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RESEARCH ARTICLE

CLINICO –BACTERIALPROFILE OF BRAIN ABSCESS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Anaerobic infection is usually polymicrobial in nature and has a significant role in mortality and morbidity of CNS infection. The bacteriology of Brain abscess is complex and consist of polymicrobialanerobic infections. We aimed to study the rate of isolation, poly microbial nature, association of predisposing factors and sensitivity pattern of obligate and facultative anaerobes in Brain abscess cases. This study was conducted in St Johns medical college, Bengaluru from January 2009 - June 2015.Total 30 samples were processed for the aerobic and anaerobic culture following the standard protocol. Out of 30 samples, 21 yielded the growth of pathogenic organisms. Obligate anaerobes were the major pathogens which were isolated from Brain abscess at the rate of 50% and *Peptostreptococcus* spp (10/26) was the most common obligate anaerobe other than *Bacteroidesfragilis,Fusobacterium*spp*, Prevotella* spp etc. Chronic suppurativeotitis media was the common predisposing factor associated with Brain abscess other than congenital heart disease (CHD), Diabetes mellitus, sinusitis, etc. Polymicrobial synergism was most commonly associated with the obligate anaerobes contributing upto (46.6%) when compared to facultative anaerobes. Three pus aspirates yielded the growth of aseptatezygomycotic fungii and two cases having a fatal outcome of death. Antibiotic sensitivity of *Bacteroidesfragilis* showed intrinsic resistance for Penicillin and Microaerophilic streptococci showed intrinsic resistance to metronidazole. This study signifies about the polymicrobial obligate anaerobic infection in brain abscess and its association with the predisposing conditions like CSOM, sinusitis, CHD etc. Also it is important to look for the sensitivity pattern in complicated cases which helps in evidence based therapy. Hence this will results into better clinical outcome decreasing the morbidity and mortality associated with CNS infections.

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INTRODUCTION

The Bacterioloy of the Central Nervous system (CNS) infection is complex and consist of polymicrobial infection of aerobic and anaerobic bacteria. Pyogenic infections of CNS which commonly involve anaerobic bacteria are Brain abscess, subdural empyema and septic thrombophlebitis (Park *et al*., 2009). Brain abscess is commonly defined as " Focal suppurative process within the brain parenchyma that begins as a localized area of cerebritis and develops into a collection of pus surrounded by a well vascularised capsule. Brain abscess most commonly originates from the contiguous site such as Chronic suppurative otitis media (CSOM), Mastoiditis, Sinusitis, Dental caries, Congenital Heart Disease(CHD) and Surgical proceedureetc (Kashi *et al*., 2006) (Brook, I1995). In upto 25% of cases no primary source of infection is apparent (Pit *et al*., 1993). Despite the advent of modern Neurosurgical technique, new antibiotics and powerful imaging techniques.

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Brain abscess continues to remain potentially fatal (LuCH Chung *et al*., 2002). So early diagnosis, appropriate antibiotic therapy based on the knowledge of the causative agent and surgery are the major prognostic factors for these infections (Louvois J de *et al*., 1977). There is a wide spectrum of etiological agents for CNS infection which could be bacterial, viral, fungal and parasitic in nature. Obligate anaerobes are strict anerobes which grow only in an absence of oxygen and facultative anaerobes are ordinary aerobes but can also grow without oxygen. The microorganisms most frequently isolated are *Fusobacterium* spp*, Bacteroidesfragilis, Peptostreptococcus* spp and *Clostridium sporogenes*etc (Finegold, 1977). Hence it becomes important to know the etiological agent as treatment differs for each of them. Very few published data is available from developing countries like India and since these conditions continues to be a major problem with a fatal outcome, there is a need for more information related to the causative agents of Brain abscess. This study was done to determine the bacterial profile with their synergism, predisposing factors, antibiotic susceptibility pattern and clinical outcome of the Brain abscess patients.

MATERIALS AND METHODS

Subjects and Methods: A study was conducted from January 2009 to June 2015 wherein the pus aspirate from Brain abscess was collected. A total of 30 pus aspirates from Brain abscess admitted in Neurosurgical ward were collected.

Collection and transport: The pus aspirate in a sterile syringe along with the inoculated Robertsons cooked meat broth and thioglycollatebroth were collected and immediatetely transferred to the Microbiology diagnostics. Pus swabs and delayed sample >48 hrswere not accepted for anaerobic culture as they were not suitable samples.

