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Prevalence and antibiotic resistance pattern of bacterial isolates among pleural fluid samples at tertiary care hospital, Surat

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Abstract

Introduction: The bacteriology of thoracic empyema has been changing since the introduction of antibiotics. Gram stain and culture has for decades been the "gold standard" for the detection of microorganisms in pleural fluid samples. The present prospective cross sectional study was designed to review our experience with the microbial causes of empyema and their antibiotic resistance patterns. The knowledge of likely prevalent strains along with their antimicrobial resistance pattern helps in the framing of antibiotic policy and better management of patients.

Aims & Objectives: To study bacteriological profile in pleural space infection and to identify the antibiotic resistance pattern of bacterial agents isolated from Pleural fluid.

Materials and Methods: All specimens of Pleural fluid from June 2021 to Nov 2021 that received for culture & sensitivity to Microbiology department in tertiary care hospital Surat, were included in the study. All the fluids were subjected to gram staining for provisional report and then inoculated on culture media and incubated overnight at 37ºC. Growth if any was noted and isolate was identified using standard protocols. Antibiotic susceptibility testing was done using Kirby bauer disk diffusion method and E-test using CLSI guidelines.

Results: Total sample of Pleural fluid received from June 2021 to Nov 2021 were 153 out of which 45 (29.41%) showed growth while 108 (70.59%) samples were sterile. Culture positivity rate was 29.41%. Majority of isolates were Gram negative bacilli 44 (97%) of which most common was Pseudomonas aeruginosa 17 (11%) followed by Acinetobacter

baumanii 10 (7%) Klebsiella pnemoniae 10 (7%), E. coli 6 (4%) and Citrobacter freundii 1 (1%) .Among gram positive spectrum, Enterococcus faecalis was isolated from 1 (1%) samples. Overall among gram negative bacteria maximum resistance was noted for Cefotaxime and ceftazidime.Overall among gram positive organisms 100% sensitivity was seen for Vancomycin, Gentamicin & Ciprofloxacin.

Conclusion: Pleural fluid examination is a useful diagnostic tool to study the aetiology of bacterial empyema. Antibiogram helps in screening resistant pathogens and selecting better drug for treatment, thereby helping to decrease the mortality and morbidity.

Keywords: Bacterial Culture Growth, Pleural Space Infection, Pseudomonas Aeruginosa, Thoracic Empyema.

Introduction

Acute respiratory tract infection is the leading cause of morbidity and mortality in critically ill patients in developing countries such as India. Lower Respiratory Tract Infections (LRTIs) are the most common bacterial infections among patients admitted in Intensive Care Units (ICUs) which result in high mortality[(1)].

Pleural cavity of normal human being contains a small amount of fluid known as a pleural fluid which lubricates the lining of the cavity[(1)].Pleural effusion is defined as an abnormal, excessive collection of fluid in the Pleural space[(2)]. There are two main types of pleural effusions - Transudative pleural effusion and Exudative pleural effusion[(2)].

Bacterial infection of the pleura was first described in ancient Greece by Hippocrates [(3)]. Empyema thoracic is a pyogenic or suppurative infection of the pleural space [(4)]. For centuries Empyema thoracic has been recognized as a serious problem for centuries with antibiotic resistance has added the gravity of the condition [(4,6)].

Since the introduction of antibiotics, the causative microbial agents of pleural cavity infections has changed significantly. This has been modified according to the patient conditions such as trauma, surgical procedures or underlying disorders, or depend on the step used for collection, transport and culture of the specimen[(5)]. Before the antibiotic era, Streptococcus pneumonia or β-hemolytic streptococci were isolated in most empyema fluid, while Staphylococcus aureus was the most common bacterial pathogen of thoracic empyema between 1955 and 1965. In the early 1970s, anaerobic bacteria were isolated most frequently [(10)]. Several studies have found that the majority of culture positive effusions are due to aerobic microorganisms, while up to 15% are caused exclusively by anaerobic bacteria and the remainders are due to multiple microorganisms[(4)]. This is the reason that difference in results found in several studies regarding the types of causative organisms causing infections in pleural cavity[(4)].

