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Research Article

ANAEROBIC PLEURO – PULMONARY INFECTIONS: IS ROUTINE CULTURE NECESSARY

Hussain JH [1]*, Hazarika NK [1], Barua N [2], Bhagawati G [1], Khandelwal F [1]

1. **Department of Microbiology, Gauhati Medical College, Guwahati, Assam**
2. **Department of Microbiology, Jorhat Medical College, Jorhat, Assam**

Corresponding Author: Dr. Jasmin Halim Hussain, Department of Microbiology, G. B. Pant Hospital, J. L. Nehru marg, NewDelhi 110002.

Abstract :

Background: Anaerobes play a major role in pleuropulmonary infections. Obligate anaerobes are the predominant constituents of normal oropharyngeal flora and produce pleuropulmonary infection in patients who are prone to aspirate. Predisposing conditions include prominent dental disease, chronic upper respiratory tract infections and reduced consciousness. **Aims:** To isolate both aerobic and obligate anaerobic bacteria implicated for causing pleuro – pulmonary infections and to evaluate the necessity of routine anaerobic culture for such infection. **Settings and Designs:** A prospective study was conducted over a period of one year. **Methods:** Specimens of pleural fluid, empyema fluid, and aspirates from lung abscess, collected through transthoracic route and blood were collected from 55 patients, clinically suspected to have pleuro-pulmonary infections. Specimens were processed for isolation of both aerobes / facultative anaerobes and obligate anaerobes using standard microbiological techniques. **Results and Observation:** Out of the 55 cases included in the study, 18 (32.7%) cases showed growth of aerobic organisms while 2 (3.63%) cases showed the growth of anaerobic organisms, the rest being culture negative. From the culture positive cases, the most commonly isolated aerobe was *Klebsiella pneumoniae* (36.84%), followed by *Staphylococcus aureus* (21.05%), *Pseudomonas* spp. (15.78%), *Streptococcus pneumonia* (10.52%), *Escherichia coli* (5.26%) and *Proteus vulgaris* (5.26%). *Prevotella* spp. was the only anaerobe isolated from 10.52% of the culture positive cases. Blood cultures revealed no growth of any organisms. **Conclusion**: To obtain proper clinical specimens for anaerobic culture is very difficult and also the process of culturing these organisms is very expensive and time consuming. Suspected anaerobic infections can be treated with empirical antibiotics guided by published studies. Therefore routine culture and susceptibility testing for such infection is rarely warranted.

Key words: anaerobes, pleuro – pulmonary infections

INTRODUCTION

Anaerobic bacteria have been implicated in aspiration pneumonia and its sequelae, including lung abscess, necrotizing pneumonia and empyema since the early 1900s [1]. Obligate anaerobes are the predominant constituents of normal oropharyngeal flora and produce pleuro – pulmonary infection in patients who are prone to aspirate [2]. Predisposing conditions include prominent dental diseases, chronic upper respiratory tract infections and reduced consciousness [3].

It has also been found that the aetiology of pleuro-pulmonary infections depends on the geographic region, patient's age and advances in the diagnosis and treatment of the underlying cause [4, 5].

Anaerobic bacteria play a relatively well confirmed role in selected types of pulmonary infections that are uncommon but distinctive, with common clinical features that include

indolent course, putrid discharge and response to antibiotics directed at anaerobes including clindamycin or β – lactam – β – lactamase inhibitors that are favoured for most cases of lung abscess [1].

The clues to the subset that do involve anaerobes include probable aspiration as evidenced by dysphagia (inability to drink water rapidly) or reduced consciousness along with infection in a dependent pulmonary segment with aspiration in the recumbent position or basilar segments with aspiration in the upright position; putrid discharge (sputum, empyema fluid), diagnostic of anaerobes; indolent course (nonspecific); necrosis of tissue with necrotizing pneumonia, lung abscess or empyema with a bronchopleural fistula [1].

Most cases of pneumonia probably do not involve anaerobic bacteria. In addition, the antimicrobials that are commonly used for community acquired pneumonia and other common lung infections like β – lactams, macrolides and flouroquinolones have sufficient activity versus upper airway anaerobes [1].

Obtaining material from these patients for culture from the site of infection that is uncontaminated by normal flora is problematic. In vitro cultivation of obligate anaerobes requires rigorous anaerobic techniques and susceptibility testing of obligate anaerobes is not standardized in many clinical microbiology laboratories. Few clinical trials of drugs have been done in patients with laboratory documented or putative anaerobic pulmonary infection. For these reasons the diagnosis and therapy of anaerobic pulmonary infection are frequently empirical and guided by published studies of in – vitro activity against collected clinical isolates [1].

Considering the above, the present work was undertaken to isolate and identify the bacterial agents causing pleuro - pulmonary infections and to evaluate whether it is actually required to carry out anaerobic cultures on a routine basis in order to manage such infections.

