

RESEARCH ARTICLE

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Increasing Blood Culture Positivity for *Streptococcus pneumoniae* Using Tryptic Soy Sheep Blood Agar with Gentamicin (TSSBA-G) in Suspected Bacteremia and Pneumonia at Universitas Sumatera Utara Hospital, Indonesia

R. Lia Kusumawati^{1*} , Mirzan Hasibuan² , Rahmita Sari² ,
Nisrina Tari³  and Cynthia Gozali⁴ 

¹Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, 20155, Indonesia.

²Universitas Sumatera Utara (USU) Hospital, Universitas Sumatera Utara, Medan, 20155, Indonesia.

³PCR-WGS Laboratory, Faculty of Medicine, Universitas Sumatera Utara, Medan, 20155, Indonesia.

⁴Division of Materials Science, Graduate School of Science and Technology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan.

Abstract

Conventional blood culture media often fail to isolate *Streptococcus pneumoniae*, a key cause of pneumonia and bacteremia. This study aimed to improve the detection rate of *S. pneumoniae* using selective media. The study involved 45 blood samples from patients suspected of bacteremia and pneumonia at the Universitas Sumatera Utara Hospital. The samples were cultured using tryptic soy to make sheep blood 5% with and without gentamicin (TSSBA-G and TSSBA) in detecting *S. pneumoniae* in the blood of patients with bacteremia and pneumonia. Blood samples were incubated in the BACT/ALERT tube to detect the presence or absence of microorganism growth in blood samples. *S. pneumoniae* was isolated in 6 of 45 samples (13%) using TSSBA-G. TSSBA without gentamicin yielded 11 cultures (24%) including potential contaminants. Routine media (blood agar and MacConkey agar) detected 12 positive cultures (27%) but failed to isolate *S. pneumoniae*. Combined use of TSSBA-G and TSSBA increased total positivity to 29/45 (64%). The use of TSSBA-G, especially alongside TSSBA, substantially increases blood culture positivity, particularly for isolating *S. pneumoniae* a key cause of bacteremia. These findings support the adoption of selective media to improve diagnostic yield.

Keywords: *Streptococcus pneumoniae*, TSSBA-G, Blood Culture, Bacteremia, Pneumonia

*Correspondence: r.lia@usu.ac.id

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INTRODUCTION

Streptococcus pneumoniae is a major cause of invasive pneumococcal disease (IPD), which includes bacteremia, meningitis, and pneumonia. Pneumococcal bacteremia, in particular, contributes significantly to global morbidity and mortality. A systematic review and meta-analysis reported an overall mortality rate of 20.8% for IPD, with higher rates observed in elderly populations. IPD is defined by the presence of *S. pneumoniae* in normally sterile sites such as blood, cerebrospinal fluid, or pleural fluid.^{1,2}

Despite its clinical importance, the isolation of *S. pneumoniae* from blood cultures remains a challenge. Blood culture remains the gold standard for detection of bacteremia caused by *S. pneumoniae*, but the sensitivity of conventional methods is often suboptimal.^{3,4} Optimal growth of this facultative anaerobe often depends on using the right media and atmospheric environments (aerobic, anaerobic, or microaerophilic) conditions, which are critical to enhancing the recovery and identification of the organism.⁵⁻⁷

The bacterium's comparatively slow growth rate under normal circumstances and the interference of another microorganism with a faster growth rate are two of the many reasons that contribute to this poor detection rate. Another important factor is that prior antibiotic treatment can significantly reduce the likelihood of successfully isolating *S. pneumoniae* from blood samples.^{8,9} Previous reports indicate that pneumococcal isolation rates from routine culture systems may fall below 10% in suspected pneumonia and sepsis cases, underscoring the need for more selective culture strategies.^{10,11}

The low sensitivity of blood culture, autolysis, and contamination with fast-growing microorganisms jeopardizes isolation of *S. pneumoniae*.^{12,13} Current routine culture media used in Microbiology Laboratories are sheep blood Agar (SBA) and MacConkey agar (MA) for isolation of *S. pneumoniae*, which has limitations such as a low positivity rate (<10%), causing frequent underdetection of pathogen.¹⁴⁻¹⁶ The routine culture media used in microbiology laboratories for the isolation of *S. pneumoniae* are sheep blood agar (SBA) and MacConkey agar (MA), both of

which have limitations, including a low positivity rate of 5%-8%, leading to frequent underdetection of the pathogen.