Diagnosis of pleural effusion is based on clinical history of the patients, physical examination of the patients, Chest X-ray and detailed examination of pleural fluid[(2,7)]. Further investigations such as Computed Tomography(CT) of thorax, thoracoscopy, pleural biopsy and sometimes bronchoscopy can be done, if needed[(2,7)]. Gram stain and culture has for decades been the gold standard tests for the detection of microorganisms in pleural fluid samples. Peripheral blood culture can increase the identification rate of the causative organism, while sputum cultures are positive less often than pleural fluid cultures[(8)] . A various techniques such as countercurrent immunoelectrophoresis, direct gas-liquid

chromatography, immunochromatographic membrane test and flow-cytometry, have not been demonstrated to be superior, because their usefulness is limited to certain bacterial groups[9]. Currently the use of nucleic acid amplification tests appears to be the method with the highest sensitivity (up to 75%) in the identification of bacteria in pleural fluid[10]. It should be emphasized, however, that pleural fluid culture is the only method that provides the sensitivity profile of the isolated microorganism to various antibiotics[4].

The present prospective cross sectional study was carried out to review our experience with the microbial causes of empyema and their antibiotic resistance patterns. The knowledge of likely prevalent strains along with resistance to antibiotic agents helpful in the development of antibiotic policy and proper care of the patients with appropriate empirical antibiotic therapy to improve the clinical outcome of pleural fluid infection.

Materials and Methods

This prospective cross sectional study was including 153 pleural fluid samples of suspected bacteriological infection of pleural fluid received in Microbiology laboratory for culture and sensitivity test.

Inclusion Criteria

1) All pleural fluid samples which were received at microbiology laboratory for bacteriological culture and antibiotic sensitivity testing during study period.

2) Patients age ≥18 years.

Exclusion Criteria

1) Patient < 18 years of age.

2) Repeat pleural fluid sample of same patient.

3) Pleural fluid samples of the patients currently suffering from tuberculosis.

4) Pleural fluid samples which show fungal growth.

Receiving of Sample

- Pleural fluid samples with completely filled lab request form were accepted such as details of patient's name, age, sex, hospital identification number, probable diagnosis were recorded.
- Laboratory ID was given after receiving the sample, to the sample as well as the form received.

Processing of Pleural Fluid Samples

Appropriate pleural fluid sample was inoculated Nutrient agar, Blood agar, Chocolate agar and MacConkey's agar.These inoculated plates were then incubated for a periodof 18- 24 hours after which they were examined for evidenceof bacterial growth. A single well separated colony

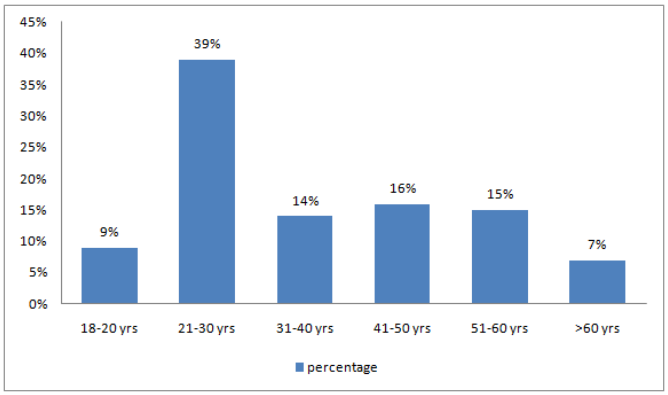
was identified. Preliminary tests like Grams staining of the colony, Hanging- drop preparation, Catalase test and Cytochrome oxidase test were done. Biochemical tests like Indole test, Methyl red test, Voges proskauer test, Citrate utilisation test, Urease test, Triple sugar iron agar and PPA (Phenyl Pyruvic Acid) test were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol were done to identify the isolate. All these tests were performed according to standard methods. Antibiotic sensitivity test of the isolates were performed by Kirby Bauer Disc Diffusion method and E-test using Mueller Hinton agar, antibiotic discs and E-strip , as described by Clinical Laboratory Standard Institute (CLSI) guidelines. The zones of inhibition were measured and sensitivities to various antibiotics were determined using CLSI guidelines, for each antibiotic. [11].

Results

Individual bacterial isolates and their resistance pattern to various antibiotics were also recorded in all 153 samples of pleural fluid.

Age Distribution

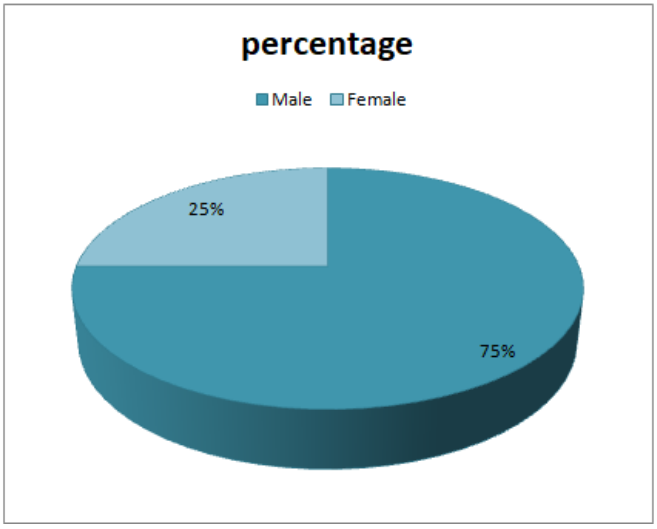
Age group wise distribution of all patients:



