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EXPLORING BACTERIOLOGICAL PROFILE OF DEEP-SEATED ABSCESS: WITH SPECIAL REFERENCE TO ANAEROBES, A NEGLECTED PATHOGEN

Microbiology

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ABSTRACT

Deep seated abscess occur at various sites like lung, brain, intra-abdominal, retroperitoneal region. Abscesses are often polymicrobial in nature involving both aerobic and anaerobic infection. It is often seen that aerobes are properly isolated and treated accordingly, but anaerobes are often missed due to their fastidious nature and difficulty in collection of sample. The aim of the current study was to identify the aerobic & anaerobic bacteria, study the antimicrobial susceptibility pattern of both aerobic & anaerobic bacteria isolated. Specimens from the clinically diagnosed deep-seated abscesses from various sites with suspected bacterial etiology were collected aseptically in a sterile syringe and were processed both aerobically and anaerobically. Total of 100 patients was enrolled in the study. Out of 100 samples, 82 were culture positive while 18 samples showed no growth on culture. Most common deep-seated abscess reported was deep gluteal abscess (22%) followed by abdominal abscess (21%) and liver abscess (16%). Out of 100 samples, 74 samples showed only aerobic growth, 4 samples showed only anaerobic growth and 4 showed mixed growth. Handling of specimen containing anaerobic organisms is very much challenging due to their susceptibility to environmental oxygen, technical difficulties in cultivation, cost, and most importantly prolonged turnaround time which leads to failure of detection of the different anaerobes responsible for various infections. So, the present study was undertaken to know the bacteriological profile of deep-seated abscesses by using conventional and a few automated methods with special reference to anaerobes and also finding their antimicrobial susceptibility pattern

KEYWORDS

Anaerobes, Deep abscess, Vitek, BACTEC

1. INTRODUCTION

An abscess is a localized collection of purulent inflammatory tissue caused by suppuration deep within a tissue, an organ, or a confined space. It is produced by the deep seeding of pyogenic bacteria into a tissue.[1] An abscess can occur in any part of the body as a superficial infection or deep-seated infection associated with any internal organs like forming intraabdominal abscess, liver, lung, brain, deep neck space abscess.[1,2] Most deep-seated abscesses are polymicrobial and may include obligate anaerobes, facultative anaerobes, facultative aerobes, or obligate aerobes as concomitant microorganisms. Because aerobic and anaerobic bacteria are frequently found in the same infected site, appropriate procedures for isolation and culture are necessary to avoid overlooking the anaerobes. Handling of specimen containing anaerobic organisms is very much challenging due to their susceptibility to environmental oxygen, technical difficulties in cultivation, cost, and most importantly prolonged turnaround time.

2. OBJECTIVES:

The aim of the current study was to identify the aerobic & anaerobic bacteria, study the antimicrobial susceptibility pattern of both aerobic & anaerobic bacteria isolated from deep seated abscess and to compare the conventional techniques of isolation of anaerobes with automated culture techniques.

3. MATERIALS AND METHODS:

A prospective observational study was carried out in department of Microbiology, BJGMC and Sassoon General Hospitals Pune, over a period of 2 years from December 2019 to December 2021.

In the present study, total 100 pus specimens were collected from patients with deep seated abscess attending surgical department as outpatients and inpatients. Two samples were collected from each patient. One was transported in a sterile test tube for aerobic culture while the second one was inoculated immediately in RCM medium and Anaerobic PLUS BACTEC bottle for anaerobic culture. The aspirated samples taken from each patient were subjected to smear preparation, aerobic and anaerobic culture.

