MICROBIOLOGICAL ANALYSIS OF PLEURAL FLUIDS: A STUDY FROM DR.RAJENDRA PRASAD GOVERNMENT MEDICAL COLLEGE , KANGRA AT TANDA(HIMACHAL PRADESH, INDIA)

Sharma Shaifali, Sood Anuradha*, Chauhan Smriti, Thakur Kamlesh, Sharma Tarun
Department of Microbiology, Department of Medicine, Dr. Rajendra Prasad Government Medical College , Kangra at Tanda (Himachal Pradesh)

Keywords:
microbial aetiology, pleural fluids.

Abstract

Introduction:The microbial aetiology of pleural space infections has changed since the introduction of antibiotics. The present study was carried out in department of Microbiology, Dr. Rajendra Prasad Medical College, Kangra at Tanda, Himachal Pradesh, Indiafrom 1st January 2017 to September 2017 with aim to get the microbiological profile of patients presenting with pleural effusion and empyema.

Methods: The pleural fluid samples collected aseptically by thoracocentesis from inpatients department of medicine, paediatrics and pulmonary medicine departments were included in this study. The specimens were processed for identification based on standard laboratory techniques followed byantibiotic susceptibility testing of the pyogenic isolates performed by Modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar according to Clinical and Laboratory Standards Institute guidelines.

Results: Over a period of 9 months 45 pleural fluid samples were received. Out of 45 pleural fluid samples only 13(28.8%) gave positivity on bacteriological culture and 5 were positive for mycobacterium tuberculosis by CB-NAAT(Cartridge Based Nucleic Acid Amplification Test).Gram stain positivity was 16.2%.Culture positivity was 28.8%.The most common microorganisms isolated were gram positive organisms and all were identified as *Staphylococcus aureus* except one isolate each of of coagulase negative *Staphylococcus species* and *Streptococcus pneumoniae*. Among gram negative organisms *Proidentiaspp, Ecsherichia coli,Nonfermentor group of organism* and *Pseudomonas spp*. were isolated..The most common antibiotic to which gram positive microorganisms were resistant was Azithromycin(71.4%) followed by Penicillin (57.1%).The gram negative isolates were sensitive to Gentamycin and Imepenem only.

Conclusion: The emergence of antibiotic resistant microorganisms ,the increase in the frequency of nosocomial infections, and the steadily increasing number of patients with a compromised immunity have combined to keep pleural infections a common entity

Introduction

Pleural effusions and empyema are frequently the primary manifestation of intrathoracic disease and are associated with poor outcome. Pleural effusion can be transudative or exudative in nature. Transudative is due to systemic diseases CCHF, liver cirrhosis ,nephrosis etc. and exudative may be due to malignancy or any inflammatory process. Empyema is usually a complication of pneumonia but may arise from infections at other sites. The microbial aetiology of pleural space infections has changed since the introduction of antibiotics.It can be modified by either specific patient factors such as surgical procedures, trauma or underlying conditions, or by methodological factors, namely the specimen collection, transport and culture. For these reasons, several studies have found discordant results in the spectrum of pathogens causing pleural infections[1].The present study was carried out in department of Microbiology, Dr. Rajendra Prasad Medical College, Kangra at Tanda, Himachal Pradesh, India with aim to get the microbiological profile of patients presenting with pleural effusion and empyema in our rural medical college hospital.

Methods

The pleural fluid samples collected aseptically by thoracocentesis from inpatients department of medicine, paediatrics and pulmonary medicine from 1st January 2017 to September 2017 were included in this study. At least 5-10 ml of samples were collected in EDTA vialsand transported without delay to the microbiology laboratory. Single or mixed growth from one patient and consecutive samples from new patients were included in this study. Repeat sample received from a patient already enrolled, patients on antibiotics and patients who did not give their consent was excluded from study. 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.Detection of Methicillin resistant *Staphylococcus aureus* (MRSA) was done for *Staphylococcal* isolates using cefoxitin (30 mcg) disks[3].

The first line drugs tested for gram positive microorganisms included penicillin, vancomycin,gentamicin, linezolid , azithromycin, clindamycin, and for gram negative microorganisms werecephalothin, ceftazidime, gentamicin, ciprofloxacin, amoxycillin-clavulanic acid, cotrimoxazole,imepenam. Second line drugs were put up when all drugs of first line were found to be resistant.

Results

Over a period of 9 months 45 pleural fluid samples were received. Out of 45 pleural fluid samples only 13(28.8%) gave positivity on bacteriological culture and 5(11.1%) were positive for *Mycobacterium tuberculosis* by CB-NAAT(Cartridge Based Nucleic Acid Amplification Test).