One promising approach is the addition of gentamicin to tryptic soy agar with 5% sheep blood, known as TSSBA-G. Gentamicin selectively inhibits Gram-negative bacteria without impairing *S. pneumoniae* growth, thus increasing recovery rates in mixed or contaminated specimens.^{10,17,18} Recent studies highlight the effectiveness of selective blood agar in improving culture sensitivity and reducing diagnostic delays, especially in high-burden clinical settings.^{16,19} To address these limitations, there is a pressing need for more selective and sensitive culture media. This study proposes the use of tryptic soy agar supplemented with 5% sheep blood and gentamicin (TSSBA-G) to enhance the recovery of *S. pneumoniae* from blood samples of patients with suspected bacteremia or pneumonia.

METHODOLOGY

Study setting

This descriptive study compared the culture positivity rates of *Streptococcus pneumoniae* using routine media and a selective medium—tryptic soy agar with 5% sheep blood, with and without gentamicin (TSSBA and TSSBA-G). Blood samples were collected from hospitalized patients with suspected bacteremia or pneumonia at the Clinical Microbiology Laboratory, Universitas Sumatera Utara Hospital, between January and August 2019. A volume of 5 mL of blood was collected from pediatric patients, and 10 mL from adults. Each blood sample represented an independent clinical case (n = 45); no technical triplicates were performed.

The study protocol was reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia (Approval No. 11/KEP/USU/2021). Written informed consent was obtained from all participants or their legal guardians prior to sample collection.

Preparation of culture media

The culture media employed in this study included Columbia agar (Oxoid CM0331B), MacConkey agar (Oxoid CM0115B), and Tryptic

Soy Agar (TSA; Oxoid CM0131B). Sheep blood, obtained from CV Karya Graha Asri, Jakarta (a certified supplier of laboratory-grade sheep blood), was used for culture preparation.

Routine Media

Blood Agar (BA) was prepared by dissolving 3.8 g of blood agar base in 100 mL of distilled water, followed by autoclaving at 121 °C for 15 minutes. Once cooled to 40-45 °C, 5% defibrinated sheep blood was added, mixed thoroughly, and poured into sterile petri dishes. MacConkey agar was prepared similarly by dissolving 5.15 g of MacConkey agar in 100 mL of distilled water, autoclaving, cooling to 50 °C, and pouring into petri dishes.

Tryptic Soy Sheep Blood Agar (TSSBA) and Tryptic Soy Sheep Blood Agar with Gentamicin (TSSBA-G)

Tryptic soy agar (4 g) was dissolved in 100 mL of distilled water, sterilized by autoclaving, and cooled to 40-45 °C. A 2.5 mg/mL gentamicin stock solution was prepared by dissolving 80 mg of gentamicin in 32 mL of sterile distilled water, filtered, and stored at -20 °C to -70 °C. To prepare TSSBA-G, 1 mL of gentamicin stock was added to 500 mL of cooled tryptic soy agar, along with 5% defibrinated sheep blood. The resulting medium was mixed and poured into petri dishes.

Culture and microscopy

The laboratory identification of *S. pneumoniae* isolates relies on the recognition of typical morphological characteristics and the results of a few phenotypic tests. During microscopic evaluation, *S. pneumoniae* appears as lancet-shaped, Gram-positive diplococci or chains of cocci.³ Each blood sample was inoculated onto Blood Agar, MacConkey agar, TSSBA, and TSSBA-G media. Cultures were incubated at 35-37 °C in 5% CO₂ for 24-48 hours. *S. pneumoniae* was identified by colony morphology and Gram staining, appearing as lancet-shaped, Gram-positive diplococci or chains.

Optochin susceptibility test

Additional confirmation of *S. pneumoniae* was performed using the optochin susceptibility test. A 5 µg optochin disk (6 mm) was placed on

a lawn culture of the isolate grown on 5% sheep blood agar. The plates were incubated at 35-37 °C in 5% CO₂ for 18-24 hours. *S. pneumoniae* was identified by the presence of a zone of inhibition measuring ≥14 mm around the optochin disk, whereas viridans streptococci exhibited resistance with a smaller or no zone of inhibition.

