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Microbiology Section

Diagnostic Performance of *Streptococcus pneumoniae* Urinary Antigen Assay: A Cross-sectional Study on Comparative Analysis of Bacterial Culture and Molecular Detection in Pneumococcal Infections

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ABSTRACT

Introduction: Pneumonia is the most prevalent infection worldwide, leading to hospitalisation and contributing to mortality rates. Among the bacterial agents associated with Community-Acquired Pneumonia (CAP), *Streptococcus pneumoniae* remains the most common. Conventional microbiological diagnostic tests have various limitations, including issues with sample collection, prior antibiotic administration, and delayed specimen transport. Urinary Antigen Testing (UAT) shows promise in rapidly identifying the causative agent of CAP, allowing for targeted therapy.

Aim: To evaluate the diagnostic accuracy of the pneumococcal UAT in identifying CAP.

Materials and Methods: A cross-sectional analytical study was conducted over a period of one year from June 2022 to May 2023 at SRM Medical College Hospital and Research Centre, Kattankulathur, Chengalpattu, Tamil Nadu, India. A total of 38 patients (>18 years of age) with clinically suspected CAP and who satisfied the clinical criteria for CAP were recruited for the study. Respiratory specimens were subjected to bacterial culture, real-time Polymerase Chain Reaction (PCR), and UAT using the Fluorescent Immunoassay (FIA) to detect *Streptococcus pneumoniae*. The sensitivity, specificity, positive and negative predictive values, and accuracy of the pneumococcal UAT for detecting CAP were assessed. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software, version 21.0.

Results: The study revealed a female predominance 22 (57.89%). Bacterial culture and real-time PCR identified 7 (18.42%) of patients with *S. pneumoniae*, while the UAT only detected 1 (2.63%). The pneumococcal UAT showed low sensitivity (14.29%), high specificity (100%), and satisfactory accuracy (84.21%).

Conclusion: The pneumococcal UAT, with its straightforward technology, ease of use, rapid results, non invasive approach, cost-effectiveness, and high specificity and accuracy, could be favoured over bacterial culture and molecular techniques for ruling out CAP.

Keywords: Diagnostic reagent and test kits, Infection, Molecular biology, Streptococcal

INTRODUCTION

The CAP, which accounts for the highest number of deaths and morbidity, carries a substantial clinical and financial burden. In 2019, CAP accounted for 14% of child mortality in those under the age of 5 and 22% of child mortality between the ages of 1 and 5 worldwide. Southern Asia and Sub-Saharan Africa were particularly affected by this condition [1]. After neonatal diseases, pneumonia continues to be the country's number one cause of infant death [2]. According to a study from Mumbai, *S. pneumoniae* and gram negative bacteria *(Klebsiella pneumoniae* and *Pseudomonas aeruginosa)* were more frequently found in severe pneumonia, accounting for 19% of all patients [3]. Koul PA et al., have elucidated the significance of invasive pneumococcal infection in their review [4]. The mortality rate ranges from 14-30% for CAP patients and reaches 47% for severe CAP [5]. The bacterial infections responsible for CAP vary according to host features and geographic distribution. Despite the geographical differences, *S. pneumoniae* continues to be a common infection in people of all ages worldwide. Other bacteria that contribute to the bulk of CAP aetiology include *Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [5].

Streptococcus pneumoniae was first discovered by Pasteur and Sternberg in 1881, who named it the pneumococcus [6]. Streptococcal transmission occurs frequently in dry climates, which are conducive to copious sputum production and are associated with upper and lower respiratory tract infections [7]. Bacterial culture and the PCR method are both utilised to diagnose *Streptococcus pneumoniae*. The aetiological agent is most often not identified in CAP due to the limitations in conventional culture techniques, such as suboptimal sample collection, delay in sample processing, and prior history of antibiotic use. It usually takes 48 hours for cultures to yield an identifiable bacterium. According to a recent meta-analysis, the diagnostic yield from blood culture and sputum culture was 4.7-16% and 50%, respectively [8].

The *S. pneumoniae* UAT method is useful in detecting the C-polysaccharide antigen found in the urine, enabling prompt diagnosis of CAP caused by all serotypes of *S. pneumoniae*. Boulware DR et al., documented a pooled sensitivity of 74% and a pooled specificity of 94% among patients with CAP, including cases with bacteraemia and empyema [9]. Antibiotic de-escalation has been proven to cause no clinical failure or mortality in patients with CAP. UAT can strengthen antimicrobial stewardship in hospitals due to its role in antibiotic de-escalation. This may reduce expenses, drug toxicity, duration of hospital stay, and the emergence of multidrug resistance [10,11].

