

**Study of Microbial Growth in Plural Fluid Culture and Its Sensitivity Pattern in Tertiary Care Center**

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**Abstract**

**Background:** Pleural infection is a major cause of morbidity or death, and its incidence continues to rise in adults and children. Identification of the infecting bacteria by culture of pleural fluid and its sensitivity pattern is important for clinical care. Hence the present study was undertaken to study microbial growth in plural fluid culture and its sensitivity pattern in Tertiary Care Center.

**Method:** A total of 50 pleural fluid samples from 50 children of both the sexes aged 0-12 years diagnosed to have empyema thoracis were collected aseptically by thoracocentesis during a period from 1<sup>st</sup> October 2018 to 31<sup>st</sup> October 2019 were included in the study. In all the cases routine investigations like chest X-Ray, blood analysis, pleural fluid analysis, ultra sound scan chest, and culture sensitivity were performed.

**Results:** Out of 50 pleural fluid samples, only 7 (14%) gave positivity on pleural fluid culture and 2 (4%) were positive for *mycobacterium tuberculosis* by CB-NAAT (Cartridge Based Nucleic Acid Amplification Test). The most common microorganisms isolated were gram

positive organisms and all were identified as *Staphylococcus aureus* (6%) and *Streptococcus pneumonia* (4%) while *Klebsiella pneumoniae* (2%) and *Pseudomonas* (2%) were gram negative organisms isolated. The gram positive isolates were sensitive to vancomycin, gentamicin and linezolid whereas gram negative isolates sensitive to Gentamicin and Imepenem only.

**Conclusion:** The present study shows variation in the bacteriological profile and antibiotic susceptibility pattern of pleural fluid it may reflect the local trends of bacterial prevalence and antibiotic sensitivity pattern in our area.

**Keywords:** Pleural fluid, Sensitivity, Empyema thoracis, Tuberculosis, Microorganisms, Gentamycin

**Introduction**

Empyema thoracis, accumulation or suppuration of pus in the pleural cavity known for centuries, is a dreaded complication of pneumonia and chest injuries prior to antibiotic era [1, 2]. It is a common condition in childhood has significant morbidity and mortality [3]. Empyema thoracis constitutes approximately 5-10% of

cases seen by paediatrician in India [3, 4]. Acute respiratory infections are the most common illness of childhood accounting 50% of all illness in under-fives and 30% in the 5-12 years age groups, largely involving the upper respiratory. However, about 5% involve the lower respiratory tract resulting in serious diseases, especially the bacterial pneumonia [5]. Forty percent of bacterial pneumonia are said to be complicated by parapneumonic effusions, 10% of whom would evolve into empyema [6]. Possible reason for this include delay in initiating treatment, prolonged oral treatment in the community with antibiotics inadequate drug level in the pleural space and delayed presentation, or unusual casual organism [7].

However, common causative organisms of empyema are *Streptococcus pneumoniae* and *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenza* and *Klebsiella pneumonia*, *streptococcus pyogenes* and uncommon causative organisms are *Mycobacterium tuberculosis* and *cryptococcus neoformans*. Immediately after infection proteinaceous fluid starts to fill the pleural cavity [8]. The clinical manifestations of empyema are high grade fever with chills and rigors, cough, breathlessness, chest pain. Bronchopleural fistulas, pyopneumothorax, purulent pericarditis, pulmonary abscess, osteomyelitis of ribs are the local complications of empyema [9]. In addition, pleural effusion and empyema are the primary manifestation of intra thoracic disease and are associated with poor outcome [10]. There has been change in the trend of spectrum of pathogens causing pleural space infections. Studies in the past shows that majority of the pleural space infections were due to gram positive organisms but over the time there has been studies showing contrasting results. Hence, the present study was

undertaken to study the microbial growth in pleural fluid and its antibiotic sensitivity pattern.

### Materials and Methods

This observational study was conducted in the Department of Pediatrics at Tertiary Care Hospital from 1<sup>st</sup> October 2018 to 31<sup>st</sup> October 2019. A total of 50 pleural fluid samples from 50 children of both the sexes aged 0-12 years diagnosed to have empyema thoracis were collected aseptically by thoracocentesis during the study period of 6 months. In all the cases routine investigations like chest X-Ray, blood analysis, pleural fluid analysis, ultra sound scan chest, and culture sensitivity were performed.