MATERIALS AND METHODS:

The study involved 55 patients suspected to have anaerobic pleuro – pulmonary infections and was done in a tertiary care hospital in Assam, India.

Two specimens of pleural fluid, empyema fluid or aspirates from lung abscess were taken from each patient who had the predisposing factors that might lead to anaerobic pleuro – pulmonary infections. The specimens were collected either transthoracically or intraoperatively.

Gram stains of all the specimens were made and processed following the standard microbiological techniques for isolation and identification of both aerobic and anaerobic organisms [6, 7].

For isolation of anaerobic organisms, ready to use Thioglycollate broth from Hi Media Laboratories Pvt. Ltd. Mumbai were used as a media for collection and transport of the specimens. The specimens were collectcted in sterile syringes and inoculated immediately to the pre reduced thioglycollate broth avoiding introduction of any air. Then the broth were incubated anaerobically for 48 hours at 37^0 C. Then the broth was subcultured on anaerobic blood agar media and incubated in an anaerobic jar at 37^0 C for 48 hours. Anaerobiasis was achieved by anaerobic gas packs commercially available from Hi Media Laboratories Pvt. Ltd. Mumbai. The organisms isolated anaerobically are further subcultured on blood agar, MacConckey agar and anaerobic blood agar media and incubated in aerobic and anaerobic conditions respectively to deduce whether the isolate is facultative or obligate anaerobe. Obligate anaerobes showed no growth in plates incubated aerobically and facultative

anaerobes were found to be grown in both aerobic and anaerobic conditions. Identification of anaerobic organisms was done manually according to standard guidelines.

For isolation of aerobic organisms, the specimens were collected in a sterile tube. Gram stains were prepared from all the specimens and were inoculated on blood agar and MacConkey agar media. Isolated organisms were identified manually according to the standard guidelines [7].

Two specimens of blood were taken from all the patients and were processed for isolation of aerobic, facultative anaerobic and obligate anaerobic bacteria.

Isolates of *Staphylococcus aureus* were screened for MRSA using standard guidelines.

All the isolated bacteria were tested against different antimicrobial agents by standard disc diffusion method (Kirby Bauer Technique).

RESULTS:

Out of the 55 cases included in the study, 18 (32.7%) cases showed growth of aerobic organisms while 2 (3.63%) cases showed the growth of anaerobic organisms, the rest being culture negative. (Table1)

Table1: Culture results of the specimens of the 55 cases included in the study

TYPE OF ISOLATES	**NO.**	**PERCENTAGE**
Only aerobes	**17**	**89.48%**
Only anaerobes	**1**	**5.26%**
Both aerobes and anaerobes	**1**	**5.26%**
TOTAL	**19**	**100%**

Table2: Various bacterial isolates of the culture positive cases

Aerobic isolates	
Gram negative bacilli	**No. (%)**
Klebsiella spp	7 (35%)
Pseudomonas spp	3 (15%)
Escherichia coli	1 (5%)
Proteus vulgaris	1 (5%)
Gram Positive cocci	**No. (%)**
Staphylococcus aureus	4 (20%)
Streptococcus pneumoniae	2 (10%)
Total aerobic isolates	18 (90%)
Anaerobic isolates	
Gram negative bacilli	
Prevotella spp.	2 (10%)
Total no. of isolates	20 (100%)

Of the four isolates of *Staphylococcus aureus*, two were found to be MRSA.

Of the enterobacteriacae group, the organisms showed maximum sensitivity to Imipenem (100%), followed by Cefotaxime (77.77%), Pipercillin – Tazobactam and Gentamicin (66.66%), Ciprofloxacin (55.55%), Cefepime (33.33%) and Cefuroxime (22.22%). All the organisms showed resistance to Ampicillin.

The isolated *Pseudomonas* spp showed maximum (100%) sensitivity to Imipenem and Polymyxin B, followed by Amikacin, Tobramycin and Ceftazidime (66.66% each) while Pipercillin – Tazobactam and Ciprofloxacin shows 33.33% sensitivity. None of the Pseudomonas isolate was sensitive to Aztreonam.

The Staphylococcal isolates showed maximum (100%) sensitivity to vancomycin and Linezolid, followed by Amoxyclav, Erythromycin and Doxycyclin (50% each) while Gentamicin and Ciprofloxacin shows 25% sensitivity. All isolates were resistant to Penicillin.

Both the isolates of *Streptococcus pneumoniae* were sensitive to Penicillin, Gentamicin, Vancomycin and Linezolid, while one isolate was found to be sensitive to Amoxyclave.

The two isolates of *Prevotella* spp. were sensitive Clindamycin, Piperacillin-Tazobactam, Cefotaxime and Imipenem while only one isolate was found to be sensitive to be sensitive to cefuroxime and Ciprofloxacin. Both the isolates were resistant to Metronidazole and Ceftriaxone.