To the best of authors knowledge, only one study conducted in India, authored by Khan S et al., has addressed the topic of pneumococcal UAT. According to this study, the performance of

the UAT is reported to be similar to that of the latex agglutination test [12].

The aim of the study was to evaluate the diagnostic accuracy of the pneumococcal UAT in identifying CAP. The central hypothesis of this study posits that the UAT is a valuable and expeditious tool for diagnosing CAP caused by *S. pneumoniae*. Additionally, this study seeks to compare the results obtained from UAT with molecular detection and bacterial culture of *S. pneumoniae* from respiratory samples to evaluate the diagnostic accuracy of the UAT.

MATERIALS AND METHODS

A cross-sectional analytical study was conducted at SRM Medical College Hospital and Research Centre, Kattankulathur, Chengalpattu, Tamil Nadu, located in Southern India, spanning a duration of one year from June 2022 to May 2023. The study was approved by the Institutional Ethics Committee (IEC) (8415/IEC/2022).

Inclusion criteria: Patients with clinically suspected CAP (according to the clinical criteria for CAP) and who provided informed consent were recruited for the study.

Definition of Community-Acquired Pneumonia (CAP): A patient with CAP is typically identified by the triad of: 1) Non specific signs of infection such as fever, chills, and leucocytosis; 2) Specific indicators of infection including symptoms such as cough, increased sputum production, difficulty breathing, chest pain, abnormal pulmonary examination findings such as crackles, signs of consolidation, identification of a pleural effusion; 3) The presence of a new or modified radiographic infiltrate [13].

Exclusion criteria: Patients diagnosed with cystic fibrosis, hospital-acquired pneumonia, Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) pneumonia, and alternate diagnoses to account for the clinical presentation were excluded from the study.

Sample size: The sample size was calculated using the formula:

$n=z^2\ p\ q/d^2$

$=\{(1.96)\ 2\times71.4\times28.6\}/(14.28)^2$

$=(3.84\times2042.04)/203.9184=38.45=38$

Where n=sample size, p=prevalence (71.4%), q=100-p, d=20% precision [14]. The study enrolled a total of 38 patients.

All study subjects were tested for *S. pneumoniae* using three methodologies: bacterial culture of respiratory specimens (sputum/bronchoalveolar lavage/endobronchial aspirate/blood culture), molecular detection using respiratory specimens, and *S. pneumoniae* UAT. The primers targeting the *psaA* gene were utilised for the molecular identification of *S. pneumoniae* in respiratory specimens. The Standard F *Streptococcus pneumoniae* Ag FIA test system was utilised for UAT detection. The sputum/bronchoalveolar lavage/endobronchial aspirate samples and urine were collected in sterile containers and sent to the laboratory for bacterial processing.

Bacterial culture of respiratory samples: The respiratory specimens were inoculated onto 5% sheep blood agar and chocolate agar (Himedia, Maharashtra, India). Following inoculation, the plates were placed in a 5% carbon dioxide incubator and maintained at a temperature of 37°C for 18 hours. Bacterial colonies with a carrom coin shape and susceptible to optochin (5 µg) were selected for bacterial identification using VITEK2 GP ID cards (bioMerieux). Gram staining of the bacterial colony was performed to observe lanceolate-shaped gram positive cocci in pairs. Additional tests such as the 10% sodium deoxycholate solubility test and inulin fermentation test were also conducted to confirm the identification of the bacteria [15].

Molecular identification of *Streptococcus pneumoniae*: Respiratory samples from study subjects were subjected to real-time PCR using the Helini *S. pneumoniae* real-time PCR kit (HELINI biomolecules, Chennai, India). The kit targets the *psaA* sequence for bacterial identification. The procedure comprises a total of 40 PCR cycles, involving denaturation at 95°C for 20 seconds, annealing at 56°C for 20 seconds, and extension at 72°C for 20 seconds. A Cycle threshold (Ct) value of <36 was considered as the criteria for detection of *Streptococcus pneumoniae* [16].

