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Microbial Spectra of Intracranial Abscesses

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Abstract

A total of 454 pus samples from various intracranial abscess cases were analysed microbiologically for aerobic and anaerobic organisms over a period of ten years. More than 85% of these were culture positive. 22.33% of culture positive cases were due to only anaerobes, 33.67% showed both aerobes and anaerobes and 44.04% yielded only aerobes. Thus the total incidence of anaerobes in brain abscess cases was over 56%, signifying their importance in these infections. Bacteroides spp. was the most commonly isolated anaerobe. Clostridia were isolated from cases secondary to trauma. Proteus mirabilis was the most frequently isolated aerobe followed by non-haemolytic Streptococci and Staphylococcus aureus.

Characterisation of the metabolites of a few anaerobic isolates was made by Gas Chromatography, for speciation of the organisms.

Rare etiological agents like Mycobacterium tuberculosis and the fungus Cladosporium bantianum were isolated from four and two cases of brain abscess respectively.

The microbial spectra as etiological agents in intracranial abscess cases and their predisposing factors are discussed.

Key words -
Brain abscess,
Intracranial abscess,
Microbiology of intracranial abscess,
CNS infections,
Anaerobes

Central nervous system (CNS) bacterial infections commonly present as pyogenic meningitis and intracranial abscesses. Frequently anaerobic bacteria are associated along with aerobes in the causation of CNS infections such as sub-dural empyema, cerebral and cerebellar abscesses and septic thrombophlebitis of the cortical veins. Rarely mycobacteria and fungi are responsible for such infections.

Prompt processing and improved techniques for isolating and identifying the anaerobes in addition to the aerobic organisms, mycobacteria and fungi have now changed the earlier concepts like "unfulfilled expectation in cerebral

abscess" [1] and the myth of "sterile brain abscess". The aerobic and anaerobic culture procedures were set up at this centre in 1978, to study the spectra of microbes, responsible for the causation of the CNS infections.

Material and Methods

Pus from intracranial abscess cases admitted to Neurosurgical services of National Institute of Mental Health & Neuro Sciences, Bangalore, were collected with sterile precautions. Care was taken to fill the specimen upto the brim of the sterile containers which were sealed air tight and transported without delay from operation theatre to the Department of Medical Microbiology . The appearance, consistency and odour of the specimens were noted and immediate cultures were put up from the depths of the specimens for anerobes, aerobes including mycobacteria and fungi, according to standard procedures [2], [3], [4]. The time taken from collection to putting up for culture was not more than 15 to 20 min in most cases. Gram's and Ziehl Neelsen's stains were performed on all specimens to know the type and morphology of the organisms and an immediate report was sent. An India ink preparation for the evidence of cryptococci and a wet cover slip preparation for the evidence of any protozoal parasites were also done.

Briefly, the methodology for isolation of anaerobes was as follows: The specimens were inoculated on to standard enriched and selective anerobic media. Anaerobiosis was attained using BBL Gas Pak and indicator in BBL anerobic jar with the catalyst. The anerobic system was incubated at 37° C for 48 to 96 hours after which the plates were examined for characteristic colonies. Subcultures were made whenever necessary. Smears were made from different types of colonies, stained by Gram's method and examined for typical morphology. An-ident discs (oxoid) were used for gross identification of common anaerobes [5], [6], [7], [8]. For further identification and speciation, biochemical reactions were put up as mentioned earlier [9].

The method used for the isolation of aerobic organisms was as follows: The specimens were plated on Mac Conkey's, Blood and Chocolate agar plates and inoculated into thiglycollate broth, incubated at 37° C aerobically for 24 to 48 hours. The colonies on the plates were identified biochemically according to standard procedures. Thioglycollate broth was subcultured both aerobically and anaerobically and further identification of the organisms was made according to standard methods.

All the specimen were also inoculated on to two slopes of Lowenstein Jensen's medium and Sabouraud's dextrose agar with and without chloramphenicol, to recover any mycobacteria or fungi.