Identification and antimicrobial susceptibility testing

The identification was started by colony microscopy with Gram staining. Colonies grown on both routine and selective media (TSSBA-G and TSSBA) were identified using standard laboratory procedures. Antimicrobial susceptibility testing was then performed with the VITEK 2 system using GP and AST-GP67 cards (bioMérieux), and results were interpreted based on Clinical and Laboratory Standards Institute (CLSI) breakpoint criteria.²⁰

Data analysis

The proportion of positive cultures for each medium was calculated and presented as percentages and bar plots. The McNemar test was used to assess the statistical significance of differences between media types with a significance threshold of $\alpha = 0.05$ (95% confidence) applied, and p-values <0.05 were considered statistically significant. Analyses were performed in R version 4.5.0, and additional visualization was performed in Python version 3.11.7.

RESULTS AND DISCUSSIONS

Out of 45 blood samples from patients suspected of pneumonia and bacteremia, various bacterial growth patterns were observed across all tested culture media, as summarized in Table 1. Using Tryptic Soy Sheep Blood Agar with Gentamicin (TSSBA-G) and incubation under anaerobic conditions with 5% CO₂, *Streptococcus pneumoniae* was successfully isolated from 6 samples (13%), as shown in Table 2. In contrast, 11 samples (24%) demonstrated bacterial growth on TSSBA without gentamicin; however, not all isolates were identified as *S. pneumoniae*. The comparative performance and detection efficiency of each culture medium, including their ability to recover *S. pneumoniae*, are summarized in Table 3.

Recent studies emphasize that media formulation, incubation atmosphere (e.g., 5% CO₂), and enriched nutrient content significantly enhance *S. pneumoniae* viability and detection.^{21,22} Additionally, antibiotic administration before sample collection has been shown to reduce culture sensitivity, leading to false negatives and underreporting.²³

TSSBA yielded 11 positive bacterial cultures (24%), but these included mixed flora such as *Micrococcus luteus* and *Staphylococcus epidermidis*, organisms often considered contaminants in blood cultures. In contrast, isolates grown on TSSBA-G exhibited the characteristic colony morphology of *S. pneumoniae*. Notably, while routine blood agar yielded 12 positive cultures (27%), none of these isolates were identified as *S. pneumoniae*. (Table 2).

The use of tryptic soy sheep blood agar, particularly when supplemented with gentamicin, significantly increased the yield of positive blood cultures, including specific recovery of *S. pneumoniae*. The tryptic soy agar (TSA) media is recommended for use as a general-purpose growth medium for the isolation, cultivation, and differentiation of a wide variety of microorganisms. The addition of 5% sheep blood from healthy and antibiotic-free donor animals. This media can also be used as a growth medium for other organisms than bacteria, namely yeast and fungi. The selective use of gentamicin in TSSBA-G was intended to suppress the growth of non-target bacteria, thereby enhancing the recovery of *S. pneumoniae* compared to TSSBA without gentamicin. No additional enrichment media, such as Todd Hewitt Broth, were employed in this

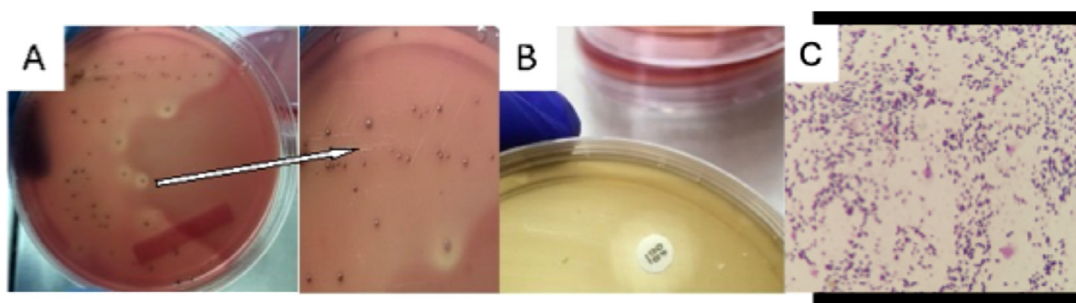


Figure 1. (A) *S. pneumoniae* Growth on TSSBA-G; (B) Optochin Disc Diffusion Test showing zone of inhibition confirming *S. pneumoniae*. (C) Microscopic appearance of *S. pneumoniae*

