Comparison of Conventional and Automated Blood Culture Methods for The Diagnosis of Neonatal Septicemia

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Abstract
Neonatal Septicemia is a serious clinical syndrome and the definitive diagnosis is based on positive blood cultures which are obtained either by conventional or automated method. Early availability of proper isolation and identification of causative bacteria facilitates the timely initiation of appropriate antibiotic therapy. Thereby the present study was conducted to identify the bacterial causes of neonatal septicemia in the fastest possible time by comparing conventional and automated blood culture methods. This cross-sectional study was done during the period from January 2018 to December 2018 and included clinically suspected cases of neonatal septicemia admitted to Neonatal Intensive Care Units of Chattogram Medical College Hospital (CMCH) and Chattogram Maa-Shishu O General Hospital (CMSOGH). Out of 178 samples, automated method detected 29 (16.3%) and conventional method detected 26 (14.6%) blood culture positive samples. The yield of bacteria by automated method was 100% and by conventional method was 89.7%. Number of bacteria isolated only by automated method were 3 (10.2%). Mean time for isolation of bacteria by automated method was 26.38 hours and by conventional method was 46.34 hours. Automated method detected 47.05% of isolated bacteria in first 24 hours but none of them were detected by conventional method within first 24 hours. Among the isolated bacteria, Klebsiella spp was most common (62.0%). Most of the isolates were resistant to Ampicillin, Cefotaxime and Ceftazidime. Analyzing the findings of the study, there was no significant difference in the rate of isolation in each time interval (p=0.157) of two methods but there was significant difference in the mean time of isolation of bacteria between two methods (p=0.000004).

Introduction
Neonatal sepsis remains the most serious problem in neonatal intensive care and results in significant morbidity and mortality¹. About 20.2% of death of newborns in Bangladesh are due to sepsis². All neonates suspected of having sepsis should have a blood sample sent for cultures³. Blood cultures, which are “gold standard” of Blood Stream Infection, are used to detect viable pathogens in blood, have the advantage of allowing the evaluation of their antimicrobial susceptibility⁴. Various manual blood culture systems are enlisted among which monophasic medium is one that consists of 50-100 ml of brain heart infusion broth/trypticase soy broth⁵. Advantages of manual blood culture system include cost-effectiveness and usefulness in small laboratories. The three main commercially available automated blood culture systems include BacT/ALERT blood culture systems, BACTEC 9000 series and the Versa TREK system. The advantages of these systems encompass higher sensitivity for organism recovery, faster time to positivity, fully automated and computerized⁶. The ideal blood culture system assembles the maximum yield of pathogen as early as possible in order to have maximum influence on patient management⁷. Different comparative studies including ours have reported different percentages of bacterial growth along with yield of bacteria by both methods⁸,⁹. In case of life threatening conditions like neonatal sepsis, irrational use of antibiotics have swayed the sensitivity pattern of microbes, which are evident in several recent studies thus making the use of unconventional drugs compulsory and lifesaving¹⁰,¹¹. So, earlier detection of bacteria is of utmost importance for facilitating the accurate treatment with the required antibiotics that minimizes the use of unnecessary antibiotics. The scarcity of relevant data among our local population have strengthen the need for an amending study regarding functionalities of both conventional and automated blood culture methods along with antibiotic susceptibility patterns.
Materials And Methods
This cross sectional study was carried out in The Department of Microbiology, Chattogram Medical College Hospital (CMCH) and the Department of Microbiology, Chattogram Maa-O-Shishu Hospital Medical College (CMOSHMC), Chattogram from January 2018 to December 2018. A total of 178 neonates admitted to Neonatal Intensive Care Units (NICU) of CMCH and CMOSHMC, who had accomplished the eligibility criteria of clinically suspected cases of neonatal sepsis were included in the study.

Methods of collection and inoculation of blood sample: After explaining the procedure and taking written informed consent to the patient parties, a single sample of 2 ml of venous blood was drawn from each patient. Strict skin antisepsis was performed following the established guidelines. After removing the syringe and needle from venipuncture site, the sampling needle were discarded and replaced by fresh sterile needle. The top of the rubber stopper of both conventional and automated blood culture bottles were disinfected by 70% ethyl alcohol swab, than 1ml of blood were introduced in the conventional blood culture bottle containing 10 ml trypticase soya broth and 1 ml of blood was introduced in the automated blood culture bottle. Immediately after introduction of blood, inoculated bottles were gently shaken a few times to mix the blood in the broth medium. These procedures were performed at the bedside of patients.

