

BACTERIOLOGY OF BODY FLUIDS WITH AN EVALUATION OF ENRICHMENT TECHNIQUE TO INCREASE CULTURE POSITIVITYAnasua Deb¹, Swati Mudshingkar², Vaishali Dohe³, Renu Bharadwaj⁴**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: BACKGROUND: Body fluids like ascitic fluids, pleural fluids, cerebrospinal fluids (CSF) etc. are sent for culture in a clinical microbiology laboratory to achieve etiological diagnosis. However the yield of such cultures is usually very low. So, ongoing monitoring of prevalent pathogenic organisms and their sensitivities help the clinicians institute therapy in absence of a culture report. **AIMS:** The study was done to identify the common pathogens isolated from body fluids along with their antimicrobial susceptibility pattern and also to evaluate the impact of enrichment on their culture positivity. **SETTING AND DESIGN:** A 3-month prospective analytical study was done in a tertiary care hospital. **MATERIALS AND METHODS:** A total of 333 Body fluids were processed; 103 of them were ascitic fluids, 71 pleural fluids, 139 CSF and 20 other fluids. They were processed by plating the direct sample and after enrichment. Enrichment was done by two methods: in Soyabean-Caesin digest broth (274 samples) and by BACTEC (59 samples). Isolates were identified by routine procedures & their antimicrobial susceptibility determined as per CLSI guidelines. The results were analyzed using Microsoft Excel® software using $p < 0.05$ as the cut-off for significance. **RESULTS:** Gram negative isolate were obtained from 21.3% of the samples. The common isolates were Pseudomonas (20.7%), Acinetobacter (11.6%), Citrobacter (10.7%) and E. coli (10.7%). The antibiotics most effective against Gram negative pathogens were Gentamicin (47.5%), Piperacillin-Tazobactam (51.6%), Amikacin (56.7%) and Cefoperazone-Sulbactam (65.3%). Gram positive isolates, obtained from 9% of the samples, mostly consisted of MSSA, Enterococcus and CONS, for which Ciprofloxacin (48%) followed by Cotrimoxazole (40%) and Erythromycin (28.6%) showed reasonable efficacy. The Culture positivity with direct plating, Soyabean-Caesin broth enrichment and BACTEC was 14.41%, 29.19% and 42.37% respectively. Increase in positivity by Soyabean-Casein broth was maximum for pleural fluids (12%) followed by ascitic fluids (11.6%) and CSF (11.52%). Using automated system the corresponding increases were 20.7% for ascitic fluids and 5.4% for pleural fluids. The mean time for identification using direct plating, enrichment method and BACTEC were 48 hours, 72 hours and 40 hours respectively. **CONCLUSION:** Gram negative isolates are commonly isolated pathogens from body fluids in our setup. Enrichment of body fluids improved yield of pathogens. In resource-poor settings simple enrichment in blood culture bottles can increase culture positivity of these precious samples.

KEYWORDS: Fluid culture, bacteriology, enrichment.

INTRODUCTION: Body fluids, such as ascitic, pleural, cerebrospinal, pericardial and synovial fluids, are commonly encountered in the diagnostic microbiology laboratory for bacterial culture in suspected cases of infection. Such infections are associated with considerable morbidity and mortality and pose a substantial burden on healthcare system. A timely diagnosis and prompt treatment of these infections are vital for the successful management of these cases. Therefore, rapid isolation and

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accurate identification of pathogenic micro-organisms along with their antimicrobial susceptibility pattern play a major role in timely management of such cases.

The relatively low counts of the bacterial pathogen in the fluid samples along with prior institution of empirical antibiotics often hinder the successful isolation of pathogens by conventional culture techniques. Another practical problem faced in the processing of these samples is that often the quantity of these samples is insufficient. All these factors contribute to lower rates of culture positivity by standard diagnostic procedures.