The metabolites of few isolates of Bacteroides spp., Fusobacterium spp. and anaerobic cocci were characterised by Gas chromotography (GC) (Pye Unicam GC 204) for speciation. The standards of volatile and non-volatile fatty acids for comparison were prepared using Sigma chemicals. Extraction of various fatty acids from the anaerobic isolates was as described by Willis [3].

The antibiotic susceptibility of aerobic isolates was tested by standard disc difficulties method of Kirbv and Bauer. The anti-microbial agents tested for aerobic gram negative organisms included Ampicillin (AM), Carabenicillin (CN), Cephaloridine (CR), Chloramphenicol (C), Colistin (CS), Cortrimoxazole (BA), Gentamicin (G), Kanamycin (K), Polymycin - B (PB), Streptomycin (S), Sulphatriad (ST), and Tetracycline (T). and for aerobic gram positive organisms included AM, CN, CR, C, BA, K, ST, T, Cloxacillin (CX), Erythromycin (E), Lincomycin (L) and Penicillin (P).

For anaerobic isolates the antimicrobial agents included for susceptibility testing were, Eyrthromycin

(E), Rifampicin (R), Colistin (CS), Penicillin (P), Kanamycin (K), Vancomycin (V), and Metronidazole (MZ). The inclusion of first six antibiotics helps also in gross identification of the anaerobes depending upon their sensitivity or resistance to these antibiotics [5], [6], [7], [8].

Results

A total of 454 pus samples collected from various intracranial abscess cases over a period of ten years were analysed for microbial pathogens, by aerobic, anaerobic, mycobacterial and fungal cultures. This revealed that 385 cases (over 85%) were culture positive (Table 1). Majority of brain abscess cases (70%) in this series were secondary to middle ear infection. Other predisposing causes included chronic paranasal sinus infections, subacute bacterial endocarditis, congenital heart diseases, trauma, septic abortion and sepsis elsewhere like lung abscess, empyema, pneumonia and cellulitis. Among the anatomical types of intracranial abscesses, temporal and cerebellar abscesses accounted for over 60% of cases followed by parietal (16%), frontal (14%), subdural (6%), occipital and deep cerebral (4%).

Table 1 - Incidence of anaerobes and aerobes in culture positive brain abscess cases (Total no. 385)

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More than 56% of culture positive cases showed the presence of anaerobes either singly or in association with aerobes (Table 1). The spectra of anaerobes isolated from the cases of brain abscess showed a variety of organisms (Table 2). Bacteroides fragilis and other Bacteroides spp. were most frequently isolated followed by anaerobic cocci and Fusobacteria. All the Clostridial isolates were from post-traumatic intracranial abscess cases. Proteus mirabilis among the aerobes was the most common isolate followed by non-haemolytic streptococci, Staphylococcus aureus and Haemophilus aphrophilus. Apart from these and the other usual aerobic organisms listed in Table 3, of special interest were, three cases of brain abscess caused by Salmonella typhi, two by Salmonell typhimurium, three cases by Mycobacterium tuberculosis and two cases by the fungus Cladosporium bantianum/trichoides. This study also reveals that in large majority of cases of brain abscess, more than two organisms can be isolated signifying their polymicrobal etiology.

Table 2 - Spectra of anaerobes isolated in culture positive brain abscess cases (n=385)

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The chromatographic study of a few representative isolates of *Bacteroides spp Fusobacterium spp* and anaerobic cocci were analysed by comparing the chromatograms with that of standard for speciation of the isolates.

Over 95% of aerobic enteric gram negative bacilli, pseudomonas and non fermenters showed multidrug resistance being sensitive only to G. All the three isolates of *S. typhi* were resistant to C and sensitive to the other antibiotics. *Staphylococcus aureus* showed a variable sensitivity pattern, all of them uniformly being sensitive to CX and L and resistant to P and AM. The other organisms isolated showed different sensitivity patterns. The Streptococci and H. aphrophilus were sensitive to the

antimicrobial agents tested, except for Sulpha in few cases.

Table 3 - Aerobic organisms isolated from 385 culture positive brain abscess cases

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The isolates of M. tuberculosis were sensitive to all the standard antitubercular drugs, namely Streptomycin, INH, Rifampicin, Ethambutol and PAS.