Methodology- Direct gram stain was done from all the pus samples by Huckers method. It was done basically for the presumptive identification of causative organisms. Also in few smears showing numerous pus cells with no bacteria, a ZiehlNeelsen stain was performed to look for Acid Fast Bacilli.

Culture: All the samples were incubated for both aerobic and anaerobic culture. For Aerobic culture 5% sheep blood agar, Mac conkey agar and Thioglycollate broth was inoculated and incubated at 37^0Celsius. For anaerobic culture Brucella Blood agar, thioglycollate broth and Robertsons cooked meat broth was inoculated and incubated anaerobically by Anaerobic Gas pack jar method for 72 hrs.

Bacterial Identification: Presumptive identification of Facultative anaerobes was done following the Standard protocol (SOP 2006) and for obligate anerobes, which were confirmed by the aerotolerance test and gram stain check smear from the anaerobic culture plate. Genus identification of obligate anaerobes was done by using antibiotic discs like kanamycin (1mg), vancomycin (10mcg), colistin (10 IU) etc (Oxoidanident disc) and species identification was done following the standard wadsworth anaerobic manual (Sutter VL *et al* 1980).

Antibiotic Susceptibilitytesting: Antibiotic susceptibility testing was performed by Kirby bauer disc diffusion method. For obligate anaerobes it was done following the wadsworthanerobic manual for commonly used antibiotics like Chloramphenicol (30mcg), Erythromycin (15mcg), Penicillin (10IU) and Clindamycin (2mcg), For Facultative anaerobes it was done as per the CLSI guidelines. Other Demographical data like Age, Sex, Predisposing factors, Clinical outcome, recurrence and other sample culture sensitivity like blood to rule out bacteremia was collected from medical records of St Johns Hospital.

RESULTS

A total of 30 pus aspirates were collected from Brain abscess cases Out of these, 24 were from males and 6 were from females with male to female ratio of 4:1 and eight samples were from paediatric patient. A total of 21(70%) showed the growth of causative pathogenic organisms. 15(68.1%) brain abscess pus samples showed the growth of obligate anaerobes and 7 (33.1%) samples showed the growth of facultative anaerobes.

The rate isolation of obligate anaerobes from brain abscess is more contributing to 50%.Polymicrobial synergism were seen more in obligate anaerobes. Monomicrobial facultative anaerobes isolated from brain abscess were MRSA, *Klebsiella* spp and MSSA. In one pus aspirate, *Escherichia coli* was also isolated with the Aseptatezygomycotic fungi. The spectrum of obligate and facultative anaerobes in brain abscess and their synergism is shown in Table 1.

A total of 26 obligate anaerobes were isolated from 15 brain abscess samples. The most common obligate anaerobic bacteria is *Peptostreptococcus* spp (38.4%). This is similar to other studies where in *Peptostreptococcus* spp is the common gram positive bacteria causing Brain abscess (Murdoch, 1998). The other commonly isolated were *Bacteroidesfragilis, Fusobacterium* spp *and Prevotella* spp etc (Fig 2a). There were few obligate anaerobes which were not completely identified as the strains were lost on subsequent subculture. Seven facultative anaerobes were isolated in which MRSA (1), *Klebsiella* spp (1) MSSA (2) and *Escherichia coli* (2). The spectrum of obligate anaerobes (26) and facultative anaerobes (7) isolated from 21 Brain abscess pus sample are shown in fig 2A and 2B. As the obligate anaerobes are endogenous in nature, they cause infection through contiguous spread when there is a breech in the protective mucosal barrier. Brain abscess with different site of infection with different predisposing factors like CSOM, Congenital heart disease, Diabetes mellitus etc have been depicted in the Table 2.

