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COMPARISON OF BACTEC VERSUS CONVENTIONAL METHOD FOR CULTURE OF STERILE BODY FLUIDS IN A TERTIARY CARE HOSPITAL

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ABSTRACT: Aim & Background: Body fluids are sent to clinical microbiology laboratory for culture to find the etiological agent causing the infection. This study was conducted to compare the culture results of sterile body fluids processed simultaneously by both conventional method and BACTEC blood culture system. **Methods:** Sterile body fluids except blood were included in the study. A total of 61 body fluid samples were received and simultaneously processed by both the culture methods-conventional and BACTEC. **Results:** In this study, overall culture positivity for BACTEC was 85.25% as compared to 59.02% by conventional system. According to the results of our study, the overall culture positivity was increased by 26.23% by BACTEC method than the conventional method. BACTEC blood culture system detected more pathogenic isolates (52.45%) than the routine conventional culture method (21.31%) and it was found to be statistically significant (*p* value=<0.005). The most common pathogenic micro-organisms detected by conventional and BACTEC methods in this study were Gram-negative followed by Gram-positive. The mean time to detection of pathogenic isolates by BACTEC and conventional method was 1.19(+/- 0.39) days and 2.00(+/- 1.00) days and the difference was statistically significant (p value=0.013). **Conclusion:** The study recommends the use of automated blood culture system for culture of sterile body fluids as it was found to improve the yield of isolates with reduced time to detection. However, cost may be a limiting factor in resource constrained settings.

INTRODUCTION: Sterile body fluid infections can be caused by both Gram-positive and Gram-negative bacteria. Gram-negative bacteria like *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumanii* and *Pseudomonas aeruginosa* are the frequently isolated pathogens followed by Gram-positive bacteria like *Staphylococcus aureus* and *Enterococcus* [1-4]. Presence of microorganisms in normally sterile body sites causes life-threatening infections [2, 5].

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For early and accurate diagnosis of those infections, sterile body fluids are required to be sent to microbiology laboratory for culture [2, 5]. The conventional method used for culture of sterile body fluids, is culture on a solid medium with or without an enrichment broth. In addition, various procedures, such as filtration or centrifugation, are required to concentrate organisms within the specimen.

However, there are many challenges for recovery of microorganisms by conventional culture methods: Low number of microorganisms; low volume of sample e.g: CSF and synovial fluids; initiation of antibiotic therapy prior to collection of sample which lead to the false negative results; fastidious organisms may be missed if proper media or supplements are not incorporated into the

culture method. Conventional methods of culture are now being replaced by automated culture systems like BACTEC (Becton Dickinson Diagnostic Instrument) because of reduced time for culture and ease of laboratory work using machines and higher isolation rate against conventional methods. The main objective of this study was to compare the culture results of sterile body fluids processed simultaneously by both conventional method and BACTEC blood culture system.

MATERIALS AND METHODS: This study was conducted after taking permission from the Institutional Ethics Committee. The IEC approval number of this study was No. BFUHS/2K22p-TH/7232. A total of 61 sterile body fluids except blood were included in this study.

Gram stain was done immediately followed by direct plating of sample onto conventional media – Blood agar, MacConkey agar, Chocolate agar and the media was incubated at 37°C for 18-24 hours as per standard protocol. Out of the remaining sample, half of the sterile body fluid was inoculated into aerobic BACTEC culture bottle (Becton Dickinson Diagnostic Instrument Systems) and remaining half was inoculated into conventional enrichment broth (Brain-heart infusion broth). Conventional broth was incubated and processed as per standard protocol. Subcultures from the broth were done at the end of 24 hour, 72 hour and 7^{th} day of incubation. If no growth was detected at the end of 7 days of incubation, it was reported negative. The BACTEC bottle was incubated in BACTEC 9120 system till signal for growth was detected. When positive signal was observed, the bottles were unloaded from instrument and Gram stain and cultures on Blood agar, MacConkey agar, Chocolate agar were performed as per the standard microbiological protocols. Identification of the micro-organisms was done by observing the colony morphology, gram staining and biochemical reactions as per standard microbiological protocol [6]. BACTEC bottles giving no signal were reported negative after 5 days of incubation.