Majority of patients were 21 to 30 years of age group (39%) followed by 41 to 50 years (16%),51 to 60 years (15%), 31-40 years (14%), less than 20 years (9%) and more than 60 years (7%).

Sex Distribution

Sex wise distribution of empyema cases



It is evident from that out of 153 pleural fluid samples, 114 (75%) were of males and 39 (25%) were of females. The ratio between male and female is 2.92:1.

Bacteriological Profile

Finding	No. of patients	Percentage (%)
No Organism	108	71 %
Acinetobacter baumanni	10	7 %
Pseudomonas aeruginosa	17	11 %
E. coli	6	4 %
Klebseilla pneumonia	10	7 %
Citrobacter freundii	1	1 %
Enterococcus feacalis	1	1 %
Total	153	100 %

Out of 153 pleural fluid samples ,45 (29.41%) showed growth while 108 (70.59%) samples were sterile. Culture positivity rate was 29.41%**.**Out of 45 pathogenic bacteria, p.aeruginosa was the commonest bacteria, isolated in 17(11%) pleural fluid sample, followed by A.baumanni and k.pneumoniae isolated in 10(7%) pleural fluid samples, E.coli in 6(4%), C.freundii and E.feacalis in 1(1%) pleural fluid samples.

Antibiotic Resistance Patterns of the Isolates

Antimicrobial resistance pattern of isolated Gram negative bacilli:

Antibiotic	P.aeruginosa (n = 17)	A.baumannii(n=10)	K. pneumoniae(n = 10)	E. coli (n=6)	C. Freundii (n=1)
Piperacillin	33	50	75	78	0
Ampicillin-sulbactam	--	42	62	78	--
Piperacillin-Tazobactam	19	50	37	44	00
Ticarcillin/clavulanic Acid	42	50	62	78	00
Cefepime	22	42	37	45	00
Ceftriaxone	--	50	62	89	00
Cefotaxime	--	67	75	100	00
Ceftazidime	69	75	75	89	00
Imipenem	33	50	25	44	00
Meropenem	50	50	37	33	00
Gentamycin	25	50	37	22	00
Tobramycin	33	50	50	00	00
Amikacin	--	42	37	11	00
Netilmycin	31	33	50	11	00
Tetracycline	--	42	12	89	00
Doxycycline	--	42	00	67	00
Minocycline	--	17	12	44	00
Ciprofloxacin	17	25	37	33	00
Levofloxacin	36	58	50	67	00
Co-trimoxazole	--	50	25	56	00
Amoxycillin / Clavulanic acid	--	--	75	56	--
Ampicilline	--	--	--	89	--
Aztreonam	28	--	62	67	00
Cefazoline	--	--	75	89	--
Cefixime	--	--	75	89	00
Cefoxitin	--	--	62	78	--
Cefuroxime	--	--	75	89	--
Chloramphenicol	--	--	37	11	00
Ertapenem	--	--	50	44	00

Most resistant antibiotic for Pseudomonas aeruginosa was Ceftazidime (69%) followed by Meropenem (50%) and Ticarcillin/Clavulanic acid (42%).

Most resistant antibiotic for Acinetobacter baumannii was Ceftazidime (75%) followed by Cefotaxime(67%) and Levofloxacin (58%).

Most resistant antibiotics for Klebseilla pneumoniae were Pipracilline (75%), Cefotaxime (75%), Ceftazidime (75%), Amoxycillin/Clavulanic acid (75%), Cefazoline (75%), Cefixime (75%) and Cefuroxime (75%).

Most resistant antibiotic for E.coli was Cefotaxime (100%) followed by Ceftriaxone (89%), Ceftazidime (89%), Tetracycline (89%), Ampicillin (89%), Cefazoline(89%), Cefixime(89%) and Cefuroxime (89%).

No antibiotic resistance was seen for Citrobacter Freundii in present study.