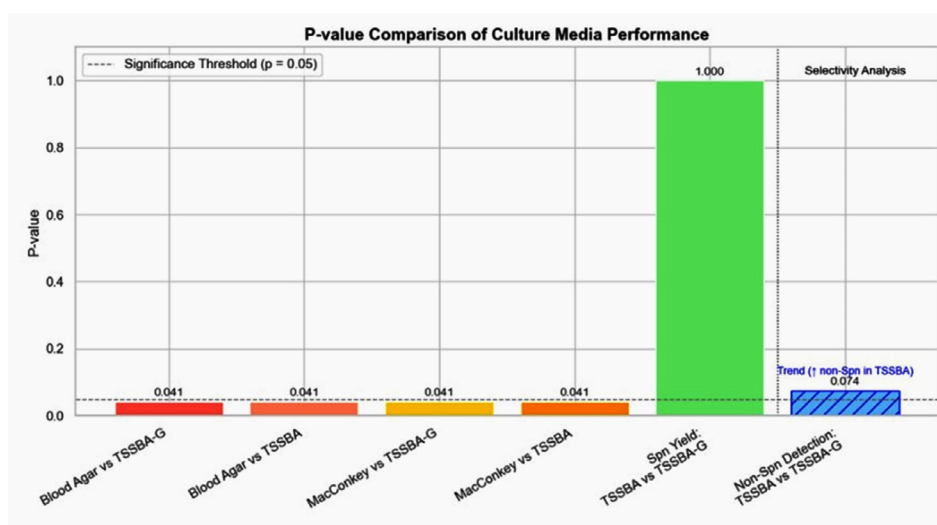


Figure 2. P-value Comparison of Culture Media Performance

Table 1. Bacterial Growth from Blood Samples Using Different Culture Media (n = 45)

Media Type	Total Positive Cultures n (%)	Total Negative Cultures n (%)	non- <i>S. pneumoniae</i> Identified Bacteria (n)
Routine Media			
Blood Agar	12 (27%)	33 (73%)	<i>S. aureus</i> (3), <i>S. haemolyticus</i> (1), <i>K. pneumoniae</i> (1), <i>A. baumannii</i> (1), <i>P. fluorescens</i> (1), <i>B. cepacia</i> (1), <i>P. mirabilis</i> (1), <i>Pseudomonas</i> sp. (1), <i>Micrococcus luteus</i> (1), <i>S. epidermidis</i> (1)
MacConkey Agar	6 (13%)	39 (87%)	*Only Gram-negatives bacilli 6 (13%)
Selective Media			
TSSBA-G	6 (13%)	39 (87%)	-
TSSBA (without gentamicin)	11 (24%)	34 (76%)	<i>S. aureus</i> (3), <i>Micrococcus luteus</i> (1), <i>S. epidermidis</i> (1)

Table 2. Detection of *Streptococcus pneumoniae* Across Different Culture Media (n = 45)

Media Type	<i>S. pneumoniae</i> detected n (%)
Routine Media	
Blood Agar	0 (0%)
MacConkey Agar	0 (0%)
Selective Media	
TSSBA-G	6 (13%)
TSSBA (without gentamicin)	6 (13%)

Table 3. Total Bacterial Growth on Routine and Selective Media

Media Type	Total Positive Cultures n (%)	<i>S. pneumoniae</i> detected n (%)
Routine Media		
Blood Agar	12 (27%)	0 (0%)
MacConkey Agar	6 (13%)	0 (0%)
Selective Media		
TSSBA-G & TSSBA (without gentamicin)	11 (24%)	6 (13%)
Total Bacterial Growth	29 (64%)	

study. The macroscopic characteristics of bacterial colonies growing on TSSBA media without gentamycin were large and slimy, whereas those on TSSBA with gentamycin were small.