DISCUSSION

The causative agents of bacterial brain abscess vary with time period, geographical distribution, age, underlying medical, surgical conditions and mode of infections. Despite the availability of improved surgical techniques, effective antimicrobial therapy and modern diagnostic modalities, Brain abscess continues to remain potentially fatal (Lu chung *et al*., 2002). The rationale use of antibiotics depends on microbial etiology of cerebral abscess in that particular geographical location with their sensitivity pattern. In our study out of 30 Brain abscess cases 21(70%) showed the growth of either obligate anaerobes or facultative anaerobes. 15(50%) showed only the growth of obligate anaerobes in which 3 sample yielded obligate and facultative anaerobes. This study signifies that the total rate of isolation of obligate anaerobes is more in cases of Brain abscess (50%) and hence there are chance of missing the causative agents if anaerobic culture is not performed. This may lead to wrong diagnosis and may hamper the treatment modalities of the patients. So in cases of an abscess in Brain parenchyma (Figure 2a&2b) we need to consider these organisms as the probable causative agents and take appropriate measures in the diagnosis and treatment.

However nine samples did not yield any growth. Among them two pus samples showed the presence of polymicrobial obligate anaerobic infection which were presumtively identified by gram staining. All the samples were negative for Acid Fast bacilli by ZiehlNeelsen stain. Among obligate anerobes a total of 26 were isolated from 15 samples in which *Peptostreptococcus* spp *(10/26)* were commonly present contributing for *38.4%.*

Table 1. Obligate and Facultative anaerobes isolated from the pus aspirates of Brain abscess(n=21).

SI NO	MICROBES	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6	SAMPLE 7 &8	SAMPLE 9	SAMPLE 10
1	Obligate Anaerobes	Peptostrepto Coccus spp Fusobacterium spp. GNCB*			B.Fragilis AGPC**		Pr.melanonogenica Peptostreptococcus Fusobacterium spp		Microaerophilic streptococci	B.Fragilis AGPC**
2	Obligate + Facultative Anaerobes		Enterococcus faecalis. Peptostreptococcus spp. BacteroidesFragilis							
3	Facultative Anaerobes			Klebsiella spp		MRSA				
4	Fungal growth							AseptateZygomycois		

SI NO	MICROBES	SAMPLE 11 12& 13	SAMPLE 14	SAMPLE 15	SAMPLE 16	SAMPLE 17	SAMPLE 18	SAMPLE 19	SAMPLE 20	Sample 21
1	Obligate Anaerobes	Peptostreptococcusspp					Prevotella spp Peptostreptococcus	Peptostreptococci + Fusobacterium	Peptostreptococci + GNCB	Bacteroidesfragilis
2	Obligate + Facultative Anaerobes		Escherichia coli Unidentified AGPC AND GNCB.	MSSA+ Peptostreptococci						
3	Facultative Anaerobes				MSSA					
4	Fungal growth					Escherichia coli + AseptateZygomycosis				

*GNCB-Gram negative coccobacilli. AGPC-Anaerobic Gram positive cocci. GNB-gram negative bacilli

Table 2. Predisposing factors of brain abscess yielding growth and its location (n= 21)

S.no	Predisposing factor	Frontal abscess	Temporo cerebellar abscess	Parieto occipital abscess	Cerebral abscess
1	CSOM (8) Sinusitis /		8		
2	Orbital cellulitis(2)	2			
3	Congenital Heart Disease(5)	2		3	
4	Diabetes mellitus(3)	2	1		
5	Cancer			1	
6	Others (Seizure disorder, Age))		1		1

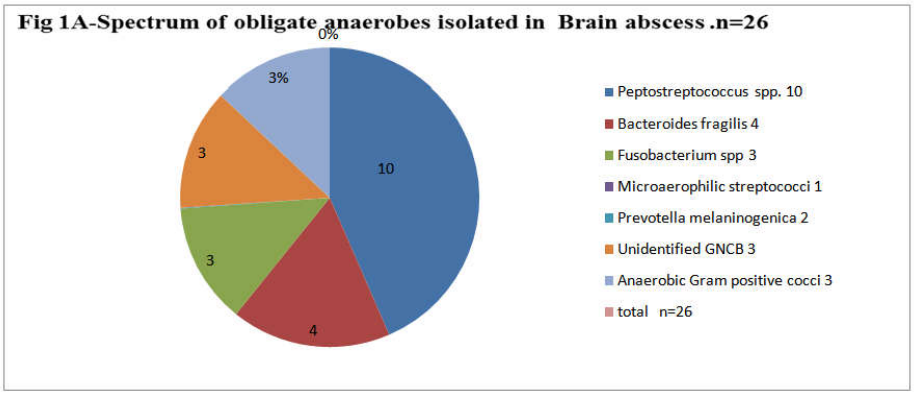
*CSOM-chronic suppurative otitis media. DM-Diabetes mellitus. CHD-Congenital heart disease

Other obligate anaerobes were *Fusobacterium* spp, *Prevotella* spp and Microaerophlic streptococci. Spectrum of obligate anaerobes isolated is shown in Figure 1. Polymicrobial infection was seen more in obligate anaerobes 7(47%) indicating polymicrobial synergism and 3 samples it was between obligate and facultative anaerobes (Table 1).

