

A Study on Microbiological Analysis of Pleural Fluid

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Abstract – Since the introduction of antibiotics, the microbial etiology of pleural space infections has changed. The current study was conducted between November 2016 and October 2018 (two years) in the Microbiology Division of the Central Laboratory, SMIH, Patel nagar, Dehradun, to obtain the microbiological profile of patients with pleural effusion and empyema. In this study, pleural fluid samples gathered aseptically by thoracentesis from the hospital departments of medication, pediatrics and pulmonary medication were incorporated. According to the rules of the Clinical and Laboratory Standards Institute, the examples were prepared for identification based on standard laboratory strategies followed by antibiotic powerlessness testing of pyogenic isolates performed by the Modified Kirby-Bauer plate diffusion strategy utilizing Mueller-Hinton agar. 45 samples of pleural fluid were gotten over a time of 9 months. The gram negative isolates were only powerless to Gentamycin and Imepenem. The development of antibiotic-resistant microorganisms, the ascent in nosocomial infection recurrence and the steadily increasing number of patients with impaired insusceptibility have consolidated to maintain a common element for pleural infections.

Keywords: Pleural Fluid, Microbiological Profile, Pleural Effusion, Empyema

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INTRODUCTION

Pleural effusion creates when more fluid enters the pleural space than is taken out. Potential mechanisms of pleural fluid accumulation include: increased interstitial fluid in the lungs secondary to increased capillary pulmonary pressing factor (for example heart failure) or permeability (for example pneumonia); increased pleural membrane permeability and hindered lymphatic stream (for example hypoalbuminemia); decreased plasma oncotic pressure (for example hypoalbuminemia) (i.e., chylothorax) decreased intrapleural pressure (for example atelectasis); decreased plasma oncotic pressure (for example hypoalbuminemia)(1, 2).

INITIAL EVALUATION'S PLEURAL EFFUSION

The chest exam of a pleural effusion patient is imperative for percussion gruffness, decreased or absent tactile fremitus, decreased breathing sounds, and no transmission of voice.

Posteroanterior and lateral chest radiographs generally confirm pleural effusion, however on the off chance that all the other things fails, ultrasound or CT scans are finished for the identification of small effusions and the differentiation between pleural fluid and pleural thickening (3). Free pleural fluid can dull

the costophrenic angle on a posteroanterior radiograph; structure a lateral meniscus; or cover up in a subpulmonary location, simulating an elevated hemidiaphragm.

Loculated effusions are commonly associated with conditions, for example, tuberculosis or empyema, hemothorax, that cause outrageous pleural inflammation. Occasionally, a focal intrafissural fluid collection may appear as though a lung mass. Most commonly, the present situation is found in patients with heart failure. The apparent mass disappearance absolutely decides the diagnosis of a pseudotumor when heart failure is treated (i.e., vanishing tumor). Heart failure is by a wide margin the most common cause of bilateral pleural effusion, however in the event that cardiomegaly is absent, different factors, for example, malignancy, ought to be investigated.

PLEURAL FLUID FORMATION

Pleural Fluid is a ultrafiltrator of plasma. In each pleural cavity, there is typically under 10 mL of fluid. At the point when the rate of fluid formation surpasses the rate of fluid removal, more than that will accumulate. Fluid production is enhanced if there is:

- Elevation of hydrostatic pressing factor gradient (for example in congestive cardiac failure, portal hypertension):
- A decrease in osmotic colloid pressure (e.g. in hypoproteinaemia).
- Increased permeability of vessel capillaries (e.g. in infection, malignancy, inflammation).
- Removal of fluid is reduced if there is: . Lymphatic drainage impaired (e.g. in some neoplasms).
- Pleural Space Pressure Decreased (for example in bronchial obstruction, atelectasis).
- Blood, discharge and chylus may also be available as fluid accumulations.