In order to improve the culture yield from the body fluids various techniques have been tried, of which enrichment in various broths have been promising. The different enrichment culture systems include trypticase-soy broth,^[1] Soyabean-Casein digest(SCD) broth, Brain-Heart infusion broth^[2] and automated systems like BACTEC^[3] and BacT Alert.^[4,5]

The present study was planned to identify common organisms isolated from various body fluids along with their antimicrobial susceptibility pattern. We also evaluated the relative utility of enrichment in blood culture bottles containing Soyabean Casein Digest broth with charcoal and in BACTEC (B.D. Diagnostics, Becton Dickenson, Maryland, USA) vis-a-vis direct plating method by the conventional technique.

METHODS: After ethical committee approval, a prospective analytical study of three months duration (June to August 2013) was done. Because the study was done on the samples already sent to the laboratory and did not involve additional intervention to the patient, individual patient consent was waived.

The study was done in two phases. In the first phase, which lasted for the initial two months (June-July, 2013) all fluid samples received were plated directly and after enrichment in Soyabean Casein Digest broth. Subcultures were done from these bottles after 24 hours of enrichment onto Blood agar, Mac Conkey agar and Chocolate agar. In the second phase all fluid samples except cerebrospinal fluid were enriched in BACTEC automated culture bottles after direct plating. Cerebrospinal fluid was excluded from the second phase of the study, since the usual volume of these samples is very less, and the BACTEC bottles that were employed mandate at least 5-6 ml of fluids.

Organisms isolated by all the methods were identified by standard identification procedures^[6] and their antimicrobial susceptibility determined as per CLSI guidelines.^[7] Appropriate clinical history was collected from each patient for clinical correlation.

Average time to identification was calculated for each of the procedures. This included time from receiving the sample till final reporting of the sample with the antimicrobial susceptibility pattern of the isolate.

The data obtained was statistically analyzed using Microsoft Excel software package taking $p \leq 0.05$ as the cut off for significant result.

RESULTS: In three months period a total of 333 fluid samples were processed the distribution of which has been shown in Table 1. In the first phase total 274 samples were processed which were enriched in soyabean casein digest broth. In the second phase 59 samples excluding CSF were enriched in BACTEC™ bottles. Table 2 demonstrates the pathogens isolated according to the different types of body fluids that were cultured. Their susceptibility patterns have been shown in the figures 1 and 2.

Type of fluid	Soyabean Casein Digest broth enrichment	BACTEC enrichment
Ascitic fluid	74	29
CSF	139	-
Pleural fluid	50	21
Pericardial fluid	1	1
Synovial fluid	1	4
Drain fluid	8	4
Perinephric collection	1	0
Total	274	59

Table 1: Distribution of total fluid samples processed (n= 333)

Organisms isolated	Sample				Total
	Ascitic fluid	Pleural fluid	CSF	Other fluids	
Gram positive organisms					
S. aureus	1	1	4	2	8
CoNS	3	2	1	2	8
Enterococcus spp	7	1	-	1	9
Streptococcus spp	2	3	2	-	7
Enterobacteriaceae group					
E. coli	5	1	6	1	13
K. pneumoniae	6	4	2	-	12
Citrobacter spp	9	1	3	-	13
Enterobacter spp	7	1	2	-	10
Proteus spp	1	-	-	-	1
Non-fermenter group					
P. aeruginosa	12	4	8	1	25
Acinetobacter spp	9	1	3	1	14
Other non-fermenter.	1	-	-	-	1
Total number of isolates	63	19	31	8	121

Table 2: Sample wise distribution of the isolates

CoNS = Coagulase negative Staphylococcus.

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Figure 1: Bar diagram showing antimicrobial susceptibility pattern of the Gram positive pathogens isolated (n = 32).