The isolation rate of pathogenic microorganisms, the time to culture positivity, *i.e.,* time taken from incubation to growth detection for conventional and signal positive for BACTEC 9120 was noted and mean time to detection was calculated for both methods. Kirby-Bauer's disc diffusion method was used for antibiotic susceptibility testing and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [6]. The antibiotics used for Gram-negative organisms were (concentration /disk in microgram)- Cefotaxime (30µg), Ceftriaxone (30µg), Amikacin (30µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Cefepime (30µg), Meropenem (10µg), Piperacillin-Tazobactam (100/10µg): The antibiotics used for Gram-positive organisms were (concentration / disk in microgram) Ampicillin (10µg), Erythromycin (15µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Linezolid (30µg), High-Level Gentamicin (120 µg) ATCC Control for Gram-negative micro-organisms: ATCC *E. coli* 25922 ATCC Control for Gram-positive micro-organisms: ATCC *S. aureus* 25923

Vancomycin Screen Agar Test: Method was considered for interpretation of susceptibility / resistance to vancomycin in Staphylococcal isolates as per CLSI guidelines.

Statistical Analysis: The data pertaining to sociodemographic and other clinical variables was entered in the form of data matrix in Microsoft "Excel" and analysed using Statistical Package for Social Sciences (SPSS).Detailed data of the patients was recorded in the proformas. Appropriate test of significance was applied using the statistical package for social sciences. At any point of time, p value of 0.05 was used as a threshold for significance.

RESULTS: The most common age group in this study belonged to 41-60 years (33%), followed by 21-40 years (28%) and </=20 years (26%). The least common age group was 61-80 years (13%). Out of 61 body fluid samples, majority were ascitic fluid (32.8%), pleural fluid (32.8%), followed by CSF (29.5%). The least were of pericardial fluid (3.3%) and synovial fluid (1.6%) as shown in **Fig. 1**. Overall 52 specimens were detected culture positive by BACTEC as compared to 36 culture positive specimens by conventional method. Exact McNemar's test was applied and according to this evaluation, the difference between the methods in respect to overall culture positive specimens was found to be statistically significant *(p* value<0.005) as shown in **Table 1**.

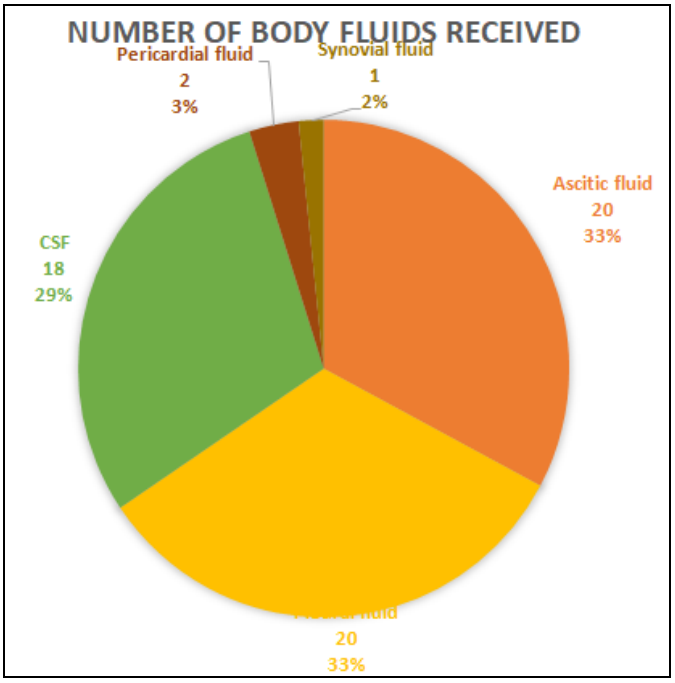

FIG. 1: SAMPLE-WISE DISTRIBUTION OF DIFFERENT BODY FLUIDS

TABLE 1: COMPARISON OF CULTURE METHODS ACCORDING TO OVERALL CULTURE POSITIVE /NEGATIVE

Method	BACTEC positive	BACTEC negative	Total
Conventional Positive	36	0	36
Conventional Negative	16	9	25
Total	52	9	61

Conventional Positive/BACTEC Positive=culture positive p value <0.005*

Pathogenic Isolates and Contaminants: BACTEC method detected 32 pathogenic isolates whereas the conventional culture method detected only 13 pathogenic isolates. McNemar-Bowker's test was applied and according to this evaluation, the difference between the methods in respect to pathogenic microorganisms was found to be statistically significant (*p* value=<0.005) as shown in **Table 2**.

TABLE 2: COMPARISON BETWEEN METHODS ACCORDING TO CONSIDERING THE MICROORGANISMS AS PATHOGENIC/ CONTAMINANT

Method	BACTEC Pathogenic	BACTEC Contaminant	BACTEC Negative	Total
Conventional Pathogenic	13	0	0	13
Conventional Contaminant	12	11	0	23
Conventional Negative	7	9	9	25
Total	32	20	9	61

p value<0.005.