For pleural fluid analysis, at least 5-10 ml of samples was collected in EDTA vials and transported without delay to the microbiology laboratory. The samples were centrifuged and processed for direct microscopy and culture. Smears were prepared from sample and Gram staining and Ziehl Neelson staining done. For culture the sample was inoculated on 5% sheep blood agar, Macconkey agar and Sabouraud dextrose agar plates to rule out fungal infections. The specimens were processed for identification based on standard microbiological techniques [2]. Also the samples were simultaneously sent for detection of *Mycobacterium tuberculosis* by CB-NAAT. Antibiotic susceptibility testing of the pyogenic isolates was performed by Modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar according to Clinical Laboratory Standards Institute (CLSI) guidelines [11]. The first line drugs tested for gram positive microorganisms included penicillin, vancomycin, gentamicin, linezolid, azithromycin, clindamycin, and for gram negative microorganisms were cephalothin, ceftazidime, gentamicin, ciprofloxacin and imepenam.

### Statistical Analysis

All the results collected were subjected to descriptive statistics like number and percentages. Microsoft excel 2007 was used for making tables, graphs and calculations.

### Observations and Results

Total sample of pleural fluid received from 1st October 2019 to 31st March 2020 were 50 out of which only 7(14%) gave positivity on bacteriological culture and 2(4%) were positive for *Mycobacterium tuberculosis* by CBNAAT. Culture positivity rate was 14%. The incidence was more common in the age group of 1-5 years (50%) followed by 5-12 years (46%). Male children were more affected (82%) than female children (18%). Fourteen patients (28%) were residents of urban areas and 36 patients (72%) belonged to rural areas and most of them belonged to low socioeconomic strata. In the present study 66% (33 cases) children were malnourished. The commonest symptoms at presentation were fever (100%), cough (96%), and breathlessness (92%) as shown in table 1.

Table 2: Demographic profile and clinical presentation of the patients

Parameters		No. of cases	Percentage
Age group	<1 year	02	04
	1-5 years	25	50
	6-12 years	23	46
Sex	Male	41	82
	Female	09	18
Residence	Urban	14	28
	Rural	36	72
Nutritional Status	Malnourished	33	66
	Normal nourishment	17	34

Manifestations	Fever	50	100
	Cough	48	96
	Breathlessness	46	92
	Chest pain	28	56

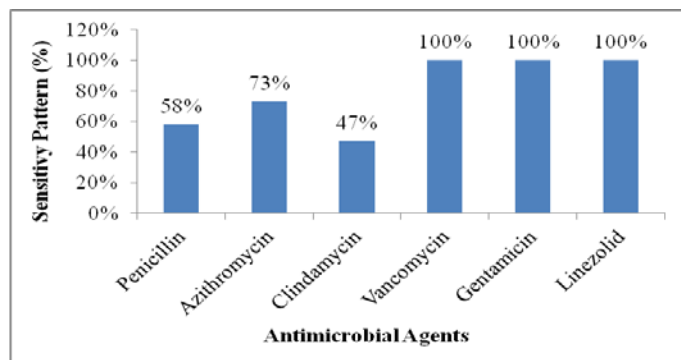
Among the culture positivity, gram positive microorganisms were more common than gram negative microorganisms [71.42% (5/7) versus 28.57% (2/7)]. The most frequently isolated micro-organism was *staphylococcus aureus* 3 (6%). Other micro-organisms were *streptococcus pneumoniae* (4%), *Klebsiella pneumoniae* (2%) and *Pseudomonas* (2%), (Table 2). Pleural fluid cultures were sterile in 82% of patients (No growth in 41 cases). Two patients showed infection with *Mycobacterium tuberculosis* as detected by CB-NAAT.

Table 2: Bacteriological Profile/ Plural Fluid Culture Results

Organism		No. of cases	Percentage
Gram positive	Staphylococcus aureus	03	06
	Streptococcus pneumonia	02	04
Gram negative	Klebsiella pneumoniae	01	02
	Pseudomonas	01	02

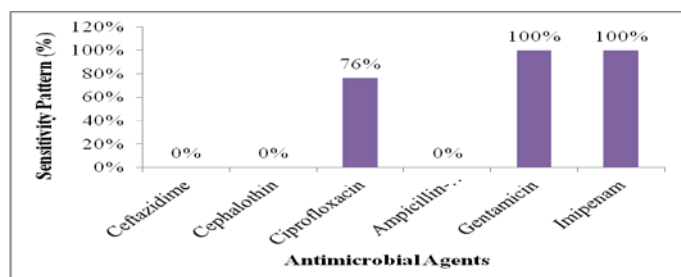
Antibiotic susceptibility test results were also compiled for both gram positive and gram negative microorganisms. Interesting trends were noticed in gram positive organisms regarding the sensitivity patterns of the isolates. Amongst gram positive microorganisms, highest resistance was seen with azithromycin (73%) followed by penicillin (58%) and clindamycin (47%) of isolates. All isolates were susceptible to vancomycin, gentamicin and linezolid, (Figure 1).