Blood culture was done from all the 55 cases of included in the study, but none of the cases revealed growth of any organisms.

DISCUSSION:

Out of the 55 cases included in the study, 18 (32.7%) cases showed growth of aerobic organisms while 2 (3.63%) cases showed the growth of anaerobic organisms, the rest being culture negative. Similarly S. Tareen et al [1] found 26% cases to be culture positive and could not recover any anaerobic organism, K. Wanjari[8] also found only 11.16% of the cases to be culture positive and could not recover any anaerobic organism.

But in comparison to some other studies, [9, 10, 11, 12, 13] the present study reveals lower isolation rate of anaerobic organisms. This might be due to administration of empirical antibiotics that are commonly instituted in such patients to stabilize or probably there may not be common involvement of anaerobic bacteria as the etiological agent in such infections in this region of the world as involvement of anaerobes may have a geographical distribution as reported by some studies [4, 5].

In our study, it was found that amongst the culture positive cases, the most commonly isolated aerobic organism was *Klebsiella pneumoniae* (36.84%), followed by *Staphylococcus aureus* (21.05%), *Pseudomonas* spp. (15.78%), *Streptococcus pneumoniae* (10.52%) and *Escherichia coli* (5.26%) and *Proteus vulgaris* (5.26%). *Prevotella* spp. was the only anaerobic organism isolated from 10.52% of the cases. Such findings were also reported by K. Y. Chen et al[9] and Jiun – Ling Wang et al[14] who reported *Klebsiella pneumoniae* to be the most commonly isolated organism. D. Panigrahi et al[15] also found *Klebsiella pneumoniae* as one of the predominant aerobic pathogen and *Prevotella* spp as the commonest anaerobic isolate.

Blood culture was done from all the 55 cases included in the study, but none of the cases revealed growth of any organism. Such findings were also reported by I. Yaacob and Z. Ariffin[16] who failed to grow any organism from blood culture in 7 out of 13 patients with empyema and could recover *Streptococcus viridans* from only one case out of 9 cases of lung abscess. But J.L. Wang et al[14] reported 18% positive blood cultures in patients with lung abscess.

The negative result for blood cultures in the present study may be attributed due to early administration of antibiotics; moreover the sensitivity of blood cultures can be increased by proper timing of specimen collection and increasing the number of specimens.

Few of the aerobic bacterial isolates were found to be resistant to third and fourth generation Cephalosporins. However all the isolates were sensitive to Carbapenems. Both the anaerobic isolates though sensitive to most of the antibiotics were resistant to metronidazole.

According to the published guidelines, for community acquired infections, the recommended antibiotics include intravenous amoxicillin – clavulanic acid or a combination of a second generation cephalosporin (e.g. cefuroxime) or clindamycin if the patient is allergic to penicillin and metronidazole [17].

Patients with nosocomial infections need adequate Gram negative coverage as Gram negative organisms are more common in nosocomial infections. For these cases coverage should include at least a carbapenem or antipseudomonial penicillin (e.g. piperacillin – tazobactam) or third or fourth generation cephalosporins (e.g. ceftazidime, cefepime) with metronidazole. If there is a strong suspicion of MRSA coinfection, vancomycin or linezolid can be added. Aminoglycosides should be avoided as these may be inactivated at low pleural fluid p^H and are ineffective against anaerobes [17].

CONCLUSION:

Microorganisms that constitute the normal oropharyngeal flora may gain access to the deeper lung tissues in individuals prone to aspirate. Oropharyngeal secretions are loaded with both aerobic and anaerobic organisms in high concentrations, therefore in an already diseased lung or in generalised immunosuppression, these organisms might overcome the defence mechanism and establish infection.

Different studies have reported that anaerobic organisms are causal factors of various types of pleuro – pulmonary infections. In the current study also two anaerobic organisms were isolated.

But routine culture of anaerobic organisms is a time consuming and expensive task. Collection of appropriate samples and their transport to the laboratory is also very meticulous and must be done properly for successful isolation of anaerobes. Moreover susceptibility testing of anaerobic organisms is not standardized. Considering the above facts it is very difficult to carry out anaerobic culture is in a routine basis.

The recommended treatment for anaerobic pleuro – pulmonary infections surgical intervention and antibiotic administration as early as possible. As culture and antibiotic susceptibility of anaerobes takes a lot of time, it always becomes necessary to start empirical antibiotic administration in order to contain such infection.

As recommended by many other published reports [2, 18] authors of this study would also like to conclude that routine culture and susceptibility testing of anaerobic organisms is not warranted. But studies on anaerobic isolates of pleura – pulmonary infection should be carried out so as to keep track on the changing trend of anaerobic isolates as causative agents of such infections and their susceptibility pattern.

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