Laboratory procedure: Both manual and automated blood culture bottles were incubated at 35°C to 37°C aerobically. For the purpose of isolation of bacteria in conventional methods, dehydrated SPS (Sodium Polyanethol Sulphonate) and TSB base were used to prepare 10 ml of broth according to standard laboratory procedure. The inoculated bottles were periodically examined for macroscopic evidences such as turbidity, hemolysis, puffballs and gas production. Initial blind subcultures were performed after 12-18 hours or after overnight incubation. Subcultures were done in blood agar, MacConkey agar and Chocolate agar medias as soon as macroscopic changes were observed and also in absence of macroscopic changes, subcultures were done at least twice during the first 2-3 days. When no growth was observed by subcultures, then a final subculture was done before discarding the bottle after 7 days of incubation. Microscopic examination of gram stained smears prepared from colonies from subcultures were done accordingly. After isolation mean time for total number of isolated bacteria was calculated, then mean deviation was calculated, after that Standard Deviation was calculated, using the formula SD=$\sqrt{\sum(x_{i}-\bar{x})^2}$. Total mean for isolation of bacteria was calculated by using the formula mean±SD.

Identification of isolated bacteria along with the antimicrobial susceptibility testing was performed by modified Kirby Bauer Disc Diffusion Method according to The Clinical and Laboratory Standards Institute (CLSI) guideline 2017 and Food and Drug Administration (FDA) 2013.

Isolation of bacteria by automated method: BACTEC FX 40 blood culture systems were used. The pediatric version of aerobic blood culture bottles of above automated systems each containing 30 ml of complex medium with inoculated 1 ml of blood samples were loaded into the respective machines and then continuously monitored at 10-24 minutes intervals for evidences of growth. They were incubated at 37°C for up to 5 days when no signal was recorded. Whenever the machines gave positive signal, Time To Positivity (TTP) was noted. Time To Positivity (TTP) is a parameter provided by the automated blood culture system and is calculated from the time of incubation until a positive signal is detected. The total time of isolation by automated method was calculated by adding TTP and time taken for positive subculture. The mean time for isolation of bacteria was calculated. Total mean for isolation of bacteria was calculated using the equations used in conventional method. After that the comparison between two means (conventional and automated) method was done by unpaired t-test. Steps of laboratory procedures after signal positive bottles were taken for subcultures.

Data analysis: The results of the experiments were recorded systematically and statistical analysis was done by SPSS for Windows version 20 software. Statistical significance was defined as P < 0.05 and confidence interval was set at 95% level. $\chi^2$(chi-square) test was done to test the significances of calculated results. Unpaired t-test was done to compare the calculated means.
Results

Table-I: Results of positive blood culture by conventional and automated methods among study population (n=178):

<table>
<thead>
<tr>
<th>Methods</th>
<th>Number</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>26</td>
<td>14.6</td>
</tr>
<tr>
<td>Automated</td>
<td>29</td>
<td>16.3</td>
</tr>
</tbody>
</table>

P-value=0.157, P>0.05, statistically no significant difference.

Table-I shows the rate of blood culture positivity by automated and conventional blood culture methods among study population. Among the study population of 178, 29 (16.3%) samples were positive by automated method and 26 (14.6%) were positive by conventional method.

Table-II: Comparison of yield of bacteria by conventional and automated methods (n=29):

<table>
<thead>
<tr>
<th>Methods</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>26</td>
<td>89.7</td>
</tr>
<tr>
<td>Automated</td>
<td>29</td>
<td>100</td>
</tr>
</tbody>
</table>

P-value= 0.157, P>0.05, statistically no significant difference.

Table-II shows among 29 samples with positive growth, automated method detected 29 (100%) samples, while conventional method detected 26 (89.7%) samples.

Table-III: Distribution of isolated bacteria by conventional and automated methods (n=29):

<table>
<thead>
<tr>
<th>Name of the bacteria</th>
<th>Number (%) of bacteria isolated by both methods</th>
<th>Number (%) of bacteria isolated by only automated method</th>
<th>Number (%) of bacteria isolated by only conventional method</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella spp</td>
<td>18 (62.0%)</td>
<td>0</td>
<td>0</td>
<td>18 (62.0%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1 (3.4%)</td>
<td>1 (3.4%)</td>
<td>0</td>
<td>2 (6.8%)</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>6 (21.0%)</td>
<td>0</td>
<td>0</td>
<td>6 (21.0%)</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>1 (3.4%)</td>
<td>1 (3.4%)</td>
<td>0</td>
<td>2 (6.8%)</td>
</tr>
<tr>
<td>Serratia spp</td>
<td>0</td>
<td>1 (3.4%)</td>
<td>0</td>
<td>1 (3.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (89.8%)</td>
<td>3 (10.2%)</td>
<td>0</td>
<td>29 (100%)</td>
</tr>
</tbody>
</table>

P-value of bacteria isolated by both methods = 0.241.
P-value of bacteria isolated by only automated method = 0.287.
P-value of bacteria isolated by only conventional method = NA. (P-value reached from chi-square test).