CHARACTERISTICS OF PLEURAL FLUID

The characteristics of pleural fluid vary according to the hidden pathological condition, however can be broadly classified into two categories, transudative and exudative, contingent upon appearance and smell, and then further into subcategories, for example, purulent, bleeding, and chylous. While assistance can also be the classic Rivalta reaction, Light's diagnostic criteria are most as often as possible used to differentiate among transudative and exudative effusions. Exudative effusion is diagnosed according to this strategy, when at least one of three criteria are met. In the event that pleural effusion, in spite of being clinically considered transudate, is diagnosed as exudate by this criterion, the distinction in albumin concentration among serum and effusion is higher than 1.2 mg/dl, at that point the effusion is diagnosed as transudate. The amount of LDH present in the pleural liquid is an unpleasant indicator of the level of pleural inflammation and is useful in the evaluation of treatment results. Often present on the different sides of the chest, transudative pleural liquid is caused by heart failure, nephrotic mix, low protein leukemia, malnutrition, hypothyroidism and other basic diseases (3).

As the condition often settle with treatment of the hidden cause or with diuretics, thoracentesis is usually not required except if ventilatory impairment or significant mediastinal displacement happens.

MATERIAL AND METHODS

Thoracocentesis aseptically collected pleural fluid samples from the Central Laboratory Microbiology Division, SMIH, Patel nagar, Dehradun between November 2016 and October 2018. (two years). In this study, were included. This study included single or mixed growth from one patient and consecutive samples from new patients. The study excluded repeat samples received from patients already enrolled, antibiotic patients and patients who did not give their

consent. For direct microscopy and culture, the specimens were centrifuged and processed. Smears from the sample and Gram staining were prepared and Ziehl Neelsen staining was done. For culture, the sample was inoculated to rule out fungal infections on 5 percent sheep blood agar, Macconkey agar and Sabouraud dextrose agar plates. The specimens were processed for identification on the basis of standard microbiological techniques. 2 The samples were also sent simultaneously by CB-NAAT to detect *Mycobacterium tuberculosis*.

According to Clinical Laboratory Standards Institute (CLSI) guidelines, antibiotic susceptibility testing of pyogenic isolates was performed using the Modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) was performed for staphylococcal isolates using cefoxitin (30 mcg) discs (4).

Penicillin, *gentamicin*, *vancomycin*, *azithromycin*, *clindamycin*, *linezolid*, and gram-negative microorganisms *cefazidime*, *meropenem*, *ciprofloxacin*, *gentamicin*, *imipenem*, *cotrimoxazole*, *amoxycillin-clavulanic acid* were the principal line drugs tried for gram-positive microorganisms.. When all first-line drugs were found to be resistant, second-line drugs were placed in place.

RESULTS AND DISCUSSION

45 samples of pleural fluid were received over a period of 9 months. Bacteriological culture energy was given by only 13 (28.8 percent) of 45 pleural fluid samples and 5 (11.1 percent) were positive for CBNAATT *Mycobacterium tuberculosis* (Cartridge Based Nucleic Acid Amplification Test).

The maximum group of patients was 10-19 years of age (24.4%), followed by >60 years of age (24.4%). (20 percent). 2:1 was the male to female ratio.

Gram stain direct detection of microorganisms was 16.2 percent . And the stain was 2/5 by Ziehl Neelsen (40 percent). On the other hand, the positivity of aerobic bacterial culture was 28.8 percent. The analysis prohibited an additional 2 patients whose pleural fluid societies indicated development of contaminant bacteria. Therefore, culture was more susceptible than gramme staining for diagnosis. No microorganism in fungal culture has been isolated.

Microbiological profile:

Gram-positive microorganisms were more prevalent than gram-negative microorganisms (57.1% vs. 28.5%). Among gram-positive organisms, *Staphylococcus aureus* was the dominant isolate, trailed by coagulase-negative *Staphylococcus species* and *Streptococcus pneumoniae*. Among the gramme negative isolates of *Providencia spp.*, *Escherichia coli*, organism group Nonfermenter,

followed by *Pseudomonas* spp. Identified were. As detected by CB-NAAT, five patients demonstrated *Mycobacterium tuberculosis* infection.