Antimicrobial resistance pattern of Gram positive isolates:

Antibiotic	**Enterococcus feacalis (n = 1)**
Penicillin G	100
Ampicillin	100
Vancomycin	00
Teicoplanin	00
Daptomycin(Mic)	00
Linezolid	100
Quinupristin/Dalfopristin	100
Ciprofloxacin	100
Levofloxacin	100
Rifampin	100
Tetracycline	100
Doxicycline	100
Minocycline	100
Erythromycin	100
Chloramphenicol	00

Discussion

In the present study, total 153 pleural fluid samples were received to the microbiology laboratory for culture and sensitivity test from June 2021 to November 2021.

In our study, the predominant age group affected with pleural effusion were from 21 to 30 years of age (39%). This was in line with the study conducted by Dhital et al[12] with most common age group affected with pleural effusion were 21- 30 years.

In the present study, pleural effusion was more common among male population.Male predominance in our study was similar to studies conducted by Solanki et al (67.8%)[4] and Khan et al (70.24%)[13].

In present study, 29% pleural fluid samples showed bacterial growth while 71% pleural fluid samples were sterile. This was similar to study conducted by Sharma et al[14] who showed culture positivity rate of 28.8%. Thus our study reported lower culture positivity rate. However study done by Mohanty et al[15] showed culture positivity rate of 15.3% which is less as compared to our studies.

Present study highlights the emergence of aerobic gram negative microbes as the predominant pathogens in empyema. A similar high rate of isolation of Gram negative bacilli from pleural fluid cultures were reported in India by Mohanty et al [15] (86.4%), Jain S et al [16] (88.5%) and Ramana B V et al [17] (95%).

The most frequent isolate in our study population was Pseudomonas aeruginosa (n=17, 37.77 % of the total pyogenic isolates), a finding in agreement with the study done by Solanki et al who concluded that most frequent isolate was Pseudomonas aeruginosa(45%)[4].

The resistance trends among the respiratory pathogens such as, Pseudomonas aeruginosa to the antimicrobial agents that have traditionally been recommended as the first line therapy, is on the rise. Our study showed Pseudomonas aeruginosa has maximum resistance to ceftazidime(69%) followed by Meropenem(50%) and Ticarcillin/Clavulanic acid (42%). Similar observation was found in study conducted by Ahmed et al [18] who concluded that Pseudomonas aeruginosa has maximum resistance to ceftazidime(40.4%). This was also in line with the study conducted by Goel et al[19] who observed that maximum resistance of Pseudomonas aeruginosa to ceftazidime(68.4%) followed by Meropenem(22.8%) and Ticarcillin/Clavulanic acid (49.1%).

In our study, among the gram negative bacilli , enterobacter species such as Escherichia coli and Klebsiella pneumoniae showed maximum resistance to Ceftriaxone were 89% and 62%, respectively. Almost similar results were found in the study conducted by Ahmed et al who showed that Escherichia coli and Klebsiella pneumoniae showed maximum resistance to Ceftriaxone were 74.1% and 76.5%, respectively[18].

Acinetobacter baumanni was highly resistant to Ceftazidime(75%) in our study which similar to study conducted by Ahmed et al who showed 100% resistance of Acinetobacter baumanni to ceftazidime[18].

The present study shows variation in the bacteriological profile and antibiotic resistance pattern of pleural fluid which may reflect the local trends of bacterial prevalence and antibiotic resistance pattern in our area, since it is a hospital based study there may be multifactorial facts that should be kept in perspective. However it is important to report differences in our 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 resistance pattern though it can not be generalized.

Conclusion

In our country, pleural space infection continues to be prevalent mainly because of delay in seeking treatment, inappropriate use, dosages and duration of antimicrobial therapy.

Inappropriate use of antimicrobial agents has led to an increase in its resistance in both gram positive as well as gram negative spectrum of bacteria. It is necessary and inevitable to increase awareness among patients about harmful effect of overuse or misuse of antimicrobial agents. There is also need to develop effective hospital based antibiotic policy with reference to sterile body fluids like pleural fluid which helps a treating physician for effective and prompt treatment to decrease mortality and morbidity significantly.

For effective management of disorders such as pleural effusion definitive bacteriological diagnostic profile and antimicrobial susceptibility testing is required.

Based on this study, a multidimensional approach is suggested to combat and to win this battle against microbial agents:

- Surveillance and monitoring of antimicrobial agents use and resistance rates.
- Conduction of appropriate Antimicrobial Stewardship Programmes.
- Application and maintenance of appropriate health care practices in health institutions.
- Appropriate antibiotic selection for critically ill patients rather than a tradition based approach for selecting antimicrobial agent.
- Better approach for care of individuals with predisposing factors so that secondary bacterial infections do not set in, thus reducing the misuse of antimicrobial agents.

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