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A Study of Bacteriological Profile of Pleural Fluid and Antibiogram of Isolates at Pandit Dindayal Upadhyay Medical College Andhospital College, Rajkot

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: 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 retrospective study was designed to review our experience with the microbial causes of empyema and their antibiotic sensitive patterns. The knowledge of likely prevalent strains along with their antimicrobial sensitive pattern helps in the framing of antibiotic policy and better management of patients.
Materials and Methods: This descriptive study was including 500 cases suspected of bacteriological infection of pleural fluid in patients admitted in ward of PDU Government Hospital, Rajkot. The performa include serial number, register number, age, sex, ward, clinical features of patients and investigation. All the samples were inoculated into Blood agar and MacConkey agar and Nutrient agar. All the plates were incubated aerobically at 37^0 C and results were read after 24 hours. If no growth present it is further incubated for next 24 hours. One smear was prepared on clean glass slide, then air dried and was heat fixed. Gram staining was done by standard technique.
Results: This study include 500 cases of pleural effusion from January 2015 to July 2016, out of

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which 87 cases show positive Bacterial culture growth and 232 cases were adenosine deaminase positive. In total 87 positive bacterial cultures, 20(22%) show bacterial pathogens in gram stain. Total positive culture found in 17.4%. Among them; most common Bacteria isolated was *Pseudomonas aeruginosa* in 40 (45%) patients, this was followed by *Klebsiella pneumonaie* in 21 (24%), *Staphylococcus aureus* in 10 (11.49%), Acinetobacter spp. in 4 (4.59%), Proteus spp.in 3 (3.44%) and Providencia in 1 (1.14%).

Conclusion: Pleural space infection continues to be prevalent in our country particularly in the lower socioeconomic strata due to the delay in seeking medical care, inappropriate antibiotics and dosages and duration of antibiotic treatment. All gram positive bacteria isolate were 100% sensitivity to Rifampicin, Vancomycin, Linezolid.

Keywords: Bacterial culture growth; pleural space infection; Pseudomonas aeruginosa; thoracic empyema.

1. INTRODUCTION

In the pleural cavity of normal human being, there is a small amount of fluid known as a pleural fluid which lubricates the lining of the cavity. Pleural effusion is always abnormal and indicates the presence of an underlying disease [1]. Pleural fluid accumulates when pleural fluid formation exceeds pleural fluid absorption. Normally, fluid enters the pleural space from the capillaries in the parietal pleura and is removed via the lymphatics situated in the parietal pleura. Fluid can also enter the pleural space from the interstitial spaces of the lung via the visceral pleura or from the peritoneal cavity via small holes in the diaphragm. The lymphatics have the capacity to absorb twenty times more fluid than is normally formed [2].

Pleural effusion is defined as an abnormal, excessive collection of fluid in the Pleural space. Two types of effusions can develop, transudative and exudative. Various kinds of pleural effusion, depending on the nature of the fluid and what caused its entry into the pleuralspace, are hydrothorax(serousfluid),hemothorax(blood), chylothorax (chyle)or pyothorax (pus).

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. For centuries Empyema thoracic has been recognized as a serious problem. The development of antibiotic resistance has also added to the gravity of the condition.

Chronic empyema is the outcome of improper management in the acute stage. The disability produced by the persistence of chronically infected pleural space is very grave. It can only be attributed to some error or neglect in early stages of pleural suppuration.

The bacteriology of thoracic empyema has been changing since the introduction of antibiotics. Before the antibiotic era Streptococcus pneumoniae or β-hemolytic streptococci were isolated in most empyema fluid, and Staphylococcus aureus was the most common pathogen of thoracic empyema between 1955 and 1965. In the early 1970s, anaerobic bacteria were isolated most frequently [4]. 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.

Gram stain and culture has for decades been the "gold standard" 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 [5]. A variety of other 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 [6].

Currently use of nucleic amplification tests appears to be the method with the highest sensitivity (up to 75%) in the identification of bacteria in pleural fluid [7]. 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.

The present retrospective study was designed to review our experience with the microbial causes of empyema and their antibiotic sensitive patterns. The knowledge of likely prevalent strains along with their antimicrobial sensitive pattern helps in the framing of antibiotic policy and better management of patients.The present retrospective study was designed to review our experience with the microbial causes of empyema and their antibiotic sensitive patterns. The knowledge of likely prevalent strains along with their antimicrobial sensitive pattern helps in the framing of antibiotic policy and better management of patients.

