among prevalent pneumococcal serotypes was identified [2]. In Brazil, Almeida *et al.,* reported sustained dominance of serotypes 19A, 3 and 6C over the COVID-19 era [16]. The local prevalence of serotype 3 in the present study consequently follows a wider pattern of some serotypes remaining clinically apparent despite vaccination pressure. Why serotyping should remain in local surveillance

Serotype data are not only decorative microbiology. They inform vaccine relevance, outbreak detection and extended surveillance. Golden *et al.,* stressed the need for laboratories to perform quality-controlled pneumococcal serotyping and antibiotic susceptibility testing [14]. Newer tools such as Cas9-targeted nanopore serotyping and CRISPR/Cas9-targeted sequencing are being developed because accurate serotype assignment is becoming more important, not less [20,21]. In the current study, eight serotypes among 30 isolates are enough to show diversity, but not enough to infer regional vaccine coverage with confidence. That line should not be crossed.

Practical Implications

The work yields three practical consequences. First, microscopy of sputum should be an actual quality gate before culture, not a cosmetic line in the operation. Second, alpha-hemolytic colonies should not be called S. pneumoniae without optochin and bile solubility testing. Third, serotyping should be kept when feasible, even if just for a subset of isolates, as the local pneumococcal ecology changes with vaccine usage, antibiotic pressure and population mobility.

Strengths and Limitations

The main strength is practical realism. The study reflects what can be done in a routine hospital microbiology laboratory without assuming constant access to molecular platforms. It also differentiates between specimen screening, presumptive selection, final phenotypic confirmation, internal agreement and serotype characterization. The restrictions, too, are plain. The investigation was single centered, 74 patients and 30 verified isolates. Age and sex were not kept in the anonymized dataset. Prior antibiotic exposure was not evaluated. No molecular reference method was used. Serotype findings were descriptive and should not be treated as regional surveillance. These limitations do not destroy the study, but they define exactly how far its conclusions may go.

Conclusion of the Discussion

In conclusion, the standard phenotypic diagnosis of S. pneumoniae remains a practical utility in regular Iraqi clinical microbiology when applied as an organized procedure. The most confirming was the combination of Gram stain morphology, alpha haemolytic culture, optochin susceptibility, bile solubility, capsule demonstration and biochemical compatibility. The study does not establish molecular diagnostic accuracy, and does not attempt to. Something more modest, and perhaps more useful for daily work: a disciplined conventional workflow can still differentiate pneumococcus from look-alike alpha-hemolytic isolates in a resource-limited hospital laboratory.

CONCLUSION

The conventional phenotypic workflow identified S. pneumoniae in 30 of 74 respiratory specimens and showed strong internal consistency when alpha-hemolysis, Gram-stain morphology, optochin susceptibility, bile solubility, capsule staining, and biochemical compatibility were interpreted together. The most useful confirmatory pair was optochin susceptibility and bile solubility. Serotype 6 was the most frequent single serotype, but the overall pattern was diverse. In Iraqi laboratories where molecular platforms are not always available, traditional identification remains relevant if used with caution and reported within its boundaries.

Limitations: This study has several limitations. It was a single-center study with a modest sample size. Age and sex were not retained in the anonymized laboratory dataset; therefore, no demographic analysis was possible. Prior antibiotic exposure was not examined. Analytical diagnostic accuracy was not calculated, since there was no molecular reference approach. The distribution of serotypes should be read descriptively and not as regional monitoring because only 30 isolates were serotyped.

Conflict of Interest: The authors declare no conflicts of interest.

Funding Statement: This study received no external funding.

Ethics and Consent: The study protocol was reviewed and approved by the Quality Evaluation Committee, Baghdad Medical City, Baghdad, Iraq (Approval No. 32-114, dated 12 May 2026). The study was conducted using respiratory specimens submitted as part of routine clinical microbiological investigation. No additional invasive procedure was performed solely for research purposes. All laboratory data were anonymized before analysis to protect patient confidentiality. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Data Availability Statement: The anonymized aggregate dataset supporting the findings of this study is provided as Supplementary File S1 with the manuscript. It contains the study flow, microscopy findings, phenotypic test results, agreement statistics, descriptive proportions, and serotype distribution reported in the article. No direct patient identifiers are included.

Author Contributions

Suadad Taha Obaid: Conceptualization, Methodology, Supervision, Data Interpretation, Writing - Original Draft, Writing - Review & Editing, and Project Administration.

Elaf Basim Noori: Investigation, Specimen Processing, Microbiological Identification, Data Collection, Laboratory Documentation, Data Curation, and Writing - Review & Editing.

Ahlam Gareeb Nhaer: Validation, Literature Review, Data Verification, Statistical Review, and Writing - Review & Editing.

All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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