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Original Research Article

Bacteriological Profile of Pleural Fluid among the Pediatric Population in a Tertiary Care Centre-A Retrospective Analysis

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ABSTRACT

Background and Objectives: Pleural effusion, mainly of infectious etiology, is one of the major causes of morbidity among children. There is a trend in changing etiology which also varies with several clinical factors. This study was undertaken to determine the bacteriological profile of pleural fluid from children diagnosed with pleural effusion in a Tertiary Care Centre in the North East of India.
Materials and Methods: Pleural fluid collected from children diagnosed with pleural effusion between January 2011 and December 2012, were subjected to standard aerobic bacteriological culture for isolation, identification and antimicrobial susceptibility of the etiological agent(s).
Results: Of the 134 samples received, 50 samples had an identifiable etiology with 2 patients having a mixed infection. The organism isolated were *Streptococcus pneumonia* (54%) followed by *Staphylococcus aureus* (16%), *Klebsiella pneumonia* (8%), *Pseudomonas aeruginosa* and *Escherichia coli* (6%) and *Candida* species (2%). Culture positivity was found in 33 of 90 samples from male patients and 17 of 44 samples from female patients. The maximum number of samples (58%) were from the age group of 1-5 years, of which 31 (40%) were culture positive. *Streptococcus pneumonia* and *S.* aureus showed 100% susceptibility to Chloramphenicol. None of the isolated *S. aureus* were Methicillin resistant. Among the Gram negative organisms, all isolates of *K pneumonia* were sensitive to Imipenem and Amikacin while all isolates of *P aeruginosa* showed 100% susceptibility to Gentamicin, Amikacin, Cefoperazone and Ciprofloxacin.
Conclusion: *Streptococcus pneumoniae* was the most common isolate from cases of pleural effusion from this tertiary care hospital.

Key Words: Antimicrobial susceptibility, Bacteriological profile, Children, Pleural effusion, *Streptococcus pneumonia.*

INTRODUCTION

Pleural effusion is the accumulation of excess quantity of fluid within the pleural space due to excess formation (from the interstitial spaces of the lung, the parietal pleura, or the peritoneal cavity) or when there is decreased fluid removal by the lymphatics. [1] This may be due to many causes including thoracic diseases, trauma, and iatrogenic injury, but the most common among these is infection. [2] Pleural effusions are nutritionally rich culture media

with poor white blood corpuscles (WBC) defenses. The pleural space is normally sterile but readily colonized once pleural fluid is accumulated. [3] Pleural effusion may be exudative or transudative in nature. Para pneumonic effusions are associated with bacterial pneumonia, lung abscess or bronchiectasis and are probably the most common cause of exudative pleural effusion. Empyema on the other hand refers to a grossly purulent effusion. [1] Para-pneumonic effusions that occur in first 48-72 hours are small, sterile polymorphonuclear leucocytes (PMNL) predominant exudates. If pneumonia remains untreated, the amount of pleural fluid increases with time due to endothelial injury increased localized permeability and edema. Bacteria invade pleural space and become persistent. [4]

Pleural infection is a frequent clinical problem with an approximate annual incidence of up to 80,000 cases in the UK and USA combined. The associated mortality and morbidity is high; in the UK 20% of patients with empyema die and approximately 20% require surgery to recover within 12 months of their infection. [5,6] Bacterial pleural infection has been a substantial clinical challenge since ancient times. The Egyptian physician Imhotep initially described pleural infection around 3000 BC, although Hippocrates has been more famously credited with its recognition in 500 BC. [7,8] The overall incidence of pleural infection is increasing. It is well recognized that pleural infection occurs most commonly in the pediatric and elderly populations and recent large-scale cohort studies concur with this finding. Farjah et al studied 4424 patients and observed an increase in incidence of 2.8% per year (95% CI 2.2% to 3.4%) in his study. [9] Similarly, in a study by Finley et al an increase in the pleural infection incidence rate ratio (IRR) of 2.2 (95% CI 1.56to 3.10) in patients aged <19 years was reported. [10]