Previous work demonstrates that gentamicin-supplemented blood agar increases yield and suppresses competing organism, thus improving detection sensitivity.¹³ Such selective media have been validated in multiple settings for enhancing recovery from polymicrobial or contaminated specimens.¹⁷ The primary culture results on TSSBA-G media incubated in a CO₂ incubator can be seen in Figure 1.

Figure 1 (A) shows that *S. pneumoniae* colonies on TSSBA-G α -hemolytic media appear within <24 hours, but the center of the pneumococcal colony becomes depressed after

24-48 hours. Figure 1 (B) shows a clear zone around an optochin disc (ethylhydrocupreine), indicating *S. pneumoniae* sensitivity. The TSSBA-G media supports the growth of pure *S. pneumoniae*, while TSSBA without gentamicin shows contamination by *Micrococcus luteus* and *Staphylococcus epidermidis*. As shown in Figure 1C, colonies grown on TSSBA-G displayed characteristic morphology consistent with *S. pneumoniae*.

These findings are further supported by Figure 2, which presents the p-values from McNemar's test comparing media performance. TSSBA-G significantly outperformed Blood Agar and MacConkey Agar ($p = 0.041$), while no significant difference was observed between TSSBA and TSSBA-G in isolating *S. pneumoniae* ($p = 1.000$). However, gentamicin appeared

to significantly reduce the presence of non-*S. pneumoniae* organisms ($p = 0.073$), indicating its value in reducing contamination.

Contamination in blood cultures can result from several sources, predominantly due to improper skin disinfection during sampling.^{24,25} The use of selective culture media, particularly those containing antimicrobial-binding agents, has proven effective in minimizing false positives caused by skin flora such as *Micrococcus* spp. and coagulase-negative staphylococci (CoNS).^{26,27} Studies on blood culture stewardship and predictive models indicate that improved protocols (sample collection, media selection, and handling) can reduce contamination and increase pathogen yield, supporting the operational feasibility of selective media such as TSSBA-G.^{16,28,29}

More than a third of septic patients had positive blood cultures, primarily due to inadequate sampling volume (50% of adults had <1.0 CFU/mL of blood) and prior antibiotic use. Additionally, 20%-30% of septic patients receive empiric antibiotics, which can reduce the likelihood of positive blood cultures.¹³ This study demonstrates that the use of TSSBA-G media, incubated anaerobically in a CO_2 incubator, can increase the rate of positive blood cultures.

Implementing selective media approaches like TSSBA-G holds promise especially in resource-limited settings where molecular diagnostics may not be accessible. Recent evidence suggests that combining selective media with faster subculture and incubation workflows can produce clinically meaningful improvements in detection without major increases in cost or complexity.

CONCLUSION

The use of Tryptic Soy Sheep Blood Agar with and without gentamicin (TSSBA-G and TSSBA) significantly increased the blood culture positivity rate for *S. pneumoniae*, the leading cause of bacteremia and pneumonia. While routine media (Blood agar and MacConkey) yielded positive cultures in 27% of samples (12/45), none isolated *S. pneumoniae*. In contrast, TSSBA-G isolated *S. pneumoniae* in 6 out of 45 samples (13%), and TSSBA detected a broader range of organisms in 24% of samples (11/45), albeit with

more contaminants. Statistical analysis using McNemar's test confirmed that both TSSBA-G and TSSBA detected significantly more pathogens than routine media ($p = 0.041$).

This study highlights the unique application of TSSBA agar, particularly the modified formulation with gentamicin (TSSBA-G), for the selective isolation of *S. pneumoniae* from blood culture specimens. The primary advantage of this approach lies in the ability of gentamicin supplementation to suppress competing flora, thereby improving the recovery of *S. pneumoniae*, which is often overgrown or masked in conventional blood agar cultures. From a long-term perspective, this method offers the potential to enhance routine laboratory detection of *S. pneumoniae* in resource-limited settings, where access to automated systems may be restricted. The simplicity, cost-effectiveness, and adaptability of TSSBA-G make it a promising alternative for broader clinical use, especially for surveillance programs and diagnostic workflows in hospitals with high burdens of pneumonia and bacteremia. Furthermore, the adoption of such selective media could support more accurate epidemiological studies and antimicrobial resistance monitoring over time.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Health Research Ethical Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, vide reference number 334/TGL/KEPK FK USU-RSUP HAM/2019.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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