Maximum patients belonged to the age group of 10-19 years (24.4%) followed by >60 years age group(20%). Male to female ratio was 2:1.

Direct detection of microorganisms by Gram stain was 16.2%. and by Ziehl Neelsen stain was 2/5(40%).
On the other hand aerobic bacterial culture positivity was 28.8%. Excluded from analysis were 2 additional patients whose pleural fluid cultures showed growth of contaminant bacteria.Hence culture was more sensitive for diagnosis than gram staining. No microorganism was isolated on fungal culture.

Microbiological profile: Gram positive microorganisms were more common than gram negative microorganisms (57.1% versus 28.5%).Among gram positive organisms*Staphylococcus aureus*was dominant isolate followed by coagulase negative *Staphylococcus species* and*Streptococcus pneumoniae*. Among gram negative isolates *Providentia spp, Escherichia coli,Nonfermenter group of organism* followed by*Pseudomonas spp*. were identified.
Five patients showed infection with *Mycobacterium tuberculosis* as detected by CB-NAAT.

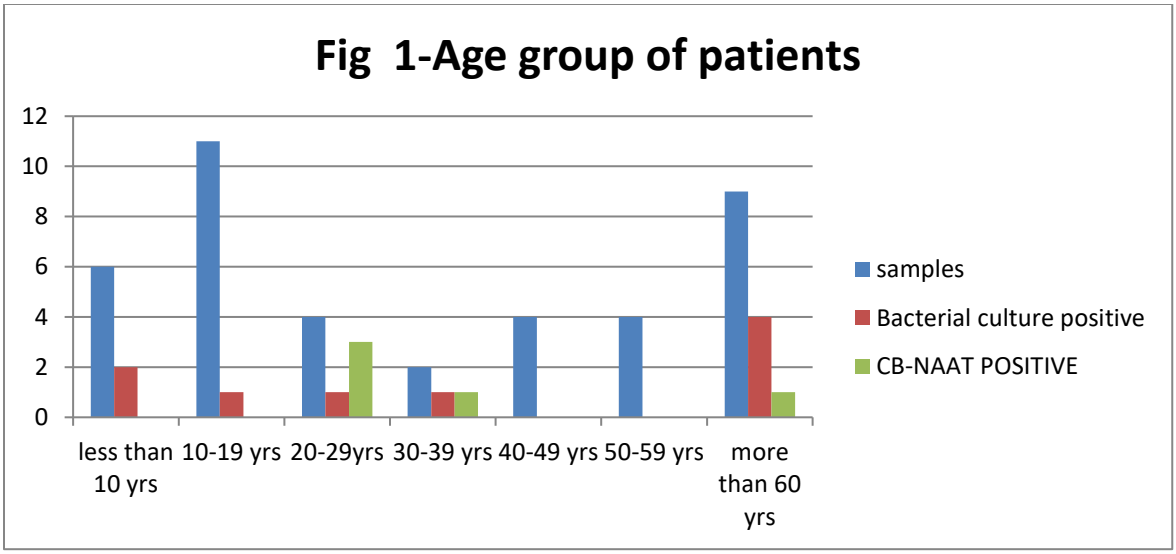
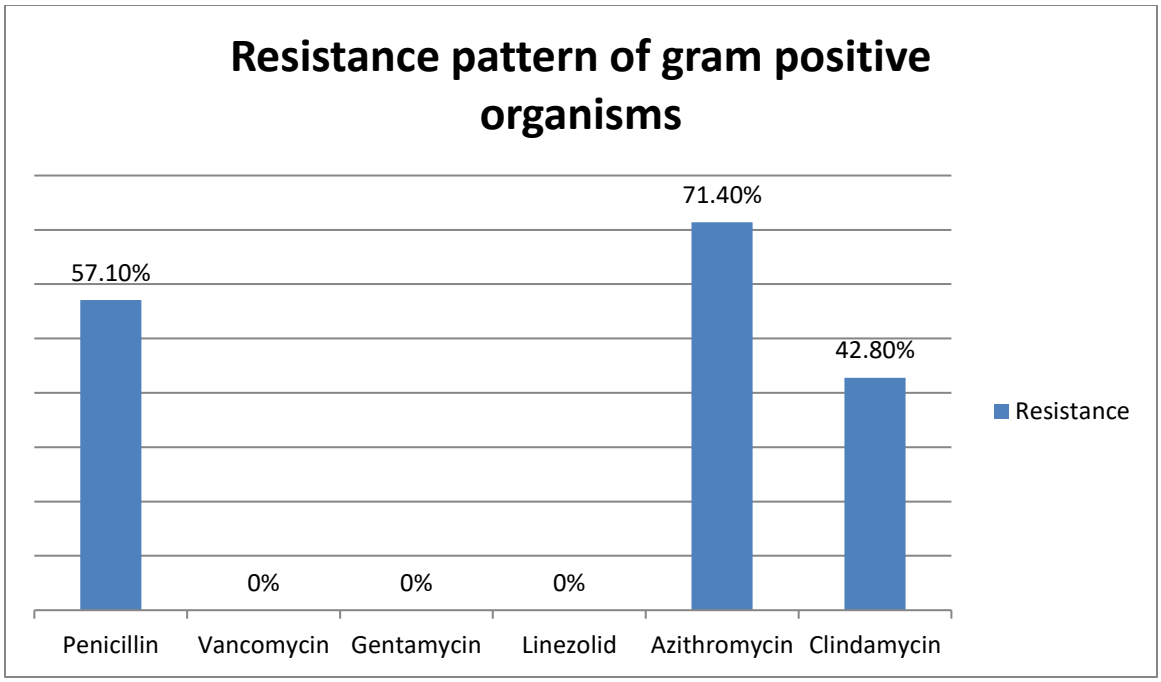