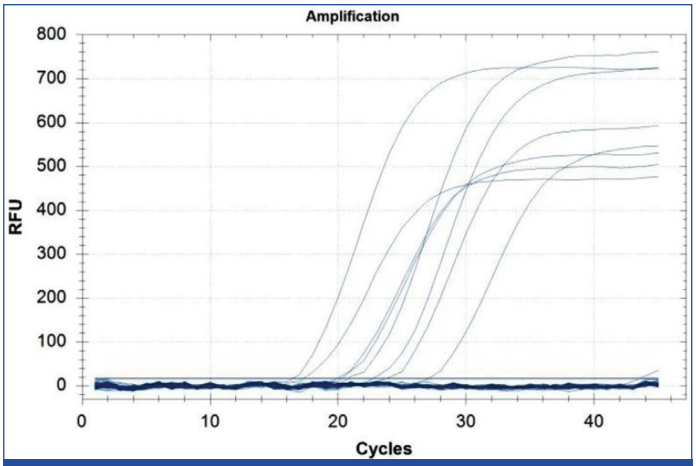
***Streptococcus pneumoniae* Urinary Antigen Test (UAT):** Patients who were suspected of having CAP provided clean catch midstream urine samples. The urine samples were tested for *Streptococcus pneumoniae* urinary antigen using the Standard F, *S. pneumoniae* Ag FIA test (SD Biosensor Healthcare Pvt., Ltd., Haryana, India). A volume of 100 µL of urine was added to the kit, which works based on the principle of fluorescent immunochromatography. The kit was read after an incubation period of 5-10 minutes by placing it inside the SD biosensor unit. The kits were stored at a temperature of 2-30°C [17].

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software, version 21.0.

RESULTS

A total of 38 patients were recruited for the study. Females constituted the majority 22 (57.89%) of the patient population. Among the study patients, 7 (18.42%) were identified to have *Streptococcus pneumoniae* using bacterial culture and molecular detection techniques. The amplification curves of the positive samples are depicted in [Table/Fig-1], which shows seven amplification curves from the respiratory samples of study subjects and one from the positive control. A Ct value of <36 was considered positive for *Streptococcus pneumoniae* according to the manufacturer's instructions.



[Table/Fig-1]: Real time PCR amplification of positive samples in the study (N=7). (Ct value <36 was considered positive for *Streptococcus pneumoniae*)
*RFU: Relative fluorescence units

The streptococcal UAT was found to be positive in 1 (2.63%) of study patients. [Table/Fig-2,3] show the comparative analysis of results obtained from bacterial culture, molecular detection techniques, and the UAT. Bacterial culture and molecular detection identified six additional cases of *streptococcus pneumoniae* CAP compared to the UAT.

Serial number	Specimen	Culture	PCR	Urinary Ag
01.	01	+	+	+
02.	02	+	+	-
03.	03	+	+	-
04.	04	+	+	-
05.	05	+	+	-
06.	06	+	+	-
07.	07	+	+	-

[Table/Fig-2]: Comparative analysis of positive results obtained from bacterial culture, Polymerase Chain Reaction (PCR) and Urinary Antigen Test (UAT) (N=7).
*PCR: Polymerase chain reaction; Urinary Ag: Urinary antigen test

PCR/Bacterial culture	Urinary antigen			Total
		+	-	
	+	1	6	7
	-	0	31	31
Total		1	37	38

[Table/Fig-3]: Distribution of positive and negative results obtained from bacterial culture, Polymerase Chain Reaction (PCR) and Urinary Antigen Test (UAT) (N=38).
*PCR: Polymerase chain reaction
Gold standard test for detection of *S. pneumoniae*: PCR

In the present study, upon analysis, there was one true positive out of 38 cases, 31 true negatives out of 38 cases, no false positives, and six false negatives out of 38 cases. The molecular detection method was utilised as the gold standard for analysis. The *S. pneumoniae* UAT demonstrated a sensitivity of 14.29%, specificity of 100%, positive predictive value of 100%, negative predictive value of 83.78%, and an overall accuracy of 84.21%. The diagnostic accuracy of bacterial culture was 100%. Hence, in comparison to the three methods, the UAT was found to be inferior to both blood culture and molecular detection in identifying *S. pneumoniae*.