Out of 32 pathogenic isolates detected by BACTEC, the most common microorganism was *Escherichia coli* followed by *Klebsiella pneumoniae, Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Methicillin-susceptible Staphylococcus aureus (MSSA), Methicillin-resistant Coagulase-Negative Staphylococci (MRCoNS), Citrobacter freundii, Enterococcus* and *Acinetobacter baumanii*. Out of 13 pathogenic isolates detected by the conventional method, the most common microorganism was *MRSA* followed by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus, Citrobacter freundii* and *Acinetobacter baumanii* as shown in **Table 3.**

TABLE 3: DISTRIBUTION OF PATHOGENIC MICROORGANISMS ISOLATED BY BOTH THE CULTURE METHODS

Microorganism	Growth in conventional method	Growth in BACTEC system
MRSA	4(30.7%)	5(15.6%)
MSSA	0	3(9.4%)
MRCoNS	0	3(9.4%)
Enterococcus	1(7.7%)	1(3%)
Escherichia coli	2(15.4%)	7(22%)
Klebsiella pneumoniae	2(15.4%)	6(18.8%)
Citrobacter freundii	1(7.7%)	2(6.3%)
Pseudomonas aeruginosa	2(15.4%)	4(12.5%)
Acinetobacter baumanii	1(7.7%)	1(3%)
Total	13	32

The comparison of time to detection of pathogenic isolates by both the methods is shown in **Table 4**. The mean time to detection of pathogenic isolates was 1.19 (+/- 0.39) days by BACTEC as compared

to 2.00 (+/-1.00) days by conventional method. Independent-Samples T test was applied and according to this evaluation, the difference between the methods in respect to mean time to detection of pathogenic isolates was found to be statistically significant (*p* value=0.013) **Table 4**.

TABLE 4: COMPARISON OF TIME TO DETECTION OF GROWTH OF PATHOGENIC ORGANISMS

Serial no.	Method	Day 1	Day 2	Day 4	Total no. of pathogenic isolates
1.	Conventional	4(30.8%)	7(53.8%)	2(15.4%)	13
2.	BACTEC	26(81.2%)	6(18.8%)	0	32

DISCUSSION: Body fluids like pleural fluid, peritoneal fluid, CSF, synovial fluid and pericardial fluid are usually sterile. There are certain common pathogenic bacteria like *Escherichia coli, Klebsiella species, Staphylococcus aureus, Neisseria meningitidis,* Non fermenting Gram-negative bacilli, *Pseudomonas, Acinetobacter species,* which invade and infect the sterile body fluids leading to morbidity and life-threatening infections [2, 5, 7]. Hence infections of sterile body fluids are a medical emergency and need an early diagnosis and effective treatment [7].

In the present study, overall culture positivity for BACTEC was 85.25% as compared to 59.02% by conventional system. The recovery rate with the BACTEC culture method was higher than with conventional culture methods and the difference was statistically significant (p value<0.005). Similar results of higher positivity by BACTEC culture method were obtained by many authors in their studies on sterile body fluids [8-12].

The results of this study showed that overall 36(59.02%) specimens were culture positive by both the methods, 16(26.23%) were positive by BACTEC only and 9(14.75%) were sterile by both the methods. Conventional culture did not detect bacteria in any instance not detected by BACTEC. Similar findings have been reported by Cetin *et al*., and Mengeloglu *et al* [9, 10]. According to the results of our study, the overall culture positivity was increased by 26.23% by BACTEC method than the conventional method. In this study, pathogenic microorganisms were isolated from 13(21.31%) specimens by both culture systems; however, for 19 specimens (31.14%), growth was detected only with the BACTEC system. BACTEC blood culture system detected more pathogenic isolates (52.45%) than the routine conventional culture method (21.31%) and it was found to be statistically significant. In other studies conducted by various authors, similar findings of increased isolation of pathogenic microorganisms by BACTEC as compared to conventional culture were seen [8-13]. In this study, the most frequently isolated microorganisms with the use of both BACTEC cultures and conventional methods were Gram-negative bacteria followed by Gram-positive bacteria. Similar findings of predominance of Gram-negative bacteria as compared to Gram-positive bacteria have been reported by various authors [2, 8]. However, Cetin *et al*., reported that the most frequently isolated microorganisms recovered were Gram-positive cocci followed by Gram-negative bacilli [9].