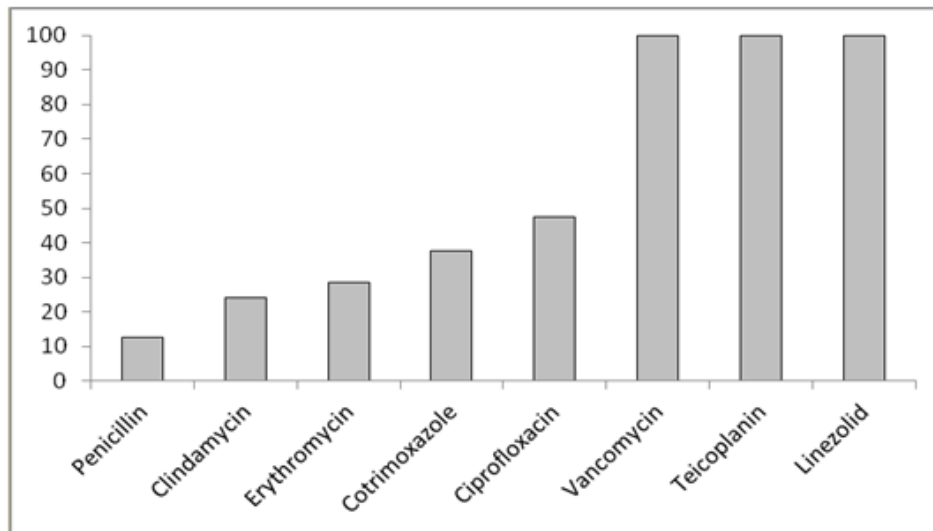


Figure 1

Figure 2: Bar diagram showing antimicrobial susceptibility pattern of the Gram negative pathogens isolated (n= 89)

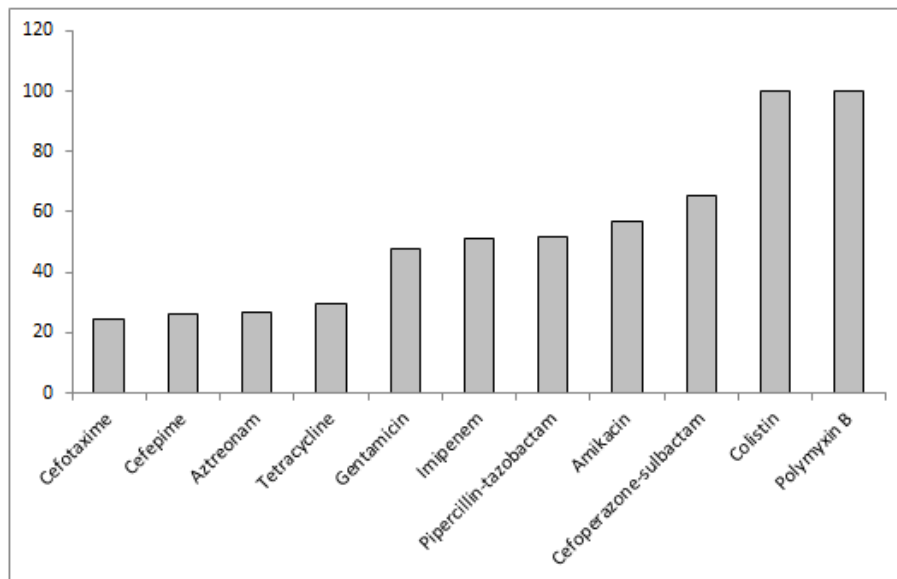


Figure 2

Table 3 demonstrates the comparative culture positivity rates using the direct plating and different methods of enrichment culture. The contaminants have been excluded while calculating the culture positivity rates.