In case of aerobes, they were inoculated on Blood agar, MacConkey and Chocolate agar. Blood and MacConkey agar plates were aerobically incubated at 37^{0}C for 18-24 hours. Chocolate agar plates were incubated in 5%-10% CO_2 at 37^{0}C for 18-24 hours. The colonies obtained were processed for biochemical reactions and other associated tests and identification was made based on that, followed by their Antimicrobial Susceptibility testing (ABST)by disc diffusion methods for all except colistin for which agar dilution method was used.[4] For anaerobic isolation, the sample in Robertson's cooked meat medium (RCM) bottle was incubated at 37^{0}C for 2 hours, after which it was inoculated on kanamycin BA, Neomycin BA and Willis and Hobbs medium for culture and then incubated under anaerobic condition in an anaerobic jar at 37^{0}C for 48 hours. All anaerobic isolates obtained were tested for their antibiotic by Agar dilution method. These were selected and tested as suggested by CLSI using antibiotics Ampicillin, Chloramphenicol, Clindamycin and Metronidazole for anaerobes and the respective Minimum Inhibitory Concentration (MIC) of the isolates were noted.[5]

The inoculated ANAEROBIC PLUS BACTEC bottles were loaded into the BACTEC system and incubated for a maximum period of 5 days. Bottles flagged as positive by the instrument were sub cultured for anaerobic growth and follow up was done same as in conventional method.[6]

Colonies which appeared only on anaerobic agar were considered as probable anaerobes and were then tested for aerotolerance by inoculating on blood agar in 5-10%CO2 which was incubated aerobically at 37^{0}C for 18-24hrs. The growth which appeared only in anaerobic conditions and were negative for aerotolerance test were considered as pure anaerobic growth and further identified by standard techniques and also by automated VITEK system for identification upto species level.

Statistical Analysis:

The data were statistically analyzed by using Microsoft Excel.

4. RESULTS:

Total of 100 patients was enrolled in the study. Out of 100 samples, 82 were culture positive while 18 samples showed no growth on culture. Thus, the culture positivity of the study was found to be 82%. The highest number of patients were of the age group 30 to 40 followed by 20 to 30 years. Number of male patients were more in number. Male to female ratio was 1.8:1. Most common deep-seated abscess reported was deep gluteal abscess (22%) followed by abdominal abscess (21%) and liver abscess (16%). Out of 82, 15 samples gave more than one

isolate. So, 18.3% samples were polymicrobial while 81.7% were monomicrobial in nature. Out of 100 samples, 74 samples showed only aerobic growth, 4 samples showed only anaerobic growth and 4 showed mixed growth. (**Graph 1**). Maximum organism isolation was seen in Abdominal abscess (24.74%), followed by deep gluteal abscess (20.6%). Most of the organisms isolated in any abscess were aerobes. Most common aerobe isolated were gram-negative (67.4%) than Gram-Positive (32.6%). Among the gram-negative *E. coli (33.33%)* was most common followed by *Klebsiella pneumonia (26.6%)*. The least common of all the aerobes was *Pseudomonas spp* **(Graph 2).** Commonest anaerobe isolated was *Clostridium spp. (37.5%).* **(Graph 3)**

Total 26 samples were flagged positive by the BACTEC bottles however only 7 cultures were recovered on culture by conventional method showing pure anaerobic growth which included 6 obligate anaerobes and 1 aerotolerant anaerobe which was grown on aerotolerance test. The other 19 bottles flagged positive, 17 showed facultative anaerobes which were also grown on aerobic media like *Klebsiella pneumoniae, Staphylococcus spp* while 2 showed no growth. While there was one obligate anerobic isolate which was recovered conventionally on culture but missed by BACTEC.

Clostridium perfringens were identified by conventional methods and automated VITEK method up to confidence level 96%. *Clostridium spp.* was identified by conventional methods which was identified as *Clostridium sporogenes* by VITEK. Out of 4 isolates identified to be *Peptostreptococcus spp.* by conventional methods, one was identified as *Peptostreptococcus anaerobius* and one as *Peptoniphilus asaccharolyticus***. (Table 1)**

Total of 60 aerobic gram-negative bacilli isolated from culture. All these organisms were highly resistant to Ampicillin and moderately to Amikacin, cefotaxime and ciprofloxacin. But most of these isolates were sensitive to Imipenem and Ceftazidime. There was overall 97 % sensitivity to Colistin as only two out of 60 isolates showed resistance to colistin**. (Table 2)**

Total of 29 isolates were gram positive aerobes. Maximum isolates were resistant to penicillin, erythromycin and clindamycin while most isolates showed good sensitivity towards gentamycin and high sensitivity towards teicoplanin, vancomycin and linezolid. **(Table 3).**