The bacteriology of pleural infection has been changing in recent years since the introduction of antibiotics in the 1940's and varies with several clinical factors, including underlying diseases, community-or hospital-acquired infections, and surgical conditions. [11-13] In the past, 60-70% of cases were due to *Streptococcus pneumonia*, which now only accounts for approximately 10% of culture-positive cases. [14] The prevalence of *Staphylococcus aureus* rose and the development of *Staphylococcal* resistance in the 1950's increased complications and mortality. [15,16] It was also postulated that pneumonia due to virulent organisms such as *Streptococcus pneumonia* often manifests with prominent symptoms at very early stage of the disease. Hence, it is often treated earlier and reducing the chance of progression to pleural infection. [17] Moreover, approximately 15% of patients with parapneumonic effusion die, and in 15to 40% surgical drainage of the infected pleural space is undertaken. The median duration of inpatient care is 15 days, with 20% of patients remaining in the hospital for a month or longer. Most parapneumonic effusions resolve upon use of appropriate antibiotics, but a significant proportion develops complicated parapneumonic effusion or empyema thoracis that will require drainage with chest tubes, administration of intrapleural fibrinolytic agents, or surgery. [18-20] Prompt evaluation and therapeutic intervention appears to reduce morbidity and mortality as well as healthcare costs. [10] Hence the present study was conducted to determine the bacteriological profile of pleural fluid from children diagnosed with pleural effusion in a Tertiary Care Centre in the North East of India.

MATERIALS AND METHODS

The study was a retrospective review of medical and microbiological records from January 2011 to December 2012. All children, up to the age of 18 years, who were clinically diagnosed to be suffering from pleural effusion, were included in the study. A total number 134 pleural fluid samples were studied and subjected to standard aerobic bacteriological culture including gram staining, microscopy, studying the cultural characteristics and biochemical reactions for isolation and identification of the etiological agent(s) and their antimicrobial susceptibility was done using the 'Kirby Bauer Disk Diffusion Method', as per standard protocol. [21-24]

A **positive finding** was defined as identification of organisms on staining procedures and growth of the organism in the appropriate culture medium and/or also identification through various biochemical tests according to standard protocol. [21-24]

***Statistical Analysis*:** Significance was evaluated by Chi Square ($\chi 2$) test and 'p' value less than 0.05 was considered as significant.

RESULTS

Of the 134 samples received, 50 samples (37%) had an identifiable etiology with 2 patients having a mixed infection. Both the patients were females with one having infection with *Pseudomonas aeruginosa* and *Acinetobacter lwoffii* and the other infected with *P aeruginosa* and *Escherichia coli.* Altogether ten (10) organisms were isolated, *Streptococcus pneumoniae* 27 (54%) followed by *Staphylococcus aureus* 8 (16%), *Klebsiella pneumoniae* 4 (8%), *Pseudomonas aeruginosa* and *Escherichia coli* 3 each (6%), and a single isolate (2%) each of *Enterococcus* species, *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Haemophilus influenzae* and *Candida* species. [Table 1]

Among the samples received, 89 were from the Pediatric ward and 45 were from the Pediatric Intensive care Unit (PICU)

Of the total samples received, 90 (67%) were from male patients of which 33 (37%) were culture positive and 44 (33%) were from female patients of which 17 (39%) showed positivity. [Figure 1]

A total of 78 (58%) samples were received from the age group of 1-5 years of which 31 (40%) were positive, followed by the 6-10 year age group in which 36 (27%) samples were received, of which 12 (33%) were positive. Six (35%) samples were positive from the 17 (13%) samples received in the age group of 11-20 years and 1 (33%) of 3 (2%) samples showed positivity among the age group of less than 1 year. [Figure 2]

Only a single isolate of *S pneumoniae* was isolated in the <1 year age group. In the age group of 1-5 year, 16 isolates of *S pneumoniae*, 4 isolates each of *S aureus* and *K pneumonia*, 3 isolates of *P aeruginosa*, 2 isolates of *E coli* and 1 isolate each of *H influenzae* and *Enterococcus* species were obtained. Among isolates obtained in the age group of 6-10 year, 7 isolates were of *S pneumoniae*, 3 isolates were of *S aureus*, and 1 isolate each were of *Acinetobacter baumannii* and *Acinetobacter lwoffii.* And in the 11-20 year age group, 3 isolates of *S pneumoniae*, 1 isolate each of *S aureus* and *E coli* were obtained. A single *Candida* species was obtained in the study in the 11- 20 year age group.