Type of fluid	Total no. of sample	Direct plating			Soyabean Casein digest broth			BACTEC		
		total	+ve	%	total	+ve	%	total	+ve	%
Ascitic fluid	103	103	34	33	74	33	44.6	29	15	51.7
Pleural fluid	71	71	9	12.7	50	12	24	21	4	17.4
CSF	139	139	19	13.6	139	28	20.1	-	-	-
Other	20	20	4	20	11	5	45	9	2	22.2
Total	333	333	48	14.4	274	75	27.4	59	25	42.4

Table 3: Culture positivity rates using the different methods

The yield of positive culture improved by approximately double after enrichment of fluids (n=48 without enrichment Vs. n=100 with enrichment, where n is the number of positive culture). For ascitic fluid the increase in yield was maximum using BACTEC method of enrichment although the difference was not statistically significant. For pleural fluids and other body fluids, recovery of pathogens was maximum using enrichment by soyabean casein digest broth and the difference was statistically significant ($p < 0.05$) for pleural fluids. Such a comparative analysis could not be done for cerebrospinal fluid since they were enriched by only soyabean casein digest broth.

The average time to identification was calculated from the receiving of the sample till the dispatch of the final report, including the antimicrobial susceptibility pattern of the organism. Using direct plating, the average time to identification was 48 hours while using soyabean casein digest broth it was 72 hours. Using BACTEC, it was 40 hours ($p < 0.05$ compared to soyabean casein digest broth). Thus automated enrichment method saves time compared to the manual method of enrichment.

DISCUSSION: In our study 21.3% of the samples grew Gram negative bacilli, the most common being those belonging to the Enterobacteriaceae family (49 isolates) followed by other Gram negative bacilli belonging to the non-fermenter group (40 isolates).

Others who have studied the bacteriological profile of fluids have also reported a similar predominance of Gram negative organisms. Daur et al^[1] reported that *Pseudomonas aeruginosa* and *Escherichia coli* were the most frequently isolated pathogens in their study. Similar finding were also reported by Lakshmi et al^[5] from Hyderabad who found that the enteric Gram negative bacilli were the predominant pathogens. Bobadilla et al^[8] and Siersema et al^[9] who evaluated culture methods in bacterial peritonitis, also reported a similar predominance of Gram negative flora. A recent Indian study on spontaneous bacterial peritonitis also reported that majority of the isolates were Gram negative bacilli.^[10]

From figure 2 it is evident that Amikacin (56.7%), Gentamicin (47.5%), Cefoperazone-Sulbactam (65.3%) and Piperacillin-tazobactam (51.6%) combinations are the most effective first line drugs against the Gram negative isolates. All the isolates were found to be sensitive to the higher drugs like Colistin and Polymyxin B. Around 23.4% of the Gram negative isolates were ESBL producer. Our setup being a tertiary care hospital, the patients already have prior exposure to

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antibiotics, which have resulted in high antimicrobial resistance. Similar increasing pattern of resistance to the Cephalosporins, fluoroquinolones and other first line drugs amongst the Gram negative isolates have been reported across the globe.^[11-13]

Overall, Gram positive isolates (32 isolates) were obtained from 9% of samples, with *S. aureus*, Coagulase negative Staphylococcus, Streptococcus, and Enterococcus being the predominant isolates. Table 2 shows that Enterococcus was commonly encountered in ascitic fluids while other Gram positive organisms were recovered mostly from CSF and pleural fluids.

Some of the studies have reported a predominant Gram positive flora isolated from the fluids. In most of these studies fluids other than ascitic fluid have contributed to the maximum number of specimens. Bourbeau et al,^[4] for example, had maximum number of synovial fluids followed by dialysis (CAPD) fluids, peritoneal fluids and pleural fluids. The organisms isolated in their study were most commonly Staphylococcus aureus followed by CoNS and Enterobactereaceae. Similar predominance of Gram positive cocci was also observed by Yoon et al^[14] who had focussed only on CAPD fluids. This difference in distribution of samples can explain the difference in the type of commonly isolated pathogens as compared to our study. Pal et al^[3] from Jaipur also mentioned a higher proportion of Gram positive isolates, viz. Co NS, *S. aureus* and Enterococcus, although the distribution of fluid types has not been mentioned in their study.