The time to culture positivity, *i.e.,* time taken from incubation to growth detection for conventional and signal positive for BACTEC 9120 was noted. Mean time to detection (MTTD) is the average of the time to culture positivity for all the isolates in a particular category [12]. Using BACTEC, majority (100%) of the pathogenic isolates were detected on/before day 2 whereas, during the same time, only eleven (84.6%) pathogenic isolates were detected by conventional method. This is similar to the studies conducted by other authors who have reported majority of their isolates being detected within 48 hours by automated systems as compared to the conventional culture methods [8, 12]. Similar reduced time to detection by BACTEC method was found in previous studies [9, 10, 12, 13]. Some authors have reported a mean detection time of 19-24 hours by BACTEC and 5-7 days by conventional method [14-16].

Since, BACTEC is a continuous monitoring system, and instrument takes readings every 10 minutes, detection occurs earlier and can be reported in hours. However, conventional method, by its very nature of processing, cannot be reported positive before 24 hours [12, 17]. There are many factors which may contribute to increased isolation by BACTEC. The resins, present in the BACTEC bottle and the dilutional effect of the liquid in the

bottles decrease the inhibitory effects of antimicrobial substances [5, 18]. BACTEC plus media are reported to effectively remove many antimicrobials. They use ion exchange and non-ionic adsorbent resins to remove antimicrobials thereby enhancing the recovery of micro-organisms.

In terms of speed, sensitivity and shortened turnaround time, BACTEC automated culture system helps in earlier presumptive reporting of the pathogen as compared to conventional culture methods [13]. This would significantly affect the change of empirical antibiotic therapy given to the patient, eventually aiding in therapy and the final clinical outcome [13].

However, cost may be a limiting factor for BACTEC in resource poor laboratories [11, 17]. Another concern is the increased risk of needle stick injury for technical staff as a result of the need to use syringes to inoculate sterile body fluids into BACTEC vials. These disadvantages should be balanced against the clinical advantage of better isolation rate of pathogens from infected body fluids [18].

In the present study, the most common isolate among Gram-positive cocci was *MRSA* and these strains showed maximum resistance to cefoxitin (100%), followed by ciprofloxacin (60%), amikacin (40%), erythromycin (40%). *MSSA* showed maximum resistance to erythromycin (100%), followed by ciprofloxacin (33%), amikacin (33%). *MRCoNS* showed maximum resistance to erythromycin (100%) and cefoxitin (100%), followed by ciprofloxacin (67%), amikacin (67%). None of the above isolate was resistant to vancomycin and linezolid in the present study.

Enterococcus showed maximum resistance (100%) to ampicillin, erythromycin, ciprofloxacin but was 100% sensitive to vancomycin, linezolid and high-level gentamicin. Most common isolate among Gram-negative bacilli was *Escherichia coli* which showed maximum resistance to cefotaxime (86%), followed by ceftriaxone (57%), ciprofloxacin (43%), amikacin (29%), piperacillin-tazobactam (29%). *Klebsiella pneumoniae* showed maximum resistance to cefotaxime (83%), followed by ceftriaxone (50%), ciprofloxacin (33%), amikacin (33%), piperacillin-tazobactam (17%). *Pseudomonas aeruginosa*, showed maximum resistance to ceftazidime (50%), cefepime (50%), ciprofloxacin (50%), followed by amikacin (25%) and piperacillin tazobactam (25%).

Citrobacter freundii showed 50% resistance to cefotaxime, ceftriaxone but was 100% sensitive to ciprofloxacin, amikacin and piperacillin-tazobactam. Only one strain of *Acinetobacter baumanii* was isolated which showed 100% resistance to ceftazidime, cefepime, ciprofloxacin, amikacin and piperacillin-tazobactam. All the Gram-negative isolates in the present study showed 100% susceptibility to meropenem.

Increased resistance to antimicrobials was observed which corroborates with other studies by different authors [19, 20]. High level of resistance to the first line drugs in the present study is a worrisome problem and it warrants routine and regular surveillance of the antibiogram.

CONCLUSION: Body fluids may be infected by both Gram-positive and Gram-negative bacteria. Conventional culture methods may not detect the causative organism from the sterile body fluids and also these are time-consuming and laborious. Automated systems significantly shorten the time to diagnosis, thus allowing rapid diagnosis and early administration of appropriate treatment. The study recommends the use of automated blood culture system for culture of sterile body fluids. Regular monitoring and surveillance of prevalence of organisms causing infection of body fluids is required for formulating an antibiotic and infection control policy so as to guide the clinicians in choosing appropriate antibiotics before a culture report is available thus preventing the development of antimicrobial resistance.

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