In all, twenty five (25) different antibiotics were used for the Gram positive and Gram negative organisms accordingly. Most of the Gram positive organisms were sensitive to Chloramphenicol followed by Penicillin, Vancomycin, Cefoperazone, Erythromycin, Ofloxacin, Ampicillin,

Levofloxacin and Gentamicin among others. [Figure 3]

TABLE 1. Age-wise Distribution of Organisms Isolated.

Age Group (in years)					
Organisms	1	1 – 5	6 – 10	11 - 20	**Total**
S Pneumoniae	1	16	7	3	27
Staphylococcus aureus		4	3	1	8
Klebsiella pneumoniae		4			4
Pseudomonas aeruginosa		3			3
Escherichia coli		2		1	3
Enterococcus species		1			1
Acinetobacter baumanii			1		1
Acinetobacter lwoffii			1		1
Hemophilus influenzae		1			1
Candida species				1	1
Total	1	31	12	6	50

[The isolation of *Streptococcus pneumoniae*, when compared to the second commonest isolate, *Staphylococcus aureus*, was statistically significant (p = 0.002)]

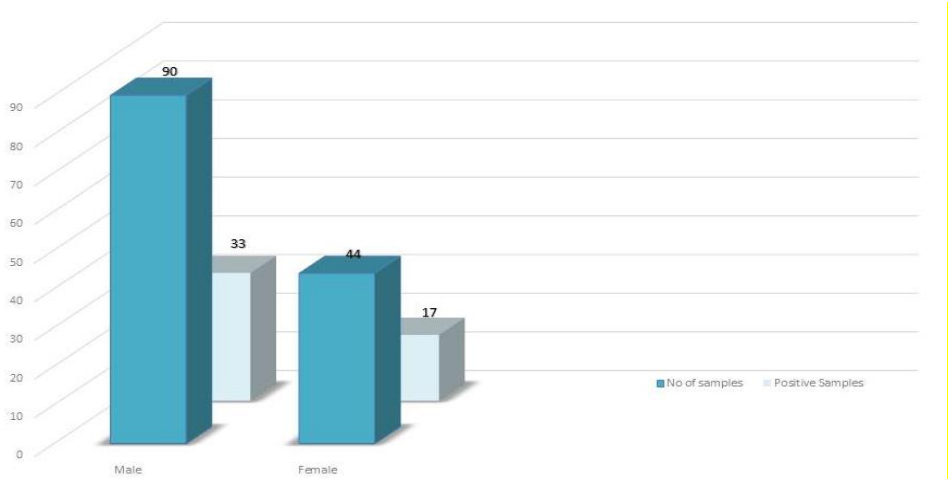

Figure 1. Sex-wise Distribution of Patient Profile [The positivity of samples among the males and the females was not statistically significant (p = 0.97)]