TABLE 1- Spectrum of various organisms isolated

Organism isolated from culture	No. Of isolates(14)	% of isolates
<i>Staphylococcal aureus</i> (MRSA)	3	21.4%
<i>Staphylococcal aureus</i> (MSSA)	3	21.4%
Nonfermentors	2	14.2%
CONS	1	7.14%
<i>Streptococcus pneumoniae</i>	1	7.14%
<i>Providentia</i>	1	7.14%
<i>Escherichia coli</i>	1	7.14%
Skin commensals	2	14.2%

For both gramme positive and gramme negative microorganisms, antibiotic susceptibility test results were also compiled. Ingram positive organisms concerning the sensitivity patterns of the isolates were noticed to be interesting trends. The highest resistance was seen among gram-positive microorganisms with azithromycin (71.4 percent), followed by isolates of penicillin (57.1 percent) and clindamycin (42.8 percent). All isolates were susceptible to vancomycin, gentamicin and linezolid. Methicillin resistant *Staphylococcus aureus* (MRSA) was detected using the cefoxitin disc method in 50 percent of *Staphylococcus aureus* isolates.

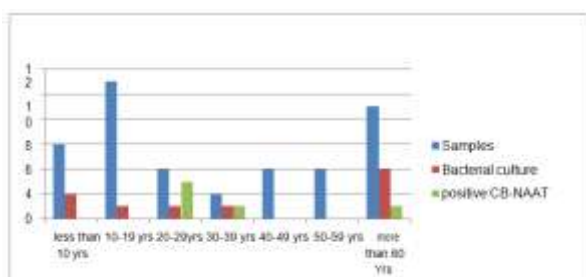


Figure 1: Age group of patients

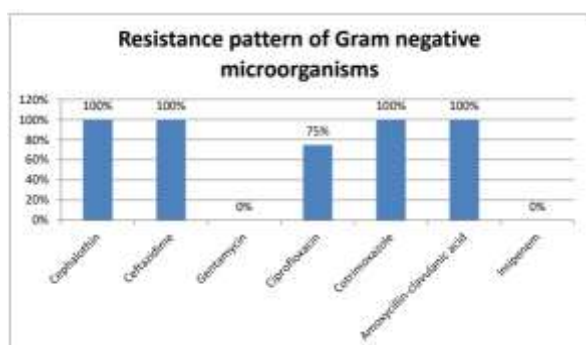


Figure 2: Resistance pattern of gram positive organisms

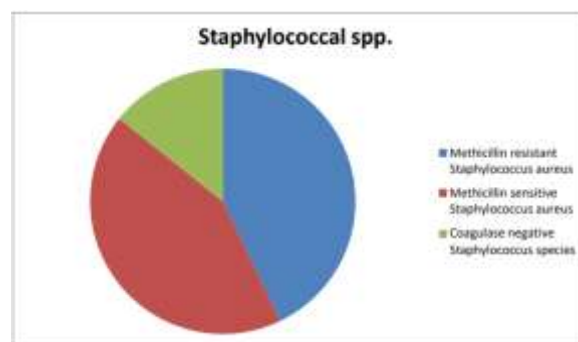


Figure 3: Staphylococcal spp.

Cotrimoxazole, ceftazidime, cephalothin, and amoxicillin-clavulanic acid were discovered to be 100% resistant to Gram-negative aerobic collection. Gentamycin and Imipenem only were powerless to these isolates. Expanded range beta lactamase (ESBL) production was confirmed in all isolates.