5. DISCUSSION:

Obligate anaerobic organisms are an important component of deep abscess, though many clinical laboratories may not recover them and report their sensitivity pattern because isolation and susceptibility testing of anaerobes is difficult and time-consuming. Empirical antimicrobial therapy against anaerobic infections is given based on susceptibility patterns derived from published reports and surveillance studies.[8,9,10]

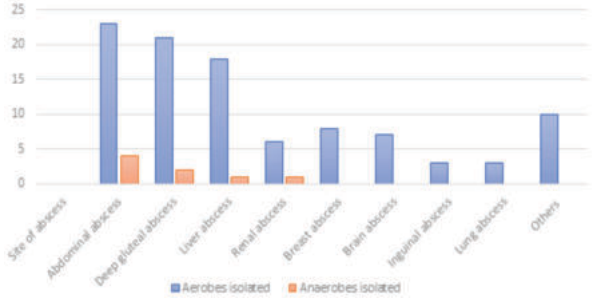
In this study, the highest organism isolation was seen in the case of Abdominal abscess (24.74%), followed by Deep gluteal abscess (20.6%), Liver abscess (17.52%). In the study by Sreekanth Basireddy *et al.,*[11] the majority of the deep-seated abscesses were obtained from the abdominal region accounting for 28% of the total samples, which correlates with Pramodini *et al.* where 31% samples were from the same region.[12]

In the present study bacteriological profile of the deep abscess were looked into regarding both aerobes and anaerobes. In the current study, out of 100 patients, samples from 82 patients yielded bacterial growth (Aerobic/Anaerobic). Culture positivity rate in our study was found to be 82% and we got an isolation rate of 1.18 organisms per sample which correlates with a study conducted by Goyal V *et al* which was 86.8%.[14] The studies conducted by Pramodini *et al.*[12] showed average numbers of organisms as 1.45 organisms per sample. Zhao-Qing Du *et al.* in a study on liver abscess culture positive rates was found to be 81.3%.[15] In our study, out of 82 positive cultures, 67(81.7%) samples showed a single isolate while 15(18.3%) samples showed two isolates per sample. The findings in our study are similar to the findings of Saini *et al.*[16] and Pramodini *et al.*[12] where single organism and polymicrobial infections accounted for 65% and 35%, 88% and 12% respectively. In our study, out of 82 positive cultures, 8 (9.8%) cultures gave anaerobic isolates. Out of which 4 were isolated as a single isolate which 4 were isolated as a polymicrobial infection along with aerobes. The most common isolate which we got was that of *Clostridium spp. (*37.5%), followed by *Peptostreptococcus spp.* (25%) and *Peptoniphilus spp.* (25%) and one isolate of *Lactobacillus spp.* which is a microaerophilic aerotolerant anaerobe. Various studies in the past have reported the prevalence of anaerobic bacteria in deep-seated abscesses to be 5% – 19% while that of aerobes is found to be much varied according to the site of an abscess (11% – 75%).[3,8,11,12] In a study by Ananth-Shenoy P *et al.*, Anaerobic bacteria were isolated along with aerobic bacteria in 71.9% of patients and only polymicrobial anaerobic growth was detected in 6.3% of patients.[17] In contrast to the present study, many studies concluded more anaerobic isolation, Saini S *et al*. isolated anaerobes in 87% of cases of secondary peritonitis.[16]

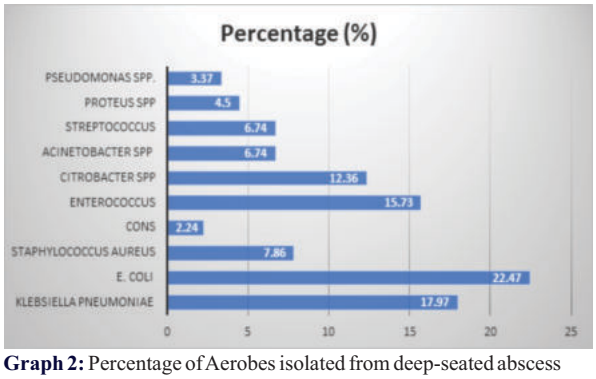
Among anaerobes, *Peptostreptococcus spp (25%) and Peptoniphilus spp (25%) was* the second most common isolated anaerobe in our study**.** *Clostridium spp.* (37.5%) are other frequent pathogens isolated from the abscesses in our study as Kamble *et al.* found as the predominant isolates in their study.[18] Gorbach *et al*. have also shown that the major source of Clostridial infections could be intraabdominal sepsis associated with trauma or prior intestinal surgery.[19]