Figure 1: Antibiotic sensitivity pattern of gram positive isolates



Among the gram-negative microorganisms, 100% resistance was observed for cephalothin, ceftazidime and amoxicillin-clavulanic acid. The isolates were sensitive to gentamycin and imipenam only, (Figure 2).

Figure 2: Antibiotic sensitivity pattern of gram negative isolates



### Discussion

Overall, 0.6 percent of pneumonia in childhood is complicated by parapneumonic effusion that can progress to empyema thoracis (ET). It predominantly involves in the right lung and 7.1% are bilateral [12]. In developing countries, more than one-fourth of hospital-admitted patients with pneumonia eventually develop parapneumonic effusion or empyema because of delayed initiation of adequate treatment. Traumatic hemopneumothorax may progress to ET following an infection with antibiotic-resistant organisms or associated with comorbid conditions (malnutrition, immunocompromised) [13]. Though the incidence of empyema thoracis has declined in the west due to effective use of broad spectrum antibiotics, but it still

remains a significant health problem in developing countries due to low socioeconomic status, malnutrition and delay in diagnosis of pneumonia, delayed referral to higher centre [14].

In the present study most of the patients were in the age group of 1-5 years (50%) with male predominance (82%) which is consistent with similar other studies [14, 15]. The higher incidence in under-fives and the slight male preponderance is in general agreement with the established pattern of acute lower respiratory infections in children [5]. A higher prevalence of empyema cases was seen more often in malnourished children in this study (66%) as similar to previous studies [16, 17]. Fever, cough and breathlessness were the most common manifestations found at admission comparable to many other studies [14, 18].

Out of 50 pleural fluid samples, only 7 (14%) gave positivity. Gram positive microorganisms were more common than gram negative microorganisms (71.42% versus 28.57%). Culture positivity rate was 14%. This culture reports were correlated with other reports, which revealed that the current study showed less bacterial growth compared to other reports [14, 15, and 19]. These variations in the culture positivity rate many factors may attribute to this like population under study, antibiotic administration practices. *Staphylococcus aureus* is the most common cause of infection in the developing world, while *streptococcus pneumonia* in the developed world [20]. The reported rates of identifying an infectious cause from pleural fluid vary from between 8% and 16% respectively [15]. In the present study, most frequently isolated micro-organism was *staphylococcus aureus* which is comparable to earlier studies [18, 19, and 21]. Other causes were *streptococcus pneumoniae*, *pseudomonas* and *Klebsiella pneumonia*. Pleural fluid cultures were

sterile in 82% of patients. The sterile sample might be due to widespread early use of antibiotics or lack of better facilities for culturing fastidious organism like anaerobes.

Antibiotic susceptibility test results were also compiled for both gram positive and gram negative microorganisms. Interesting trends were noticed in gram positive organisms regarding the sensitivity patterns of the isolates. Amongst gram positive microorganisms, highest resistance was seen with azithromycin (73%) followed by penicillin (58%) and clindamycin (47%) of isolates. All isolates were susceptible to vancomycin, gentamicin and linezolid. In contrast, previous study found very high level of resistance penicillin derivate, approximately one half isolate in infants and young children [22]. Sarangi et al [23] and Singh et al [24] were found that vancomycin and linezolid had highest antibiotic susceptibility NICU setting. Among gram-negative group, 100% resistance was observed for cephalothin, ceftazidime and amoxicillin-clavulanic acid. The isolates were sensitive to Gentamycin and Imipenam only. Limited population in specimen is the major limitation in current study; multicenter prospective studies are needed to validate our finding.

### Conclusion

In the present study, culture positivity rate was 14% in pediatrics patients. *Staphylococcus aureus* is the most prevalent etiological agent. The study shows variation in the bacteriological profile and antibiotic susceptibility pattern of pleural fluid it may reflect the local trends of bacterial prevalence and antibiotic sensitivity pattern in our area, since it is a hospital based study there may be multifactorial facets that should be kept in perspective. However it is important to report differences in current study from the previous

studies done by other research scholars as it may reflect recent trends of shift in the bacteriological profile in pleural fluid and antibiotic sensitivity pattern in children.

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