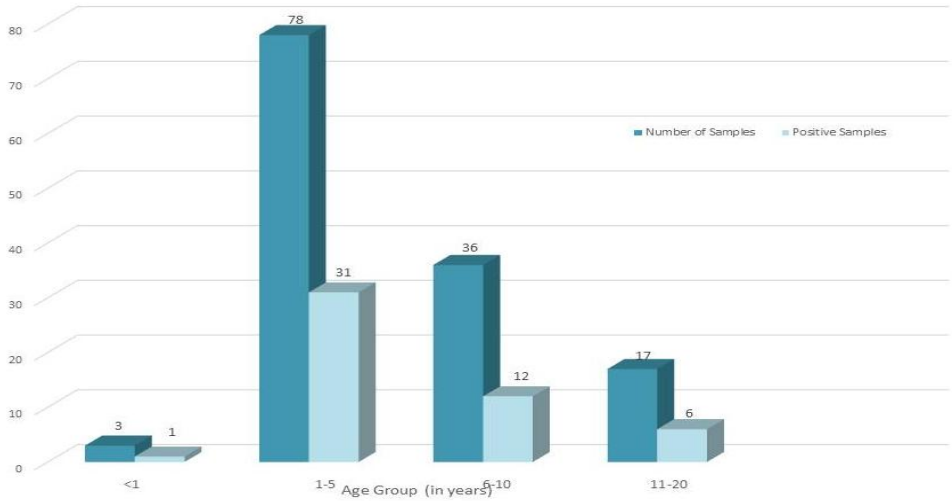

Figure 2.Age-wise Distribution of Positive Samples

The Gram negative organisms showed maximum sensitivity to Amikacin followed by Cefoperazone, Imipenem, Gentamicin, Levofloxacin, Chloramphenicol and Ciprofloxacin among others. [Figure 4]

There was no *Enterococcus* species with High Level Aminoglycoside Resistance and none of the *Staphylococcus aureus* isolated were Methicillin resistant. *Streptococcus pneumonia*, the most isolated organism, was susceptible mostly to Chloramphenicol and Penicillin.

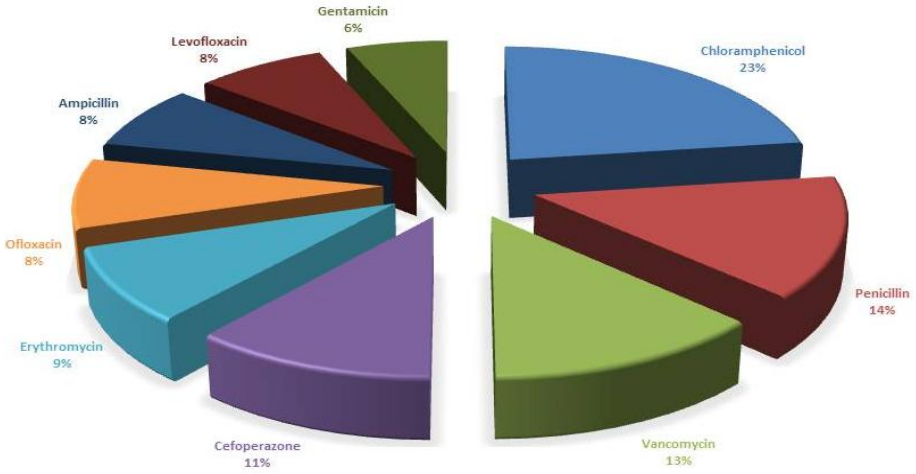

Figure 3.Antibiotic Susceptibility of Gram Positive Organisms (% indicates the percentage of resistance of the isolates)

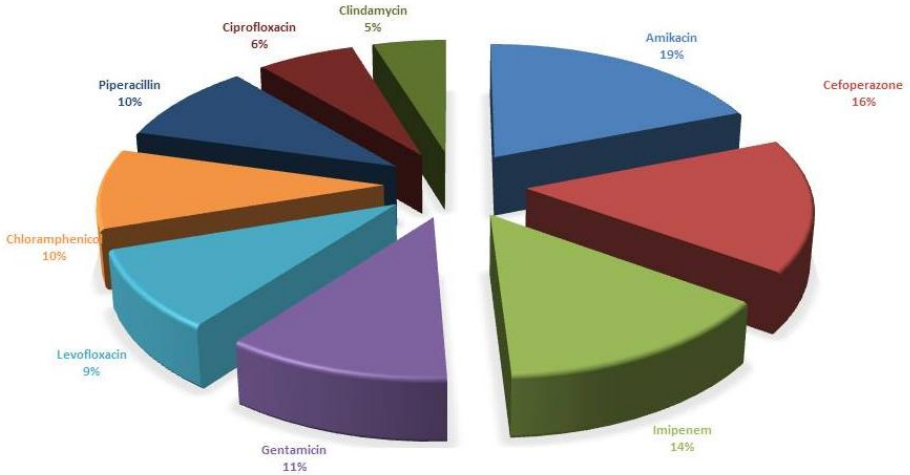

Figure 4.Antibiotic Susceptibility of Gram Negative Organisms (% indicates the percentage of resistance of the isolates)

