Original Article

Physical method to yield increased positivity of sterile body fluid bacterial cultures

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Abstract

Background: Infections of the body fluids has increased morbidity and mortality. Recovering causative organisms in these situations are a challenging task for any microbiologist as conventional methods culture positivity is low when compared with automated system which is very expensive and not frequently seen in small labs. **Aim**: To evaluate the mechanical glass bead agitation method for the isolation of organisms from body fluids and compare the results with the conventional method. **Materials and Method**: A pilot study was conducted in microbiology department at Sri Ramachandra medical college and research institute between September and December 2010. A total of 100 body fluid samples obtained from various sites like Cerebrospinal fluid (CSF) 31, Continuous Ambulatory Peritoneal Dialysis (CAPD) 2, pleural fluid 16, synovial fluid 19, ascitic fluid 29 and bile 3 were subjected to culture by normal conventional method and also by physical disruption of the body fluids using sterile glass beads simultaneously. Culture medium used for the both methods were 5% sheep blood agar, Chocolate agar, Mac-Conkey agar and thioglycolate broth. **Results:** Out of the 100 samples processed by conventional method and glass bead agitation method, culture positivity was 6(6%) and 15 (15%) respectively. All the cultures that showed positive in conventional method were also positive in mechanical glass bead agitation method with the growth of the same organism. By conventional method the breakup of isolates were *Escherichia coli* 2 in Bile fluid, 1 *Klebsiella* spp in Cerebrospinal fluid, 1 *Acinetobacter* spp in Ascitic fluid, 1 *Enterobacter* spp in Pleural fluid and 1 *Streptococcus spp* in Bile.. The breakup of isolates by glass bead method were *Klebsiella spp* 1 in Cerebrospinal fluid, 2 *E.coli* and 2 *Enterobacter spp* in Pleural fluid, 2 *Pseudomonas spp* and 1 *E.coli* in Synovial fluid, 2 *Acinetobacter spp*, 1 *Enterobacter spp* and 1 *Klebsiella spp* in ascitic fluid, 2 *E.coli* and 1 *Streptococcus spp* in Bile fluid. **Conclusion**: Mechanical glass bead agitation method culture positivity is better than the convention method, hence can be adopted as a method of choice where expensive automated culture facilities are not available.

Keywords: Glass beads, lysis centrifugation and Automated culture system.

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INTRODUCTION

Body fluid like acitic, pleural, synovial, cerebrospinal fluid, aqueous humor, bile etc., are generally sterile, but can be invaded and infected with microbes. Presence of even a single organism in these sites are considered significant and may result in increased morbidity and mortality if not identified in time. Especially in the present day scenarios where many invasive procedures are done for both therapeutic and diagnostic purpose, the chances of infections in these sites are increased. Isolation of microbes from these sites conventionally is of a great challenging task for the microbiologist, due to less number of organisms present in the specimen and many times in situations like culture of continuous ambulatory peritoneal dialysate (CAPD) where the volume of specimen is the specimen that it has to be concentrated and the number of organisms are also relatively low. To overcome this difficulty many techniques were tried to get a positive yield in the culture. Commonest method adopted by most of the laboratories is centrifugation of

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the large volume of the samples and processing the pellets for the cultivation. Most of the studies done have proven that automated system are superior in isolation over conventional methods [1and2]. However due to the expensive equipment's and the cost price of automated cultures becomes an unaffordable investigation for many of the microbiological lab and patients, at least in the developing countries, so we have to look into cheaper methods which could increase the yield of positivity in the body fluid culture. Keeping this in the mind we have decided to carry out this physical method, usage of sterile glass beads for increased yield of the bacterial cultures.

MATERIALS AND METHODS

A pilot study was conducted in microbiology department at Sri Ramachandra medical college and research institute between September and December 2010. A total of 100 body fluid samples obtained from various sites like Cerebrospinal fluid (CSF) 31, Continuous Ambulatory Peritoneal Dialysis (CAPD) 2, pleural fluid 16, synovial fluid 19, ascitic fluid 29 and bile 3 were subjected to culture by normal conventional method and also by physical disruption of the body fluids using sterile glass beads simultaneously. Culture medium used for the both methods were 5% sheep blood agar, Chocolate agar, Mac-Conkey agar and thioglycolate broth. In the conventional methods the specimens were directly inoculated in to the culture medium. For physical agitation method 3ml of the body fluid is taken in a sterile glass tubes. Six sterile glass beads were also dropped inside the tube aseptically and agitated using vortex mixer at 2500 rpm for 5 minutes. After which the fluid was inoculated into Blood agar, Chocolate agar, Mac-conkey agar and Thioglycolate broth and incubated at 37˚C for overnight. Gram smear from the specimens were also performed before and after agitations and the findings were recorded. The plates were observed for the growth at the end of 24 hours and 48 hours. Plates which did not any growth after 48 hours were recorded as no growth and the plates which showed the presence of bacterial colony was further processed to identify the bacteria along with the susceptibility pattern as per CLSI guidelines version 2010[3].