The susceptibility pattern as depicted in Fig 1 showed that the efficacy of the first line drugs against Staphylococcus and other Gram positive cocci was limited. Fortunately, we did not encounter any Methicillin resistant strain of Staphylococcus. Ciprofloxacin (48%) followed by Cotrimoxazole (40%) and Erythromycin (28.6%) showed reasonable efficacy against the Gram positive isolates. All the isolates were sensitive to higher drugs like Vancomycin and Linezolid. Thus, we observed an overall increasing trend of resistance to the first line drugs; more so among the Gram positive organisms, which warrants regular surveillance studies. This observation corroborate with other studies reporting antimicrobial susceptibility patterns of Gram positive organisms isolated from body fluids.^[11,13,15]

Overall we observed a general pattern of Gram negative isolates and Enterococci being more commonly isolated from ascitic fluid, while Gram positive isolates were more frequently isolated from CSF and other fluids. This can be explained by the fact that in bacterial peritonitis, there is increased intestinal permeability, which in turn contributes to higher number of enteric pathogens being isolated because of their transmigration across the intestinal wall.^[16] The Gram negative organisms being a part of the normal gut flora are thus more often found in ascitic fluids.

Our study also demonstrated the usefulness of enrichment culture to improve the yield of pathogens from all types of body fluids. Table 3 shows that using BACTEC, there was an overall increase in culture positivity rate to 42.4% as compared to direct plating technique which had a culture positive yield of 14.4%. Other studies using automated culture systems have also reported a significant increase in culture positivity rates after enrichment. Pal et al. from Jaipur have reported a 36.96% isolation rate from body fluid using BACTEC system.^[3] Other automated culture systems like BacT/Alert have also proved helpful in other studies. A Study from Hyderabad^[5] found an increased culture yield by 10.33% using BacT/ALERT culture over direct culture of body fluids like ascitic, pleural and pericardial fluid. Menzies et al^[17] in 2011, have recently shown that for pleural fluids, enrichment in blood culture bottles was useful. In another recent study utilizing the BacT/Alert system, Yoon et al^[14] have also reported a significant increase in culture positivity of peritoneal

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dialysis fluid (78.6%) as compared to the conventional method (50%). Bourbeau et al^[4] have found that for pleural fluids, automated BacT/Alert system was not useful although for other fluids it was beneficial; a finding that is consistent with our study.

We also observed significant increase in culture positivity using simple enrichment in blood culture bottles containing Soyabean Casein Digest broth. Overall the culture positivity increased by 13% using Soyabean Casein Digest broth as compared to direct plating. This enrichment method proved particularly more useful for pleural fluids for which it doubled the isolation rate. Even for CSF it showed a substantial increase in culture positivity. Although it is more time-consuming as compared to automated methods, the technique being simple and less expensive, can be easily instituted in resource-poor settings. It can also be used for fluids sent in small volumes (eg: CSF) as the enrichment broth can be aliquoted in smaller volumes.

The reasons for increased isolation after enrichment are manifold. Charcoal in the SCD broth containing bottles acts as an adsorbent of antibacterial substances and provides a favourable environment for bacterial multiplication. In the conventional culture, bacteria may die due to delayed inoculation and the endogenous antimicrobial activity of the body fluids which is avoided by bedside inoculation of sample into enrichment media. The continuous monitoring in automated systems without the need for invasive technologists manipulation further reduces the time to detect of positive cultures and chances of extraneous contamination.

In conclusion, Gram negative bacilli was predominantly recovered from most body fluids while for CSF, the Gram positive organisms were the main culprits. The raising menace of antimicrobial resistance highlights the necessity for continuous epidemiological monitoring so that appropriate antibiotic policy can be formulated. Furthermore, we also found that inoculation of body fluids into automated enrichment media improves the positive culture rate compared to the direct plating. Automated systems significantly shorten the time to diagnosis, thus allowing rapid diagnosis and early administration of appropriate treatment; thereby curtailing the overall healthcare expenditure and morbidity of the patient. However, in resource-poor settings simple enrichment can be an effective alternative and help in improving the culture positivity of the fluid samples.

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