In the present study, the isolation rate of anaerobes was seen to be less which may be attributed to various factors. The samples may not be collected properly by the clinician as instructed by the microbiologist, the clinical specimens had been through the normal hospital transport system, and in many cases, several hours had elapsed between taking and cultivating the samples. Many more organisms would probably have been recorded if the specimens had been speedily submitted under anaerobic conditions. Abundant use of high-spectrum antibiotics like metronidazole, clindamycin, chloramphenicol in most of the cases even before sending samples for culture and sensitivity can mark as other probability leading to low isolation rate of anaerobes. Thus, due to the less isolation rates of anaerobes there is a need of conducting more studies about the proper collection of samples for better isolation of anaerobes.

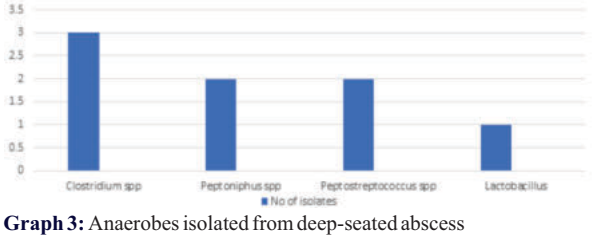
6. Conflicts of interest : None



Graph 1: Distribution according to sites in percentage



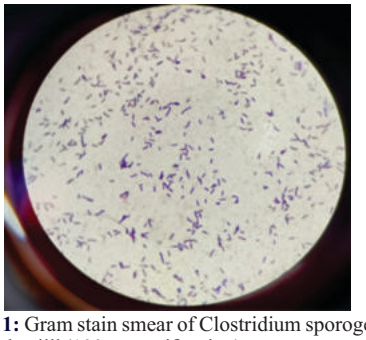
Graph 2: Percentage of Aerobes isolated from deep-seated abscess



Graph 3: Anaerobes isolated from deep-seated abscess

Table 1: Identification of Anaerobes by Vitek-2 and comparison with conventional methods

Organism identified by conventional method	No of organism identified by conventional method (n)	Identified with direct VITEK ANC ID	Identification upto species level by VITEK	Confidence level
Peptostreptococcus spp.	4	2	Peptoniphilus asaccharolyticus Peptostreptococcus anaerobius	93% 94%
Clostridium perfringens	2	2	Clostridium perferingens	96%
Clostridium spp.	1	1	Clostridium sporogenes	98%
Lactobacillus spp.	1	0	NA	NA



Photograph 1: Gram stain smear of Clostridium sporogenes showing gram positive bacilli (100x magnification).

Table2: Antibiotic resistance patterns in gram negative aerobes (showing percentage of sensitivity of organisms)