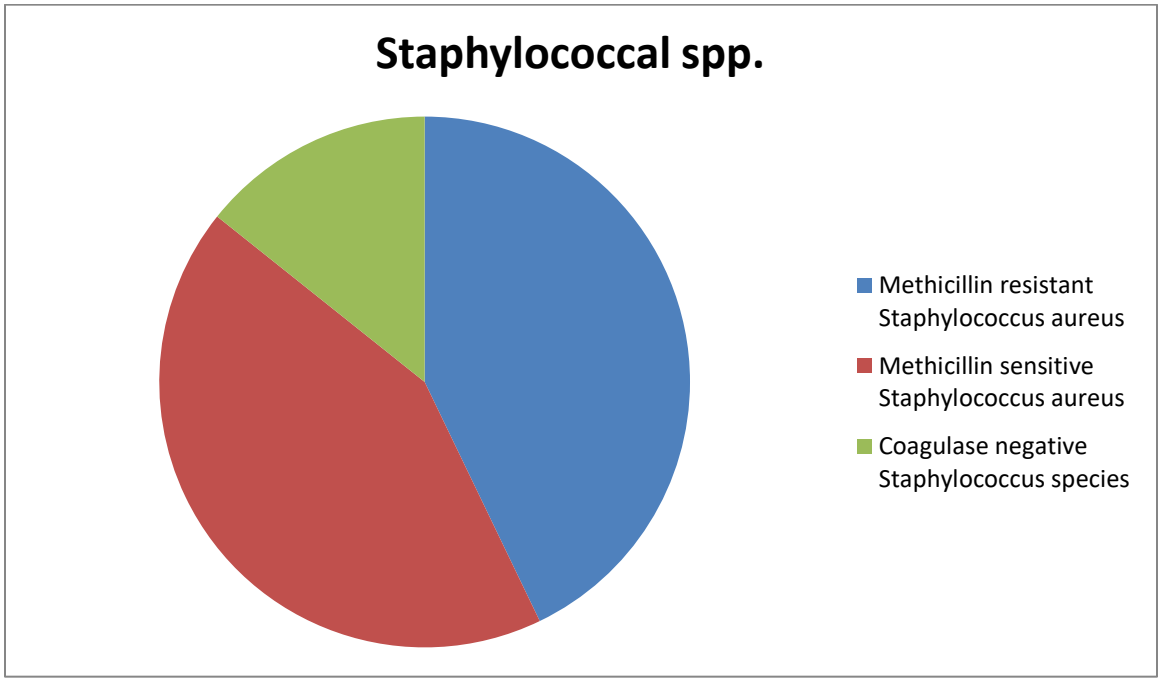
TABLE 1- Spectrum of various organisms isolated