2. MATERIALS AND METHODS

This descriptive study was including 500 cases suspected of bacteriological infection of pleural fluid in patients admitted in ward of PDU Government Hospital, Rajkot.

2.1 Inclusion Criteria

All pleural fluid samples received at the Microbiology Department from the P.D.U Government Hospital, Rajkot was accepted for the study. The Performa include serial number, register number, age, sex, ward, clinical features of patients and investigation.

2.2 Sample collection

It is a method of removing pleural fluid through a needle inserted across the skin and chest wall.

Preparation of patient: Inj. Atropine 0.6 mg should be given IM ½ hour before the procedure to prevent vasovagal shock.

Position of the patient: Patient sits on the side of the bed with his arms and head resting on one or more pillows on a bedside table. In debilitated patient it is performed with the patient lying on the side of the pleural effusion in the lateral decubitus position with his back near the edge of the bed.

2.3 Selection of the Site and Technique

Thoracocentesis should be attempted, one interspaces below the spot where tactile fremitus is lost and percussion note become dull. It should usually be performed posteriorly several inches from the spine, where the ribs are easily palpated. The exact location for the thoracentesis attempt should be just superior to rib to avoid injury to neurovascular bundles.7th or 8th ICS in midaxillary or scapular line is frequently used. Once the site for thoracentesis is detected, the skin over the site is cleaned thoroughly with an antiseptic solution.

The next step is to obtain local anaesthesia. It is necessary to anaesthetize the skin, the periosteum of the rib and the parietal pleura by using a 23 gauge needle and syringe filled with 2% lignocaine.

Once the needle is superior to the rib, it is slowly advanced toward the pleural space with aspiration followed by the injection of 0.1 to 0.2 ml lignocaine every 1 to 2 mm. This frequent aspiration and the injection of lignocaine guarantee anesthesia of the parietal pleura.

When pleural fluid is obtained through this needle into the syringe containing lignocaine, the needle should be withdrawn from the pleural space and should be reattached to a 50 to 60 ml syringe containing 1 ml heparin to prevent clotting of the pleural fluid.

2.4 Bacteriological Processing and Identification

2.4.1 Method for gram staining

One smear was prepared on clean glass slide, then air dried and was heat fixed. Gram staining was done by standard technique.

2.4.2 Processing of sample for culture

All the samples were inoculated into Blood agar and MacConkey agar and Nutrient agar. All the plates were incubated aerobically at 37^0 C and results were read after 24 hours. If no growth present it is further incubated for next 24 hours.

2.4.3 Media for cultivation of bacteria:

A: MacConkey Agar
B: Blood Agar
C: Muller Hinton Agar
Colony characters
On MacConkey agar

2.4.4 Lactose fermenting organism - pink color colonies

- ⇨ *Escherichia coli*- Pink, large, opaque, non mucoid, circular, smooth, glossy with entire margins.

- ⇨ Klebsiella- Pink, large, dome shaped, mucoid.

- **Non Lactose fermenting organism-pale or colorless**

  - ⇨ Proteus- smooth, large, raised with fishy or seminal odour.
  - ⇨ Pseudomonas-large, opaque, irregular with serrated edges, distinctive musty, earthy or grape like odour.
  - ⇨ Acinetobacter – convex entire, faint pink tint.

On Blood agar:

- *Staphylococcus aureus*- B hemolytic, 2 -4 mm large, circular, convex, smooth, shiny and easily emulsifiable.
- Acinetobacter – 0.5 -2.0 mm in diameter, translucent to opaque, convex and entire.

2.4.5 Gram staining of smears prepared from colonies

Smears were prepared and stained with grams stain to study the morphology of bacteria. A thin smear of bacterial colony to be examined was made, air dried and fixed by heat. Gram staining was done by standard method (Give references). By gram staining we can differentiate between Gram negative and Gram positive bacilli and cocci.

By this staining the organism isolated were broadly classified into:

- Gram Positive cocci:
  - ⇨ Staphylococcus aureus (in groups or cluster)
- Gram Negative bacilli:

- ⇨ E.coli (straight, 1-3*0.4*0.7 um singly or in pairs)
- ⇨ Kleibsiella (short plump,straight)
- ⇨ Proteus (pleomorphic, 1-3*0.5 µm)
- ⇨ Pseudomonas (slender,straight 1.5*0.5 um)
- ⇨ Acinetobacter (Gram negative coccobacilli often appearing as diplococci)

2.4.6 Test for Gram Positive Cocci

1) Coagulase Test

Procedure: The enzyme coagulase is demonstrated in vitro by two methods

a) The Slide coagulase test
b) The Tube coagulase test

2.5 Result and Interpretation

- ⇨ Positive reaction –Appreance of a granular precipitate or formation of white clumps within 10 seconds.
- ⇨ Negative reaction –No clumping within 2 minutes.

b) The Tube Coagulase Test

Result and interpretation:

Positive – Any degree of clotting of tube contents - staphylococcus aureus.
Negative – free flow of the contents and no clotting.