DISCUSSION

The CAP is a frequently encountered and potentially fatal infection that has significant effects on healthcare systems worldwide. CAP is the leading cause of mortality among children in India. Each year, out of the 151.8 million cases of CAP that visit hospitals, 13.1 million cases require hospital admission [18]. *S. pneumoniae* remains the most prevalent pathogen in CAP globally, and it is observed across all treatment settings, including outpatient, general ward, and intensive care units. In addition to *S. pneumoniae*, other bacterial agents commonly associated with CAP include *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*. Unlike other regions, Asia also experiences a high prevalence of gram negative infections in CAP patients, primarily caused by *Burkholderia pseudomallei* and *Klebsiella pneumoniae* [19-21].

The available diagnostic tests to determine the cause of CAP include Gram stain, bacterial culture, and identification from blood and respiratory specimens. Additionally, detection of bacterial antigens and PCR-based techniques is also utilised for this purpose. There are various reasons why diagnostic tests must be performed to identify the cause of CAP. The outcome of these tests can influence the antibiotic treatment plan for an individual patient. Based on the diagnostic test results, the antibiotic regimen may be adjusted broadened, narrowed, or completely changed-to better suit the patient's needs. Inappropriate antibiotic medication is more likely to result in increased mortality and clinical failure [22]. The discovery of the UAT against *S. pneumoniae* has helped in the rapid identification of the aetiological agent of CAP, facilitating targeted therapy and decreasing the morbidity and mortality associated with the disease [23,24]. Enzyme Immunoassay (EIA), Immunochromatographic membrane assay (ICT), FIA, and luminex xMAP bead technology are some of the techniques used to identify the urinary antigen of *pneumococcus*. Despite their potential implications, the present guidelines from the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA) discourage the utilisation of *S. pneumoniae* UAT for diagnosing CAP, except in situations of severe CAP [8]. This study was conducted with the intention of identifying the diagnostic accuracy of the FIA UAT in the diagnosis of CAP.

Throughout the duration of the study, a total of 38 patients exhibiting symptoms suggestive of CAP were identified. Females accounted for 57.89% (22/38) of the study population. Bacterial culture and molecular detection techniques identified *S. pneumoniae* infection in seven out of 38 patients (18.42%), while the UAT detected *S. pneumoniae* in only one out of 38 patients (2.63%). This reflects the poor sensitivity (14.29%) of the test. The test, however, has the advantage of being rapid, easy to perform, and having high specificity (100%) and accuracy (84.21%). The findings support the hypothesis of the study, indicating that UAT can effectively diagnose CAP caused by *S. pneumoniae*. However, due to its lower sensitivity and higher specificity, its primary utility lies in its advantageous role in ruling out the disease from the differential diagnosis for CAP. In contrast to the present study, a meta-analysis conducted by Boulware DR et al., documented a pooled sensitivity and specificity of UAT of 74% and 94%, respectively [9]. Sinclair A et al., documented a sensitivity of 74% and specificity of 97.2% in their meta-analysis [25]. Other studies have also documented moderate sensitivity and high specificity for UAT [26-28]. Burgos J et al., conducted a comparison between FIA and ICT tests for detecting *S. pneumoniae* UAT. They found that the FIA exhibited higher sensitivity (78.6% vs. 50%) than the ICT test, while both tests demonstrated similar specificity (83.3% versus 85.5%) [29]. These studies indicate that UAT is a valuable and cost-effective tool for the diagnosis of CAP and is extremely useful for excluding pneumococcal pneumonia.

Limitation(s)

There are several limitations in present study, including the short duration of the study, a small number of participants enrolled, and a lack of available data to assess the severity of CAP in the enrolled patients. These factors could have contributed to the reduced sensitivity of UAT observed in the study. However, considering the study's high specificity, positive and negative predictive values, and overall accuracy of the test, the use of UAT could be a valuable approach to rule out pneumococcal pneumonia, especially in resource-constrained settings.

CONCLUSION(S)

The CAP is the leading infectious cause of mortality worldwide and is associated with significant morbidity rates. Identifying the specific cause of CAP is crucial for administering targeted antibiotic treatment. UAT offer a promising alternative for excluding respiratory infections caused by *S. pneumoniae*. UATs are rapid, user-friendly, non invasive, cost-effective, and exhibit high specificity and accuracy, providing substantial advantages over traditional microbiological methods in excluding *S. pneumoniae* infections.

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