So in these condition it is is an indication for the treatment with the combination of two or three antibiotics in order to overcome the polymicrobial infection. Predisposing factors were identified among all the samples with growth in brain abscess cases. Among the list of predisposing factors (Table 2).

The most common predisposing factor associated with brain abscess was chronic suppurative otitis media (8) causing temporo cerebellar brain abscess. The other common predisposing factors associated were Congenital heart disease (5), Diabetes mellitus (3), sinusitis (2) etc. This signifies the secondary infection in the brain parenchyma by a contiguous spread of endogenous facultative and obligate anaerobes. According to Ingham HR study, Otogenic cerebral abscesses constitute a major proportion of all cerebral abscesses (Ingham HR 1972). Brain abscess most commonly originates from the contiguous site of an existing infection as CSOM, mastoiditis, sinusitis or dental caries but it can also occur from penetrating head injury, neurosurgical proceedures or by hematogenous spread as in children with congenital heart diseases.

In our study the chronic suppurative otitis media which was associated with the complication of temporo cerebellar abscess by contiguous spread. We also found one child with brain abscess secondary to CHD in which Microaerophilic streptococci was isolated. Aseptatezygomycotic fungi were isolated from three cases where in two cases the site of infection was frontal, one in temporal region and the associated predisposing factor was diabetes mellitus. Two patients died because of associated complications of fungal infection. According to a similar study done by Kashi *et al* (Kashi *et al*., 2006), Predisposing conditions were found in 79.7% cases, otogenic infection being the most common.



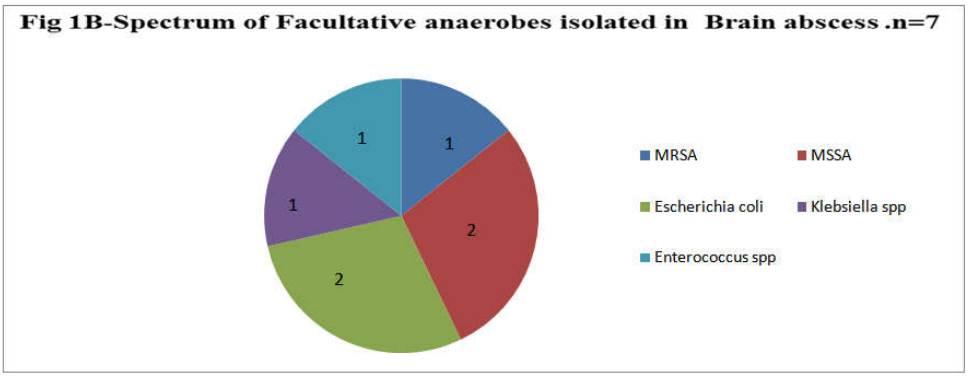


Fig 2A CT Brain showing an Abscess in brain parenchyma due to polymicrobial anaerobic infection

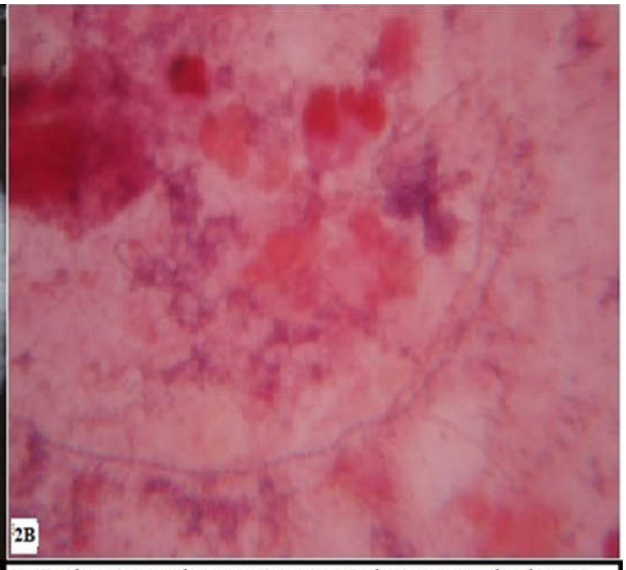


Fig2B-Direct Gram stain smear of pus from Brain abscess showing fine GPC chains and slender GNB suggesting polymicrobial obligate anaerobic infection