DISCUSSION

In the present study, an etiological diagnosis of pleural effusion/empyema in our paediatric population was obtained for 37% among the clinically diagnosed cases, which was lower compared to previous studies, which varied from 57% to 82%. [25-27] The reason for this could be due to prior use of antibiotics before sampling and the lack of use of molecular methods for diagnosis, which greatly enhances the chances of detection of the etiological

agents, even in previously treated patients. [28]

Streptococcus pneumonia was the most common pathogen isolated in the present study (54%), which is similar to the findings by Monnier et al and Langley et al. [28,29] The isolation of *Streptococcus pneumoniae*, when compared to the second commonest isolate, *Staphylococcus aureus* (16%), was statistically significant (p = 0.002), though many studies have found *Staphylococcus aureus* as the commonest cause of pleural effusion. [25,30,31]

There was no significant difference (p = 0.97) in the positivity of samples among the males and the females (37% and 39%, respectively), though the samples were more from the male population (Male: Female = 2.25: 1). It has been postulated that the admission rates of females in seeking prompt medical care might affect the outcome of positivity in certain socially backward population, but this was not the scenario in the present study. [30]

The incidence of pleural fluid infection was highest in the age group of 1-5 years and *Streptococcus pneumonia*, the commonest isolated organism, was also from the same age group. The findings were similar to previous studies. [25,28,31] Pediatric age group is an important risk factors for invasive pneumococcal diseases, with incidence being highest in young children aged <2 years. According to the World Health Organization, pneumococcal infections are the leading cause of death from a vaccine-preventable illness in children aged <5 years. [32] *Streptococcus pneumoniae* is the most common causative organism in pediatric empyema, accounting for >50% of cases. [28,33]

The resistance of *Streptococcus pneumoniae* isolates to Penicillin was very low (< 1%) and hence was not of much concern in the present study. However, *Streptococcus pneumoniae* with high level of resistance to Penicillin (Minimum Inhibitory Concentration(MIC)>2 µg/ml) and to Cephalosporins (MIC >4mg/ml) has been reported and should be treated with Vancomycin.3 There were neither incidence of High Level Aminoglycoside Resistance among the *Enterococcus* species nor of Methicillin Resistance among the *Staphylococcus aureus* isolates, though some studies have stated the rise of Methicillin Resistant *Staphylococcus aureus* (MRSA) as the etiology of pediatric pleural effusion. [25,34]

Our study is limited by the facts that conventional methods were used for identification of the organisms, which may be missed in the event of use of antimicrobials prior to sampling and also that tests to determine the MIC of the antimicrobials were not carried out. The lack of clinical data, the treatment and outcome of the patients are also limitations of the present study and hence further studies are to be undertaken for better understanding of the burden of pediatric pleural effusion in this part of the country.

CONCLUSION

Pleural infection is a major healthcare problem, with rising incidences especially in the pediatric age group. Microbiological profile of pleural infection is complex and is also changing with time and varies between various geographic regions, and this can lead to variations in clinical presentation, antibiotic response and outcome. Hence it is imperative for the microbiologists and the clinicians to know the local prevalence of microbes in pleural effusion, along with their antimicrobial susceptibility to guide appropriate antimicrobial therapy. Progress in the field of pneumococcal vaccine development may significantly reduce invasive pneumococcal diseases like pleural effusion and empyema and hence the appropriate use of vaccines in

the susceptible population should be propagated.

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