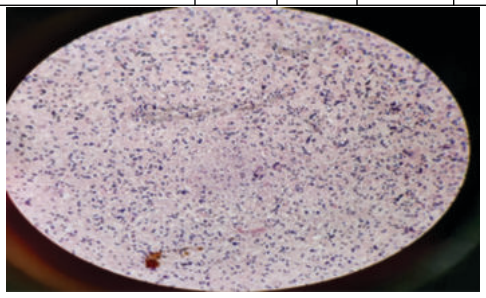
Gram negative aerobes (n= 60)	Ampicillin	Amikacin	Ciprofloxacin	Cefotaxime	Gentamycin	Piperacillin-Tazobactam	Imipenem	Meropenem	Amoxicillin Clavulanate	Ceftazidime	Colistin
E.coli (20)	86.9% (17)	40% (8)	65% (13)	45% (9)	65 % (13)	25% (5)	20% (4)	20% (4)	30% (6)	35% (7)	10% (2)
Klebsiella pneumoniae (16)	-	50% (8)	72.22% (11)	56.5% (9)	61.1% (11)	31.3% (5)	37.5% (6)	25% (4)	75% (12)	31.2% (5)	12.5% (2)
Citrobacter spp (11)	81.81% (9)	27.3% (3)	36.6% (4)	63.6% (7)	18.2% (2)	27.3% (3)	9% (1)	18.2% (2)	63.6% (7)	0%	0%
Acinetobacter spp (6)	100% (6)	50% (3)	66.66% (4)	66.6% (4)	50% (3)	50% (3)	66.6% (4)	33.3% (2)	16.6% (1)	16.6% (1)	0%
Proteus spp (4)	75% (3)	50% (2)	25% (1)	25% (1)	0%	0%	0%	25% (1)	0%	25% (1)	-
Pseudomonas spp (3)	100% (3)	66.6% (2)	33.3% (1)	100% (3)	66.6% (2)	0%	33.3% (1)	33.3% (1)	66.6% (2)	0%	0%

Table 3: Antimicrobial resistance patterns in gram positive aerobes

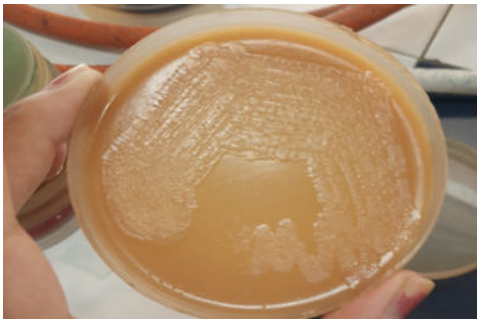
Gram positive aerobe (n=29)	Penicillin	Erythromycin	Clindamycin	Cefoxitin	Gentamycin	Ceftriaxone	Cotrimoxazole	Linezolid	Vancomycin	Tiecoplanin	High level gentamycin
Staphylococcus aureus (7)	71.42% (5)	57.14% (4)	42.8% (3)	57.1% (4)	57.1% (4)	42.8% (3)	57.1% (4)	28.5% (2)	14.28% (1)	14.2% (1)	__
CONS (2)	100% (2)	50% (1)	100% (2)	50% (1)	0% (0)	100% (2)	50% (1)	50% (1)	0%	0%	__
Streptococcus spp (6)	50% (3)	66.7% (4)	66.7% (4)	50% (3)	-	66.6% (4)	50% (3)	16.6% (1)	0%	0%	__
Enterococcus spp (14)	71.4% (10)	85.7% (12)	78.5% (11)	57.1% (8)	-	57.14% (8)	35.7% (5)	14.2% (4)	28.5% (2)	14.2% (2)	7.1% (1)

Table 4: Antimicrobial sensitivity pattern for anaerobes

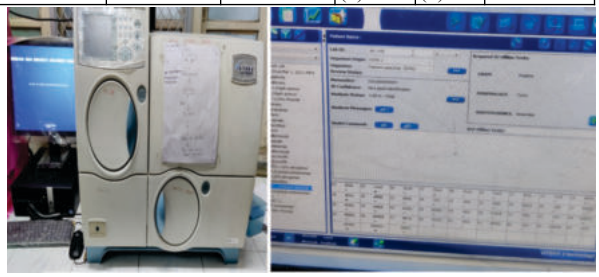
Anaerobes (n=8)	Ampicillin	Clindamycin	Chloramphenicol	Metronidazole
Clostridium spp. (3)	33.3%	33.3%	33.3%	0%
Peptostreptococcus spp. (2)	50%	50%	0%	0%
Peptoniphilus spp. (2)	50%	50%	0%	50%
Lactobacillus	0%	100%	100%	0%



Photograph 2: Gram stain smear of Peptoniphus asaccharolyticus showing gram positive cocci (100x magnification).



Photograph 3: WH agar showing feathery growth of Clostridium perferingens.



Photograph 4: Vitek report showing organism identified to be Peptoniphus asaccharolyticus.

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