Table-III shows distribution of isolated bacteria by conventional and automated blood culture system. The highest number of bacteria isolated were *Klebsiella* spp 18 (62.0%), followed by *Acinetobacter* spp 6 (21.0%), both *Pseudomonas* spp and *Escherichia coli* each 2 (6.8%) and *Serratia* spp 1 (3.4%). Among the 29 isolated bacteria, 26 (89.8%) were isolated by both conventional and automated methods and 3 (10.2%) were isolated only by automated method. No bacteria was isolated only by conventional method. The bacteria isolated only by automated methods 3 (10.2%) were *Escherichia coli*, *Pseudomonas* spp and *Serratia* spp.

Table-IV: Comparison of two methods depending on time taken to be blood culture positive:

<table>
<thead>
<tr>
<th>Time</th>
<th>Number (%) of isolated bacteria</th>
<th>Mean±SD (in hours) (n=29)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional Automated</td>
<td>Automated</td>
<td></td>
</tr>
<tr>
<td>12-24 hours</td>
<td>0</td>
<td>15 (51.7)</td>
<td>0.157</td>
</tr>
<tr>
<td>24-48 hours</td>
<td>13 (50.0%)</td>
<td>14 (48.3%)</td>
<td>0.157</td>
</tr>
<tr>
<td>48-72 hours</td>
<td>13 (50.0%)</td>
<td>14 (48.3%)</td>
<td>0.157</td>
</tr>
<tr>
<td>&gt;72 hours</td>
<td>0</td>
<td>0</td>
<td>0.157</td>
</tr>
</tbody>
</table>

P-value of rate of isolation in each time interval was 0.157 (reached through chi-square test), P>0.05; no significant difference in the rate of isolation.

P-value of two means was 0.000004, (reached through unpaired t –test), t value= 5.100, P<0.05, so difference between two means is statistically significant.

Table-IV shows the time (in hours) interval for the isolation of bacteria. Mean time for isolation of the bacteria in automated method was 26.4±4.67 hours, whereas in conventional method the mean time was 46.3±20.53 hours. 15 (51.7%) of the bacteria were identified within 24 hours and 14 (48.3%) by > 24-48 hours in automated method. So, up to 48 hours, total 29 (100%) bacteria were isolated in automated method. In conventional method, no
bacteria could be isolated before or at 24 hours. Equal numbers of bacteria were isolated by conventional method in > 24-48 hours interval and > 48-72 hours interval which was 13 (50.0%).

**Table-V: Rate of contamination of blood cultures by conventional and automated method (n=178):**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>7</td>
<td>3.9</td>
</tr>
<tr>
<td>Automated</td>
<td>5</td>
<td>2.8</td>
</tr>
</tbody>
</table>

P- value = 0.157. P > 0.05, statistically no significant difference.

Table-V shows the rate of contamination in conventional method were 7 (3.9%) and automated blood culture method were 5 (2.8%) among 178 blood samples.

**Table-VI: Antimicrobial susceptibility pattern of important isolated bacteria against different antimicrobial agents:**

![Antimicrobial susceptibility pattern chart](chart.png)

Table-VI shows the susceptibility pattern of important isolated bacteria against different antimicrobial agents. Among the 18 isolated *Klebsiella* spp, 18 (100%) were resistant to Ampicillin, Cefotaxime and Ceftazidime, followed by 17 (94.4%); resistant to Amikacin and 16 (88.89%) to Piperacillin-tazobactum, 14 (77.78%) to Gentamicin, 11 (61.1%) to Meropenem and 5 (27.81%) to Tigecycline. Among the 6 isolated *Acinetobacter* spp, 6 (100%) were resistant to Ampicillin and Cefotaxime, followed by 5 (83.4%) to Amikacin, Gentamicin and Ceftazidime each, 4 (66.7%) to Meropenem and 2 (33.3%) to Piperacillin-tazobactum.