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

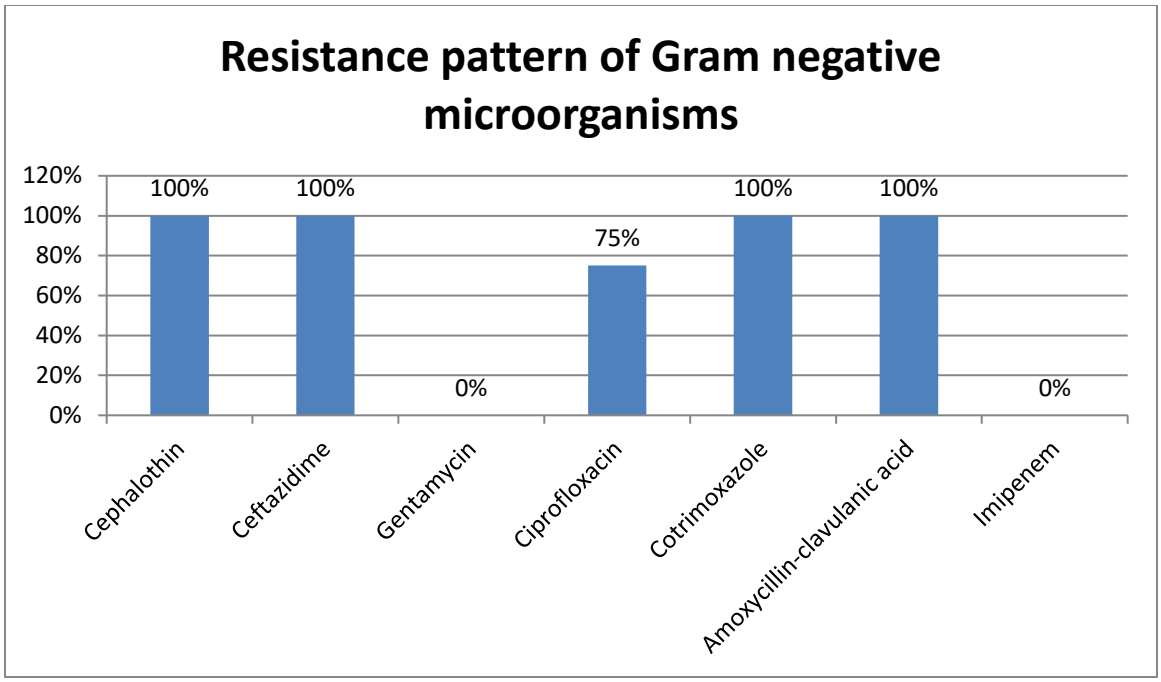
Antibiotic susceptibility test results were also compiled for both gram positive and gram negative microorganisms. Interesting trends were noticed ingram positive organisms regarding the sensitivity patterns of the isolates.Amongst gram positive microorganisms, highest resistance was seen with azithromycin(71.4%) followed by penicillin (57.1%) and clindamycin (42.8%) of isolates. All isolates were susceptible to vancomycin, gentamicin and linezolid .



Methicillin resistant *Staphylococcus aureus* (MRSA) was detected in 50% of *Staphylococcus aureus* isolates using cefoxitin disc method.



Among the aerobic Gram-negative group, 100% resistance was observed for cephalothin, ceftazidime, cotrimoxazole and amoxicillin-clavulanic acid.The isolates were sensitive to Gentamycin and Imepenem oniy. Extended spectrum beta lactamase (ESBL) production was confirmed in all isolates.



Discussion

Pleural effusion and empyema is estimated to occur in 44% of patients with community acquired pneumonia. Gram stain and culture of pleural fluid is essential part of evaluation of patients with parapneumonic pleural effusion. The emergence of antibiotic resistant microorganisms,the increase in the frequency of nosocomial infections, and the steadily increasing number of patients with a compromised immunity have combined to keep pleural infections a common entity[4].

In the present study conducted at ourtertiary care rural medical college hospital comprising of 45pleural fluid samples been received in the microbiology laboratory, the percentage of positive cultures was 28.8%. Rates of microbiological diagnosis in earlier studies have shown a wide variation. A lower positive culture rates has been observed in works of Mohanty et al(15.3%)[5].On the other hand a high positivity rate of cultures from 31-89% have reported by various workers across the world like works of Alfageme et al.[6] The reason for this wide disparity in positivity rates of empyema fluids has been attributed to differences in microbiological techniques and due to difference in prevalence of effusions caused by infective processes[7].Another important factor in the low culture yield of isolates of pleural fluid samples could be the empiric administration of antibiotics to the patients before thoracocentesis[8].In our casesmost patients had already been treated with rampant use of antibiotics from the peripheral health care institutes before being referred here.