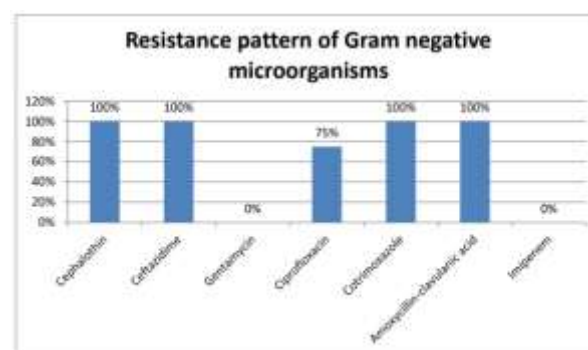


Figure 4: Resistance pattern of Gram negative microorganisms

In 44 per cent of patients with community-acquired pneumonia, pleural effusion and empyema are estimated to occur. Gram stain and pleural fluid culture are essential components of the evaluation of parapneumonic pleural effusion patients. The emergence of antibiotic-resistant microorganisms, the rise in nosocomial infection frequency and the steadily increasing number of patients with impaired immunity have combined to maintain a common entity for pleural infections (5).

In the current study conducted at our tertiary care rural medical school hospital, the proportion of positive societies was 28.8 percent, consisting of 45 pleural fluid samples got in the microbiology laboratory. A wide variation has been shown in microbiological diagnosis rates in earlier studies. In works by Mohanty et al (15.3 percent) (6), a lower positive culture rate has been observed. On the other hand, multiple workers around the world have reported a high positivity rate of cultures from 31-89 percent, such as works by Alfageme et al. (7) The explanation for this broad disparity in empyema fluid inspiration rates is because of contrasts in microbiological methods and contrasts in

microbiological procedures (8). Another significant factor in the low culture yield of pleural fluid sample isolates could be the empirical administration of antibiotics to patients preceding thoracentesis.(9) In our cases, prior to being alluded here, most patients had already been treated with widespread utilization of antibiotics from peripheral health care foundations.

After the revelation and widespread utilization of antibiotics, *Streptococcus pneumoniae* and *Streptococcus pyogenes* *Staphylococcus aureus* prevailing as the principal cause of empyema during the 1940s. Empyema polymicrobial etiology in Indian settings has been found to vary from as low as 7.5 percent (9) to up to 40.4 percent in western settings (10). MRSA was accounted for in our study at a rate of 21.4 percent. The prevalence revealed ranges from 30-85 percent of Gupta from various parts of the country and colleagues announced a 29 percent rate of tuberculosis empyema in 1989 (11, 12). A few studies from India such as Banga et al dore report a high incidence of tuberculosis empyema similar to the western figures where *Mycobacterium tuberculosis* isolation rates from pus have been very high (13).

This study highlights the continuing importance of parapneumonic effusion and empyema of *Staphylococcus* spp. *Staphylococcus aureus* was the most common organism in our study (42.8 percent), of which 50% of isolates were accounted for to be resistant to methicillin (MRSA). In cases of septicaemia, the predominance of gram-positive microorganisms can be correlated with other culture reports from our laboratory, such as blood culture reports. In comparison with gramme negative bacilli, this also demonstrates gramme positive microorganisms as dominant organisms.

Extensive use by family doctors and consultants of quinolones and 3rd generation cephalosporins in the community has led to an increase in extended spectrum beta lactamases in gram-negative organisms and methicillin-resistant *Staphylococcus aureus* (MRSA). The resistance to azithromycin in 71.4 percent isolates is notable, which can be explained not only in tertiary hospitals but also in secondary care institutes due to rampant use of azithromycin.

It is possible that these results reflect a phenomenon at the level of a local institution and cannot be generalised. The limitations of the current report are that it is a single centre study and there is a lack of anaerobic data, so the results cannot be widely extrapolated.

CONCLUSION:

Bacteria are constantly developing new mechanisms of resistance in the battle between bacteria and humanity, making the latest group of antibiotics ineffective. Prompt microbiological analysis, proper implementation of antimicrobial stewardship programmes (ASP) and active surveillance of antibiotic

use and resistance rates are the strategies to win this battle.

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