Short Communication

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Perform or perish: laboratory optimization to avoid false negative blood cultures in pneumococcal bacteremia

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Abstract: We present the laboratory operations influencing the culture yield of pneumococci and suggest an alternate solution to the problem. Blood cultures of 136 adults and 19 pediatric patients were analyzed in two phases. In phase I, the laboratory operated only during weekdays but in phase II the laboratory provided 24-h services on all days. In phase I, successful bacterial isolation leading to testing of antimicrobial susceptibility was possible only in 51% of cases, while a significant 49% had failed to grow in subcultures due to autolysis resulting from delayed processing time. These false negative blood cultures were further confirmed as *Streptococcus pneumoniae* by commercial antigen tests. In phase II, we did not observe any false-negative results due to prompt subculturing protocols ($p < 0.001$). We also found that taking blood cultures on two occasions increases the yield by 46% among adults.

Keywords: false negative blood culture; laboratory optimization; pneumococcal antigen test; pneumococcal bacteremia.

Streptococcus pneumoniae is a well-known and serious cause of bacteremia in all age groups of patients. Bacteremic pneumococcal infections exhibit higher mortality compared to those without bacteremia. Early diagnosis and testing of susceptibility have an important role in a

favorable outcome [1]. Blood culture automation is a great advancement in the detection of pneumococcal bacteremia due to its higher recovery rate and shorter detection times [2]. Although *S. pneumoniae* grows rapidly in blood cultures, autolytic properties may distort the appearance in Gram stain or prevent growth on subculture jeopardizing the sensitivity of cultures [3]. We herein present a brief analysis of laboratory operations influencing the culture yield of *S. pneumoniae* in blood cultures and an alternate solution to the problem.

As we did not collect any patient related information, informed consent was not required. The study was exempted from IRB approval.

We analyzed the blood culture yield of *S. pneumoniae* during two time periods, between January 2012 and December 2014 (36 months – phase I) and between January 2015 and December 2017 (36 months – phase II), in a 2030-bedded tertiary care center in South India. The total cultures and blood culture incidence are detailed in Table 1. During phase I, clinical microbiology laboratory functioned only between 9 am and 5 pm (Monday–Saturday) and between 9 am and 12 pm (Sunday and public holidays). Thereafter, in phase II, the facility started to provide 24-h services on all days. Two blood cultures (each with 10 mL blood) were done in adults in a gap of 2–4 h and single in children (3–5 mL) using the BacT/ALERT FA Plus and BacT/ALERT PF (bioMérieux, Inc., Marcy l’Etoile, France) media, respectively. Bottles were preincubated at 37 °C in a satellite laboratory before entering into the continuous monitoring system in phase I during the laboratory closure hours and all the bottles were entered within 2 h of collection in phase II. Bottles flagged positive were processed by Gram stain, cultured on 5% sheep blood agar and incubated in an environment containing 5% CO₂. Identification and susceptibility of the isolates were done using Vitek-2 or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the VITEK MS system (bioMérieux, Inc., Marcy l’Etoile, France). Additionally, positive

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Table 1: Description of overall blood cultures and the results of *S. pneumoniae* bacteremia.

	Phase I <i>S. pneumoniae</i> N = 59, n (%)	Phase II <i>S. pneumoniae</i> N = 96, n (%)	Total <i>S. pneumoniae</i> N = 155
No. of pediatric patients undergoing blood cultures (N = 6411)	2758 (43)	3653 (57)	
No. of adult patients undergoing blood cultures (N = 78,444)	34,490 (44)	43,954 (56)	
The incidence of blood cultures in pediatric patients (per 1000 admissions)	102.3	116.6	
The incidence of blood cultures in adult patients (per 1000 admissions)	174.5	211.6	
Percentage of patients with pneumococcal bacteremia	0.158	0.201	0.179
Successful pneumococcal isolation $p < 0.001$ (CI: 36.11–61.42)	30 (51)	96 (100)	126 (81)
False negative blood culture results for pneumococci	29 (49)	0	29 (19)
Median TTP in hours ($\pm$ SD) (time from entry of the bottle until positive signal)	NA	10.24 ($\pm$ 2.24)	
Minimum TTP	NA	3.3	
Maximum TTP		16.2	
25th percentile		8.9	
50th percentile		10.2	
75th percentile		11.3	
90th percentile		12.2	
Time to entry into the system after collection	3–22 h	1–2 h	
Time to processing after flagging positive	1–22 h	30 ($\pm$ 5) min	
Occasions of positivity in adult blood cultures ^a N = 136			
Positive in the single bottle (one occasion)	14 (25)	23 (29)	37 (27)
Positive in both the bottles (both occasions)	42 (75)	57 (71)	99 (73)

^aData calculated only for adults where two blood cultures were done. TTP, time to positivity.

signaled bottles that did not show any bacteria or showed distorted bacteria in Gram stain and did not grow bacteria upon subcultures were further tested for pneumococcal antigens using the BD Pneumostrip™ *S. pneumoniae* Latex Test Kit (BD Biosciences, USA) (stopped later due to non-availability since 2014) and BinaxNOW® *S. pneumoniae* (Alere, USA) immuno-chromatographic antigen test kit [4] (since 2014), both targeting the capsular polysaccharide antigen of *S. pneumoniae*.

Descriptive statistics were used to present the data on successful bacterial isolation in both phases. Median time to positivity (TTP) was determined for the cultures in phase 2 using SPSS version 16. Comparison of two sample proportions was done to present the differences in blood culture yield in two phases using the two-sample z test; $p \leq 0.05$ was considered significant at 95% confidence interval (CI).