Quality Control

Positive: *Staphylococcus aureus*(ATCC25923)
Negative: *Staphylococcus epidermidis*

Test for Gram Negative Bacteria: Gram negative bacteria grown on MacConkey media identified as lactose fermenting bacteria and non lactose fermenting bacteria.

1) Catalase Test :

All members of Enterobacteriaceae are catalase positive except shigella dysentriae type 1.

2) **Oxidase Test :**

This reaction is due to a cytochrome oxidase which catalyses oxidation of reduced cytochrome by oxygen. This is used for screening species of pseudomonass(positive) and exclusion of enterobacteriaceae(negative) .

Kovak Method: A strip of strip paper soacked with 1% solution of tetramethyl p-phenylene diamine dihydrochloride is placed in a petridish and colony to be tested is smeared on the paper in a line about 5 mm long

Result:

Positive – smeared area turns deep purple in 10 seconds

Negative - absence of coloration later than 60 seconds.

Delayed positive - Deep purple color in 10 – 60 sec.

Quality Control:

Positive: Pseudomonas aeruginosa(ATCC27853)
Negative: Escherichia coli (ATCC25922)

Indole test:

Result

Positive: Pink colored rink after addition of appropriate reagent e.g. E.coli
Negative: No color change even after the addition of appropriate reagent. e.g. Klebsiella pneumonia

Quality Control

Positive: *Escherichia coli* (ATCC25922)
Negative: *Klebsiella pneumonia* (ATCC13883)

Methyl Red (MR) test:

Result

Escherichia coli: MR test positive- appearance of red color after the addition of methyl red reagent.
Enterobacter aerogenes: MR test Negative- the lack of color change after the addition of methyl red.

Quality Control

MR positive: *Escherichia coli* (ATCC25922)
MR negative: *Enterobacter aerogenes*

Citrate test:

Result
Positive- blue colour along with growth e.g *Klebsiella pneumonia*

Negative – green colour and no growth e.g. *E. coli*

Quality Control

Positive: *Enterobacter aerogenes*—growth, blue color
Negative: *Escherichia coli* (ATCC25922)—little to no growth, no color change

Urease test:

Result

- ⇨ Organisms that hydrolyze urea rapidly (e.g. *Proteus* spp) may produce positive reactions within 1 or 2 hours; less active species (e.g. *Klebsiella* spp) may require 3 or more days.
- ⇨ If organism produces urease enzyme, the color of the slant changes from light orange to magenta.
- ⇨ If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

Quality Control

Positive: *Proteus vulgaris*
Weak positive: *Klebsiella pneumonia* (ATCC13883)
Negative: *Escherichia coli* (ATCC25922)

Phenyl Alanine Deaminase Test:

Result
Positive- green color in the slope and in the fluid e.g. proteus vulgaris and mirabilis.
Negative- no green color e.g. E.coli, kleibsiella.

Quality Control

Positive: *Proteus mirabilis*
Negative: *Escherichia coli* (ATCC25922)

Triple sugar iron test (TSI)

Chart 1. Interpretation

Slant/butt	Color	Utilization
Alkaline/acid	Red /Yellow	Glucose only fermented
Acid/Acid	Yellow/Yellow	Glucose fermented,lactose and/or sucrose fermented
Alkaline/No change	Red /no change	No fermentation of glucose,lactose

H_2S production is recorded by the black coloration of medium

Quality Control

A/A, gas production: *Escherichia coli* (ATCC25922)

K/A, +/− gas production, H2S+: *Salmonella typhimurium*

K/K: *Pseudomonas aeruginosa*(ATCC27853)
K/A, H2S+: *Proteus mirabilis*
K/A: *Shigella flexneri*

2.6 Sugar Fermentation

2.6.1 Method

This test is performed in a liquid media containing 1% peptone water with 1% respective sugar(10% aqueous solution).To this durhams tube is added. Andrades indicater (pH indicater) is also added sugar to sugar tubes.incubate at 37 ^{0} C for 24 hours and check the results.