After the discovery and widespread use of antibiotics in the 1940s, *Staphylococcusaureus* succeeded *Streptococcus pneumoniae* and *Streptococcus pyogens* as the major cause of empyema. Since the advent of beta-lactamase resistant semi-synthetic penicillins in the 1960s, the incidence of *Staphylococcal* empyema has decreased and infections due to aerobic gram negative bacteria(*Escherichia coli, Klebsiella spp, Pseudomonas spp, and Proteus spp*) and anaerobes have increased markedly. Polymicrobial etiology of empyema has been documented to be varying from as low as 7.5%[8] in Indian settings to up to 40.4% in the west[9]. MRSA was reported at the rate of 21.4% in our study. Reported prevalence from different parts of the country varies from 30-85%[10].

Tubercular etiology was found in 5 patients (27.7% 0f the total 18 positive patients). Gupta and co-workers have reported the incidence of tubercular empyema to be 29% in 1989[11]. A few studies from India like Banga et al do report a high incidence of tubercular empyema akin to the figures from the west where isolation rates of *Mycobacterium tuberculosis* from pus has been very high[12].

This study highlights the continuing importance of *Staphylococcus spp*in parapneumonic effusions and empyema. The most common organism in our study was *Staphylococcus aureus* (42.8%), amongst these methicillin resistance

(MRSA) was reported in 50% of isolates . The alarming incidence of MRSA is a cause of concern. The predominance of gram positive microorganisms can be correlated to other culture reports of our laboratory e.g. the blood culture reports in cases of septicaemia. This also shows gram positive microooganisms as dominant organisms as compared to gram negative bacilli.

Extensive use of quinolones and 3rd generation cephalosporins in community from family physicians and consultants has contributed to increase in extended spectrum beta lactamases in gram negative organisms and methicillin resistant *Staphylococcus aureus* (MRSA). Notable is the resistance to azithromycin in 71.4% isolateswhich can be explained due rampant use of azithromycin not only in tertiary care hospitals but also in secondary care institutes.

It is possible that these findings reflect a local institute level phenomenon and cannot be generalised. Limitations of the current report are that it is a single centre study and there is a lack of data on anaerobes so the results cannot be widely extrapolated.

Conclusion

In the battle between bacteria and mankind, bacteria are constantly evolving newer mechanisms of resistance which makes the latest group of antibiotics ineffective. The strategy to win this battle is prompt microbiological analysis, proper implementation of antimicrobial stewardship programmes (ASP) and active surveillance of antibiotic use and resistance rates

References

1. Everts RJ, Reller B. Pleural space infections. Microbiology and antimicrobial therapy. *Semin Respir Infect* 1999;14:18-30.
2. Betty A Forbes, Daniel F Sahm, Alice S Weissfeld. *Bailey & scott's diagnostic microbiology*.12 edition .Mosby.2007.
3. M100-S23, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement, CLSI
4. Delikaris PG, Conlan AA, Abramor E, Hurwitz SS, Studii R. Empyema thoracis.A prospective study on 73 patients.*S Afr Med J*. 1984 Jan 2014;65(2):47-49.
5. Srujana Mohanty, Arti Kapil, Bimal K Das.Bacteriology of parapneumonic pleural effusions in an Indian hospital.*Trop Doct 1 October* 2007; vol. 37(4):228-229.
6. Alfageme I, Muñoz F, Peña N, Umbría S. Empyema of the thorax in adults. Etiology, microbiologic findings, and management.*Chest.* 1993 Mar;103(3):839-43
7. Barnes TW, Olson EJ, Morgenthaler TI, Edson RS, Decker PA, Ryu JH. Low yield of microbiologic studies on pleural fluid specimens. *Chest*. 2005 Mar;127(3):916-21.
8. Preetam Rajgopal Acharya and Kusum V. Shah, Empyema thoracis: A clinical study;*Ann Thorac Med*. 2007 Jan; 2(1): 14–17
9. Tsang KY, Leung WS, Chan VL, Lin AW, Chu CM .Complicated parapneumonic effusion and empyema thoracis. microbiology and predictors of adverse outcomes. *Hong Kong Med J*. 2007 Jun;13(3):178-86
10. Peter M. Hawkey, Annie M Jones. The changing epidemiology of resistance. *Journal of antimicrobial chemotherapy* .2009; 64: i3-i10
11. Gupta S K, Kishan J, Singh S P. Review of one hundred cases of Empyema thoracis.*Indian J Chest Dis Allied Sci*.1989 Jan- Mar;31(1):15-20
12. Amit Banga, GC Khilnani, SK Sharma, AB Dey, Naveet Wig and Namrata Banga, A study of empyema thoracis and role of intrapleural streptokinase in its management.*BMC Infectious Diseases* 2004; 4:19