During the study period, we observed 155 cases of pneumococcal bacteremia, 59 (38%) in phase I and 96 (62%) in phase II in 136 adults and 19 pediatric patients. All children were below 8 years of age and adults between 18 and 92 years of age. The majority (85, 55%) of patients were over 50 years of age and 51 (33%) were between 18 and 50 years of age. There was an increase in pneumococcal detection during phase II by 0.043%. In phase I, detection of bacteria suggestive of pneumococci in Gram stain and culture isolation was successful in 30 (51%) cases, while failure to isolate in culture despite being flagged positive termed

as false-negative results in this study was experienced in 29 (49%) cases. However, all culture bottles had flagged positive within 24 h of entry into the automated blood culture system and shown dark yellow color of the sensor of the bottle at the time of unloading. All the false-negative bottles were tested positive for pneumococcal antigen by commercial tests. In the second phase, we did not observe any false-negative results. The median TTP in this phase was 10.2 ± 2.2 h. Among adult patients, pneumococci were detected by any method only in one blood culture bottle in 27% of cases but 73% of cases had evidence of pneumococci in both the blood cultures. Thus, the practice of two blood cultures showed a 46% increase in the overall yield. The details of blood culture results are shown in Table 1.

Prompt and appropriate antimicrobial therapy reduces mortality in bacteremia. Blood cultures are an essential, valuable and timely tool in the diagnosis of pneumococcal bacteremia. Our results endorse the challenges in the pre-analytical and analytical phases of this important test and suggest a workable solution for clinical microbiology laboratories with various workforce constraints.

Invasive pneumococcal disease, although a serious health concern, still remains underreported in many developing nations of Africa and Asia including the Indian subcontinent [5], which may be attributed to low utilization of diagnostic tools and limited access to care. Knowledge of serotype groups, antimicrobial resistance and disease burden of invasive pneumococcal disease

(IPD) is sparse in these regions [6]. While a large reduction in IPD and pneumonia has been seen in countries that have introduced pneumococcal conjugate vaccines (PCVs), developing nations still show low immunization rates with pneumococcal vaccines both in children and adults. Mortality in pneumococcal bacteremia is greatly influenced by time to initiation of appropriate therapy [7]. Given the fact that a significant number of pneumococcal bacteremia present with no detectable source, the favorable clinical outcome is still dependent on successful and early blood culture results [8].

Although current quality indicators (QIs) in laboratory science focus on the performance of analytical processes, there is an increasing body of evidence suggesting the risk of errors in the pre-analytical phase as well [9]. In the blood culture technology, less attention has been paid to several logistic factors that influence the time elapsing between blood sampling to the start of blood culture processing in the laboratory [10]. Our study draws attention to several QIs of blood culture testing such as turnaround time, pre-analytical and analytical processes that have serious consequences on patient safety when dealing with serious bacterial infections such as pneumococcal bacteremia. We also emphasize the importance of laboratory working hours for successful culture isolation of rapidly perishable bacteria such as pneumococcus.

Ideally, blood culture bottles should be entered into the automated systems for continuous monitoring. However, many laboratories do not provide 24-h services and remain closed during weekends forcing the blood culture bottles to be held at room temperature or pre-incubation at 37 °C. The latter process induces the bacteria to enter into the log phase early followed by the stationary phase, especially among fastidious bacteria [11]. In pneumococcal bacteremia, due to a very rapid growth phase, sometimes as early as 3 h, and autolytic property, bacteria disintegrate and become non-viable undesirably affecting identification and antimicrobial susceptibility testing. We observed that the 90th percentile of pneumococcal bacteremia cases flagged positive by 12.3 h and all the cases were detected by 16.30 h when standard recommendations of pre-analytical processes are followed. The golden time for optimum detection of pneumococcal bacteremia hence lies within the first 8–12 h. In the right clinical settings, immediate testing of blood culture fluid by rapid antigen tests would be the best approach to initiate early appropriate therapy in these cases. In the first phase of the study, delayed entry into continuous monitoring systems, pre-incubation at 37 °C and delayed processing of positive blood culture bottles during laboratory closure hours were the major limiting factors for

pneumococcal isolation leading to a significantly high (49%) false negative results. However, understanding of clinical conditions and application of right alternative tests could lead to filling the diagnostic gaps in our study. Interestingly, all the cases of pneumococcal bacteremia in this phase, both true positive and false negative, were diagnosed within 24 h of entry into blood culture systems and all bottles had shown yellow color of the sensor at the bottom of the culture bottle. In the second phase, when all the standard practices of blood culture were followed and the laboratory was operational 24 h, we did not observe any false negative results.

Throughout the study, we did blood sampling on two occasions in a gap of 2–4 h among adult patients for the diagnosis of pneumococcal bacteremia [12]. Pneumococci were detected in both the occasions in 73% of cases, while in 27% of cases the result was evident in one of the two occasions. Although sufficient evidence shows that the number of blood cultures and volume of blood drawn greatly influence the culture yield, this is not practiced in several centers in resource-limited countries. Our findings reiterate the practice of multiple blood cultures wherein we could have missed the diagnosis of pneumococcal bacteremia significantly (nearly one third) had we done culture only on one occasion.

We report a few limitations in our study findings, especially in phase I. We could not accurately measure the lapses in the pre-analytical and analytical phases such as time to blood collection and entry into the automated blood culture system and secondly, time to processing delay due to the retrospective nature of the study. TTP in phase I was unavailable as the laboratory did not maintain regular retrieval of such data from the automated system, while in phase II, introduction of the VITEK MS system could provide the details. However, we highlight the importance of optimizing operations in a clinical laboratory.

Under circumstances when the laboratory hours of operation cannot meet the clinical urgency of results, alternate methods such as rapid antigen testing should be sought to increase the clinical relevance of microbiological testing.

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