**Discussion**

In our study, out of 178 blood culture samples, 29 (16.3%) isolates were culture positive by automated method and 26 (14.6%) of them were positive by conventional method. These findings are similar with a comparative study that stated 24.1% positive blood culture detected by automated method and 17.9% positive blood culture by conventional method. The yield of bacteria by two methods was also compared in our study. It showed that yield of bacteria by automated method was 100% (29/29) as compared to conventional method which had 89.7% (26/29) yield of bacteria. These findings are similar to a study that showed yield of bacteria by automated and conventional methods were 96.9% and 80%. Our study showed among the isolated bacteria, *Klebsiella* spp (62.0%) was predominant, followed by *Acinetobacter* spp (21.0%), *Escherichia coli* and *Pseudomonas* spp (6.8%) each and *Serratia* spp (3.4%). Another recent study had the same finding of highest number of *Klebsiella* spp (30.66%) followed by *Acinetobacter* spp (20.0%). The present study showed among the 29 culture positive isolates, 3 (10.2%) were positive only by automated method but none was positive only by conventional method. This may be due to composition of automated vials that contain either resin or charcoal which are responsible for effective removal of antimicrobial agents from blood whereas conventional bottles do not contain these ingredients. So removal of antimicrobial agents is not possible in conventional method. Another congruous study had the findings of 32% blood culture positive samples only by automated method but none were positive by conventional method. The rate of isolation of bacteria in relation to time has been calculated in our study. The earliest time of isolation of bacteria by automated method was within 12-24 hours interval and the rate of isolation was 51.7% but no bacteria was isolated in 12-24 hours interval by conventional method. The similar findings of 45% isolated bacteria by automated method but none by conventional method in 12-24 hours interval was found in another study that correlated with our study. In the present study highest rate of isolation of bacteria by conventional method was 50.0% in > 24-48 hours. It correlates with a finding of 57.73% isolated bacteria in a comparative study. Another study stated 34% of isolated bacteria within 48 hours by conventional method. In our study, mean time for isolation of bacteria by conventional and automated methods were 46.34 hours and 26.38 hours which is similar to a study that showed mean time for conventional and automated methods as 51.09 hours and
28.09 hours\textsuperscript{32}. Another study stated that mean time for conventional and automated methods were 66.95 hours and 15.83 hours\textsuperscript{33}. In the present study, bacterial isolates were tested for antimicrobial susceptibility by Modified Kirby Bauer Disc Diffusion technique according to CLSI guideline 2017 and FDA guideline 2013. Among the 18 isolated Klebsiella spp, all were resistant to Ampicillin, Cefotaxime and Ceftazidime which is similar to a relevant study\textsuperscript{34}. In our study, most Klebsiella spp were sensitive to Tigecycline which is concordant to a similar study\textsuperscript{35}. Another similar study showed that Klebsiella spp was mostly sensitive to Meropenem\textsuperscript{36}. Among the 6 isolates of Acinetobacter spp, all of the isolates (100\%) were resistant to Ampicillin and Cefotaxime, followed by 83.4\% to Amikacin, Gentamicin and Ceftazidime. Similar resistance against Ampicillin and Cefotaxime were observed in a study in Pakistan\textsuperscript{34} and against Cefazidime, Amikacin, and Gentamicin 85.7\% each in a recent Bangladeshi study\textsuperscript{31}. Our study showed that Acinetobacter spp were mostly sensitive to Piperacillin-tazobactum (66.66\%) which is similar to a recent study\textsuperscript{37}.

A high level of resistance was observed against Ampicillin, Cefotaxime and Ceftazidime in our study against isolated bacteria which is very alarming. In our study, automated system of blood culture had significantly shorter meantime for isolation of bacteria than conventional blood culture system. Many of the laboratory facilities dealing with large number of samples in our country are still based on conventional blood culture system which is labor-intensive for the manpower of the laboratories and also consumes more time and thus delivery of antibiotic sensitivity reports of the patients are further delayed.

**Conclusion**

Conventional method of blood culture was found to be as efficient as automated blood culture method in respect to rate of isolation of bacteria and yield of bacteria though automated method had significantly shorter mean time of isolation of bacteria than conventional method. However, it is impossible to assume a complete picture of comparison between conventional and automated blood culture methods for the diagnosis of neonatal septicemia with different constraints such as limitation of time period and samples. Klebsiella spp was the commonest bacteria isolated by both methods. The isolated bacteria were resistant to most of the antimicrobial agents. So, establishment of automated blood culture system in hospitals where large numbers of patients get admitted can be an alternative to reduce the workload of microbiology laboratory. For this purpose, focusing on maintaining cost effectiveness of automated method along with the accessibility of other requirements should be accepted as areas of concerns.

**Disclosure**

The author reports no conflicts of interest in this work.

**Acknowledgements**

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**References**


Culture systems are enlisted among which monophasic blood, have the advantage of allowing the evaluation of cultures in blood, which are “gold standard” of and mortality. About 20.2% of death of newborns in Bangladesh are due to sepsis. All neonates suspected of commercially available automated blood culture systems computerized. The ideal blood culture system assembles incubation. Microscopic examination of gram stained puffballs and gas production. Initial blind subcultures culture bottle containing 10 ml trypticase soya broth and inoculated 1 ml of blood samples were loaded into the systems each containing 30 ml of complex medium with significance was defined as \( P < 0.05 \) and confidence inter-
taken for subcultures.

Isolation of bacteria by automated method:

178, 29 (16.3%) samples were positive by automated method and 26 (14.6%) were positive by conventional method. The bacteria isolated only by automated method were observed in a study in a level 2 neonatal care unit in India. Sr Lanka journal of child health. 2017;46(3):259-261.


Ampicillin and Cefotaxime were observed in a study in an intensive care unit in Pakistan armed forces medical journal. 2018;68(6): 1654-1658.