2.6.2 Antibiogram

- ⇨ The antibiotic sensitivity test of isolates was performed by disc diffusion method recommended by Kirby Bauer.
- ⇨ Mueller Hinton agar plates were used for antibiotic sensitivity tests. Before using, the plates were dried for 10-30 minutes at 37^{0}C by placing them in an upright position in the incubator.
- ⇨ After the plates were dried, broth suspension of the organism was made and adjusted to Mcfarlands opacity factor 0.5. A lawn culture was made over the surface of the media using a sterile swab, then appropriate antibiotics discs were placed after 3-5 min and incubated at 37^{0}C for 24 hours after which readings were taken.
- ⇨ The zone of inhibition was measured and reported as per the CLSI2014 guidelines:

Test for tuberculosis pleural effusion
Ziehl Neelsen Stain:

Adenosine Deaminase Assay (ADA):

Result

ADA level(in U/L)

Reference Range:

- <30 U/L: Normal
- >30 U/L:Positive

2.7 Statistical Analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

3. RESULTS

This study include 500 cases of pleural effusion from January 2015 to July 2016, out of which 87 cases show Positive Bacterial culture growth and 232 cases were ADA positive.

In this study majority of cases were in the age group of 41-50 years (5thdecade) constituting of 124 patients that is 24.8 % of the total. This was followed by the 51-60 year group where there were 114 patient sconstitu ting 22.8 % of the total. Puttogether 41-60 year sage interval has 47.6% of the total patients.

The results of drug sensitivity revealed that among the gram negative isolates, higher sensitivity was obtained to amikacin among the aminoglycosides, cefepime among the cephalosporins and to various combinations like piperacillin-Tazobactam, Ampicillin-sulbactam and Ceftazidime–clavulanic acid and Levofloxacin among the quinolone.Among gram negative bacilli, one strain (10%) of E.coli is extended spectrum beta lactamase(ESBL). All gram positive bacteria isolate were 100% sensitivity to Rifampicin, Vancomycin, Linezolid.All gram-positive bacteria isolated were 100% resistant to penicillins; this can be alleviated by combining it with betalactamase inhibitors like clavulinic acid or sulbactum, which are resistant to some of beta-lactamases produced by bacteria.In gram positive staphylococcus,in 1 strain(10%) showing Methicillin resistant staphylococcus aureus and 1 strain(10%) erythromycin induced clindamycin resistant.

Out of 232 positive samples of ADA, 18 samples were (7.7%) also positive for bacterial culture growth.

4. DISCUSSION

In the present study Pleural effusion was most common in 41 to 50 years of age group which was comparable with the study of Somenath

Kundu et al**.** [7] and Murthy M, et al**.** [8] In present study male predominance was found. Male predominance also found in all other studies mentioned in the above table. Most common complains was cough (87.8%) followed by fever (78.3%),dyspnea (64%) and chest pain(42%) which was comparable with the study of Murthy M, et al**.** [8].

Chart 2. For Gram Positive organisms

Antibiotics	Dosage(µg)	Antibiotics	Dosage(µg)
Clindamycin	2	Tetracycline	30
Cefoxitin	30	Cotrimoxazole	1.25/23.75
Penicillin-G	10	Vancomycin	30
Gentamycin	10	Linezolide	30
erythromycin	15	Rifampin	5
Levofloxacin	5		

Chart 3. For Gram Negative organisms

	Antibiotics	Dosage (ug)		Antibiotics	Dosage (ug)
Penicillin	Ampicillin	10	Aminoglycosides	Amikacin	30
	Piperacillin	100		Gentamicin	10
	Ampicillin-sulbactam	10/10	Tetracyclines	Tetracycline	30
	Piperacillin-tazobactam	100/10	Monobactam	Aztreonam	30
3rd Genaration Cephalosporin	Ceftazidime	30	Carbapenam	Imepenam	10
	Ceftazidime-clavulanic acid	30/10		Cotrimaxole	1.25/23.75
	Cefoperazone	75		Chloramphenicol	30
4th Genaration Cephalosporin	Cefepime	30		Polymyxin B	300
Fluroquinolone	Levofloxacin	5			

Table 1. Age distribution

Age Group(year)	Number of case	Percentage
<1	6	1.2
1 to 10	27	5.4
11 to 20	31	6.2
21 to 30	85	17
31 to 40	66	13.2
41 to 50	124	24.8
51 to 60	114	22.8
61 to 70	29	5.8
71 to 80	14	2.8
81 t0 90	4	0.8
TOTAL	**500**	**100**

Table 2. Gender distribution

Gender	Number of case	percentage
Male	339	67.8
Female	161	32.2
Total	500	100

Table 3. Socio-economic status (modifiedKuppuswamy'sclassification)

Socioeconomic status	Number of cases	%
Upper class	0	-
Upper Middle class	37	7.5
Lower Middle class	75	15
Upper Lower class	75	15
Lower class	313	62.5
Total	**500**	**100**

388/500 (77.5%) belonged to lower socio-economic status. None of them belonged to upper class.