With respect to anerobes, all the isolates of B fragilis were sensitive to E and MZ, and resistant to the others. B. melaninogicus isolates were sensitive to E, R, P, MZ, and variable to K, CS. Fusobacterial spp. were sensitive to CS, P, K, MZ. Peptococci and peptostreptococci were sensitive to E, R, P, K, V and MZ. The clostrida were sensitive to E, R, P, K, V and MZ.

Discussion

The microbial spectra of intracranial abscess cases is often complex. These intracranial abscesses can occur in various anatomical sites such as temporal cerebellar, parietal, frontal, subdural occipital and rarely deep cerebral like thalamic and pontine. This study over a period of ten years has emphasised the importance of anerobes and the polymicrobial nature in the etiopathogenesis of these abscesses. It has also shown clearly that the most frequent and significant predisposing factor for the causation of intracranial abscesses in chronic middle ear infection. An important clue to the presence of anaerobes in pus is its watery consistency and foul odour [4]. In almost all our cases this was noted including in those cases with a mixture of aerobic and anaerobic organisms. This stresses the importance of the so called "sniff test", to know the presence of anaerobes in a given specimen.

The most important clue to the polymicrobial nature of pus is given by Gram's smear of specimens. A careful interpretation of the Gram's stain by an experienced and vigilant microbiologist is very useful and immediately gives a fairly accurate impression of the types of organisms present depending on the morphological characters on a combination of antibiotics based on past experience until the culture and sensitivity reports are available.

The spectra of bacteria, both aerobic and anaerobic in brain abscess has been studied by various workers since 1943 [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20]. This study clearly brings out the spectra of aerobic bacteria, anaerobic bacteria, mycobacteria and fungi in a large number of cases of intracranial suppurative lesions over a period of ten years. Previous studies stress the importance of anerobes in such infections [17], [18], [19]. Earlier there have been reports of infrequent isolation of anaerobes [4] and sterile brain abscesses [12], [14], [15]. Garfield [21] has expressed that factors needed for an accurate assessment of bacteria in cases of brain abscess were very delicate. Improper collection and delay in culture of specimens, incorrect temperature and gaseous atomosphere often make the isolation of anaerobes difficult.

Stringent procedures to collect the specimen with sterile precautions, speedy and accurate culture techniques both for aerobes and anaerobes and reliable identification procedures especially for anerobes, reduce the number of the so-called entity of "sterile abscess".

The authors also stress the importance of study of antimicrobial susceptibility of the various organisms isolated, especially in these days of emergence of multiple drug resistant strains of aerobic gram

negative bacilli like Proteus spp., Enterobacter spp., Pseudomonas, S. typhimurium and Morganella morganii. Antimicrobial susceptibility testing of anaerobes should also be done routinely to identify an occasional strain deviating from the usual sensitivity pattern. It was interesting to note that all the 3 strains of S. typhi isolated from brain abscess cases were resultant to chloramphenicol, unlike the strains isolated from cases of enteric fever of from cases of meningitis [22].

No doubt, gas chromatographic analysis of metabolites of anerobic isolates is a fairly accurate mode of speciation of most of the non-sporing gram negative anaerobic bacilli and anaerobic cocci. In a routine diagnostic microbiology department, where rapid diagnositc services are needed for gross identification of the various anaerobes, an-ident disc method and biochemical speciation is quite satisfactory. For rapid assessment of presence of anaerobes in brain abscess pus direct extraction by chloroform or ether and analysis by GC may be applied when facilities are available; but often this is complicated by the polymicrobial nature of brain abscess pus. Adequate staff, a proper equipment with all the accessories, standard volatile and non-volatile fatty acids of high quality and easy availability of gases like hydrogen and nitrogen are important factors for routine GC analysis in a separate anaerobic bacteriology division.

This study also stresses the importance of looking for mycobacteria and fungi, in brain abscess pus. Without these procedures, the three cases due to M. tuberculosis and two cases due to C. bantianum would have been missed and the patients would not have been put on proper line of treatment.