RESULTS

Out of the 100 samples processed by conventional method and glass bead agitation method, culture positivity was 6(6%) and 15(15%) respectively. The breakup of the specimen and their growth is shown by both the methods are shown in the Table 1. By Conventional method the breakup of isolates was 1 Gram positive and 5 Gram negative organisms. They are *Escherichia coli*[2] in Bile fluid,[1] *Klebsiella* spp in Cerebrospinal fluid,[1] *Acinetobacter* spp in Ascitic fluid and[1] *Enterobacter* spp in Pleural fluid. The one Gram positive isolate was *Streptococcus spp* in Bile. The culture positivity after mechanical glass bead agitation method was 15 (15%) out of 100 samples. Among the 15 isolates 1 was Gram positive and 14 were Gram negative organism. The breakup of Gram negative isolates were *Klebsiella spp*[1] in Cerebrospinal fluid,[2] *E.coli* and[2] *Enterobacter spp* in Pleural fluid,[2] *Pseudomonas spp* and[1] *E.coli* in Synovial fluid,[2] *Acinetobacter spp*,[1] *Enterobacter spp* and[1] *Klebsiella spp* in ascitic fluid,[2] *E.coli* and[1] *Streptococcus spp* in Bile fluid. All the cultures that showed positive in conventional method were also positive in mechanical glass bead agitation method with the growth of the same organism. Antibiotic susceptibility pattern of both isolates were similar indicating probable same strain. In the mechanical glass bead agitation method, good amount of positivity was noted in Pleural fluid, Synovial fluid and Ascitic fluid. There was no growth in any of Synovial fluid by conventional method, but 3 out of 19 showed positivity by mechanical glass bead agitation method (15.7%). There was only 1(3.44%) culture positivity in Ascitic fluid by conventional method but 4 out of 29 (13.7%) showed positive by mechanical glass bead agitation method. In Bile specimen 1(33.3%) was positive by conventional method, but in mechanical glass bead agitation method all three turned positive (100%). The result showed enhanced culture positivity in specimens like Pleural fluid, Synovial fluid and Ascitic fluid, but there were no difference in positivity in CSF and CAPD fluid. All the isolates which were positive was identified by conventional biochemical analysis.

Table 1: Break of Specimen and culture positivity by both methods

Specimen	Total	Conventional Method		Glass Agitation method	
		Growth	No Growth	Growth	No Growth
Bile	3	3	0	3	0
CAPD*	2	0	2	0	2
Ascitic fluid	29	1	28	4	25
Synovial fluid	19	0	19	3	16
Pleural fluid	16	1	15	4	12
CSF*	31	1	30	1	30
Total	**100**	**6**	**94**	**15**	**85**

CAPD*= Continuous Ambulatory Peritoneal Dialysate, CSF*= Cerebrospinal Fluid

Figure 1

Figure 2

DISCUSSION

The problem in recovering the organism from body fluids is very difficult because of low load of organism, organism phagocytosed or organism entrapped in the organic materials. There are various methods to disrupt the organic materials and to lyse the cell to release the organism thereby increasing the positivity of the culturing such specimens. P C Taylor[4] in his study demonstrated sonication of clinical specimen prior to culturing or culturing on saponin containing media resulted in increased positivity. In our study comparing with the conventional technique which yielded 6% positivity, the glass bead agitation technique resulted in 15% of positivity. This positivity was significantly seen in Pleural fluid, Ascitic fluid and Synovial fluid which may be due to glass beads disrupting proteineceous organic material. Many studies have demonstrated very significant positivity (89%) by lysis centrifugation of blood specimen[5,6]. Daur A V *et al*[7] had compared isolation of organism from body fluids by both conventional and enrichment culture technique and found 9.7% of specimens showed positive with conventional method and 23.1% with enriched broth culture technique, using BacT/Alert blood culture bottle. In our study we also encountered a significant number of positivity 15% using glass bead agitation technique which requires no extra cost when comparing to enriched broth culture technique which are costly. Wong S F[8] has demonstrated mechanical disruption of fungal cell for yielding whole cell protein from yeast cell which according to the author inexpensive technique. Based upon the above principle (even though we have not disrupt the fungal cell for protein yield) we also got a significant culture positivity. Bourbeau P[2] performed a study with 1,157 sterile body fluid samples other than blood by culturing routine as well as BacT/Alert standard aerobic anaerobic bottles and BacT/Alert FAN anaerobic bottles and reported recovery of organisms in 82%, 96% and 81% respectively standard bottles, FAN bottles and routine cultures. We in our study used sterile glass beads to look enhanced culture positivity. In their study, they got a significant recovery of gram positive organisms, but in our study gram negative organisms were predominately isolated from mechanical disruption and also we have not done isolation of anaerobic organisms. Elston H R[9] reported 94% of culture positivity by isolator detection system in comparing with 64% positivity by large volume centrifugation method. They did their study with 155 body fluid samples other than Blood, CSF and Urine. However in our study we got 15% positivity with mechanical glass bead agitation in comparing with 6% by conventional method. So in set up which cannot afford for expensive automated culture system glass bead agitation method can be cheapest and efficient method to retrieve culture positivity. The limitations of the study is automated cultivation method also if performed simultaneously would have given more significance to the findings.

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