Rate of isolation was 30.5% with the common isolation of *Peptostreptococcus* spp and *Bacteroidesfragilis.* According to a study done by (Ingham, 2006), Otogenic cerebral abscesses constitute a major proportion of all cerebral abscesses. Metronidazole is of prime importance in the chemotherapy of anaerobic infection esp *B. fragilis.*

Clinical outcome of brain abscess cases

Most of the cases recovered with surgical treatment. Clinical outcome was also looked from the medical records where in death was seen in 4 cases due to recurrence, associated complications and fugal zygomycosis. In one patient who had Cerebellar abscess with CSOM under went craniotomy with abscess drainage came with recurrence and died due to anaerobic gram negative septicaemia by *Bacteroidesfragilis*. In other case which was Frontal abscess with pansinusitis and orbital cellulitis died due to many other associated complications.

Susceptibility Pattern of Obligate and Facultative anaerobes

Antibiotic susceptibility testing was performed by disc diffusion method on Brucella blood agar following Wadsworth manual, which showed sensitivity to all the commonly used antibiotics like Clindamycin (2 mcg), Penicillin (10 IU), Erythromycin (15mcg) and Chloramphenicol (30mcg). owever intrinsic resistance was seen in Microaerophilic streptococci for Metronidazole (10mg) and for Penicillin (10 IU) in *B.fragilis* as they commonly produce Beta lactamase enzyme. Eventhough no resistance was seen in obligate anaerobes for commonly used antibiotics, several reports of emergence of resistance to various antibiotics has been reported (Jung wust *et al.,* 1977). There are reports of 5% *Bacteroidesfragilis* resistant to metronidazole in France (Wexler HS 1991).

According to CLSI guidelines the recommended methods are Agar dilution & Broth dilution methods. It is not routine done but is significantly recommended in Brain abscess, vascular graft infection & recurrent bacteremia ie is in complicated cases and also to monitor the resistance trends in particular area (CLSI 2012). So there is a need for more and more studies regarding obligate anaerobes and their sensitivity pattern among different geographical areas. This provides enough information for the clinicians to treat these kind of infections with effective and appropriate antibiotics along with surgical measures which will be an evidence based therapy resulting in a good clinical outcome. In this study, sensitivity test done for obligate anaerobes was by disc diffusion method but as per CLSI guidelines (CLSI 2012).

Agar dilution method is recommended for obligate anaerobes. However this was an attempt to look at the sensitivity pattern for obligate anaerobes for epidemiological purpose, following the standard protocol as per wads worth anaerobic manual (Sutter, 1980). Hence this study highlights the need for more and more studies related to the sensitivity pattern in obligate anaerobes. Other facultative anaerobic sensitivity pattern were, *Enterococcus faecalis*, sensitive to Teicoplanin. Vancomycin and resistant to Penicillin and High level gentamicin. *Klebsiella* spp was sensitive to antibiotics like aminoglycosides, cephalosporins and quinolones.

MRSA and MSSA were isolated from Brain abscess which were monomicrobial in nature and one MSSA which had grown with *Peptosreptococcus* spp. *Escherichia coli* were sensitive to aminoglycosides, carbapenemspiperacillin + tazobactam but resistant to ampicillin and cephalosporins. In conclusion, the obligate anaerobes were isolated commonly in Brain abscess cases (71.4%). Most common organism being *Peptostreptococcus* spp & *B. fragilis*. Commonly associated with the predisposing conditions like CSOM, Sinusitis etc. Polymicrobialinfection were seen more with obligate anaerobes in Brain abscess.

If not treated adequately, it may lead to neurological complications like recurrence, septicaemia & death. Antibiotic susceptibility testing is necessary for complicated cases to give an accurate treatment and same has been recommended by the CLSI guidelines. This supports evidence based treatment and better clinical outcome. Acknowledgement-Department of Neurosurgery. St Johns Medical college and Hospital. Bengaluru.

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