Once a proper microbiological analysis of the specimens is known, prompt and early institution of specific antimicrobial chemotherapy, in addition to the necessary surgical procedure, helps to minimise the mortality and morbidity in intracranial abscess cases. Hence, an abscess of the central nervous system is no less an emergency to the microbiologist than it is to the neurosurgeon.

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1., Unfulfilled expectations of cerebral abscess, Editorial
British Medical Journal Page: 1: 25, 1969
2.Baily and Scott's, *Diagnostic Microbiology 4th edition, 1974 6th edition 1982 (Eds) Sydney M, Finegold & William J Martin. The C V Mosby Company U S A*
3.Willis A T, in Anaerobic Bacteriology
*Clinical and Laboratory Practice, 3rd Edn Butterworth London*1977
4.Sydney M, Finegold, *in Anaerobic Bacteria in Human Disease, New York San Francisco London, Academic Press*1977
5.Sutter V L & Fineglod S M, Antibiotic disc susceptibility tests for rapid presumptive identification of gram negative anaerobic bacilli
Applied Microbiology Page: 21: 13, 1971
6.Finegold S M, *Screening for anaerobes Paper presented at the IMLS Meeting, Liverpool*Aug 1977
7., Standardised disc susceptibility test
*Codes of Federal Regulation, 21, Food and Drugs Part 460 Revised April 1*1978

8.Leigh D A & Simmons K, Identification of nonsporing anaerobic bacteria
Journal of Clinical Pathology Page: 30: 991, 1977
9Gokul B N, Chandramukhi A, Neelam Khanna, Ravikumar R & Muralidhar K Katti, Roleof anaerobes in brain abscesses and other central nervous system infections
Proc I Asian Congress on Anaerobic Bacteria in Health and Disease (Eds) Ajitha Mehta & Nina Koche Page: p 168, 1987
10.McFarlane A M, The baceteriology of brain abscess
British Medical Journal Page: 21: 643, 1943
11.Heinman H S & Braude A I, Anaerobic infection of brain. Observations on 18 consecutive cases of brain abscess
The American Journal of Medicine Page: 35: 682, 1963
12.Barlet J G & Gorbach S L, Anaerobic infections of head and neck
Otolaryngology Clinic of North America Page: 9: 665, 1970
13.Heinman H S, Brude A L & Osterholm J L, Intracranial suppurative disease
JAMA Page: 218: 1542, 1971
14.McClelland J, Charles F & Crockard A H, Brain abscess
Journal of Neurosurgery Page: 36: 1, 1972
15.Bhatia R, Tandon P N & Banerjee A K, Brain abscess - An analysis of 55 cases
International Surgery Page: 58: 565, 1973
16.Ingham I R, Selkon J B & Roxby C M, Bacteriological study of otogenic cerebral abscess. Chemotherapeutic role of metronidazole
British Medical Journal Page: 2: 991, 1977
17.Chandramukhi A, Hegde A S & Narayana Reddy G N, Anaerobic brain abscess - Role of metronidazole in chemotherapy
Neurology India Page: 28: 213, 1980
18.Chandramukhi A, Narayana Reddy G N & Gourie-Devi M, Anaerobic infections of central nervous system, in
Anaerobic Infection in Man (Ed) Usha Gupta, Everyman's Press Page: p 89, 1982
19.Chandramuki A, Narayana Reddy G N & Hegde A S, Anaerobes and brain
Indian Journal of Surgery Special supplement Page: 43: 90, 1981
20.Bhatia R, Brain abscess in children
Indian Journal of Paediatrics Page: 50: 591, 1983
21.Garfield J, Brain abscess and focal suppurative infection in
*Handbook of Clinical Neurology Vol 33, Ch 9, p 107, (Ed) Vinken P J and Bruyn G W, North Holland Publishing Co*1973
22.Gokul B N, Chandramukhi A, Ravikumar R & Neelam Khanna, [Salmonella infections of central nervous system]
NIMHANS Journal Page: 6 : 115, 1988