Table 4. Showing symptoms of study participants

Symptoms	Number of cases	percentage
Fever	393	78.30%
Cough	439	87.80%
Dyspnea	320	64%
Chest pain	210	42%

Inthepresentstudythecommonestpresentationwascough(87%),followedbyfever(78.3%), dyspnoea(64%)chestpain(42%).

Table 5. Gram stain and ZN stain

Gram stain		ZN stain	
Positive	**Negative**	**Positive**	**Negative**
20	480	0	500

In total 87 positive bacterial cultures, 20(22%) show bacterial pathogens in gram stain

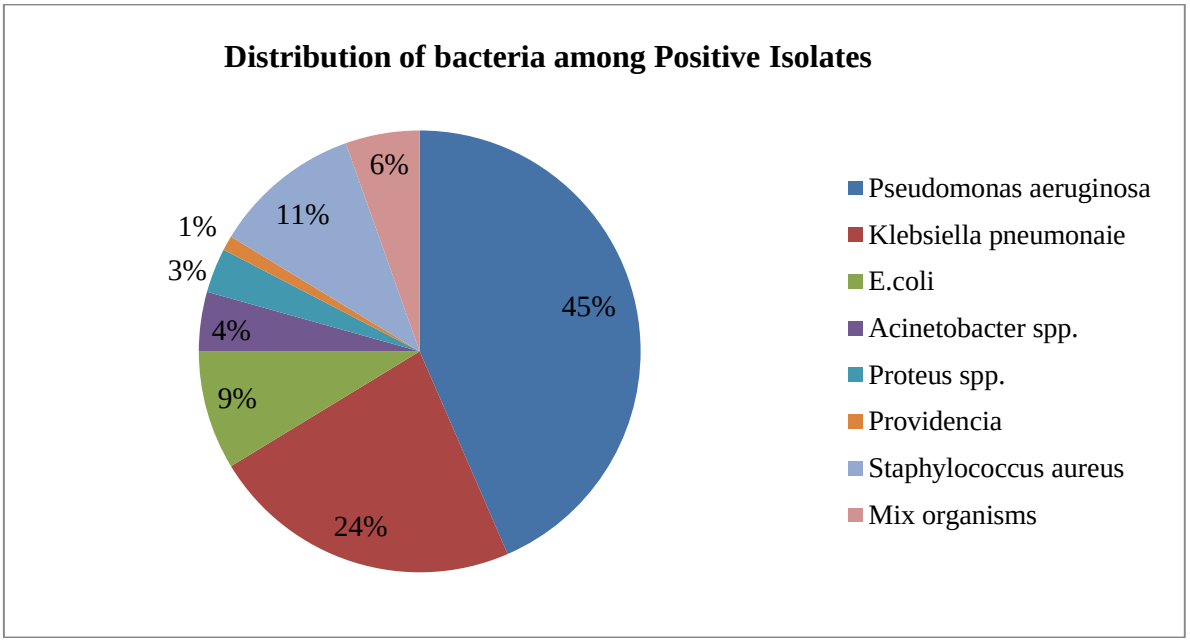

Fig. 1. Distribution of bacteria among Positive Isolates

More than one isolate was seen in 6 patients (6.8%) and they were combination of isolates from the Enterobacteriaceae group and Pseudomonas aeruginosa.

In the present study, comprising 500 pleural fluid samples received in the microbiology laboratory the percentage of positive cultures was 17.6%. Rates of microbiological diagnosis in earlier studies have shown a wide variation. A lower positive culture rates similar to ours has been observed in Indian studies like that of Mohanty et al. [9] (15.3%), [10] and western studies like

Ferrer et al. [11] (15.5%) and Barnes et al. [12] (1.4%).

A higher positive culture rates has been observed in studies like that of Dass R et al [13] and Ramana B V et al [14].

Given the low positive yield of pleural fluid cultures, we tried to determine the factors associated with a higher likelihood of positive results. Microbiological studies performed only on exudative effusions could definitely enhance the yield of the samples. This however, is not a practical solution since the exudative nature of the pleural fluid can't be ascertained prior to the fluid result being available. Second important factor in the low yield of pleural fluid samples could be the empiric administration of antibiotics to the patients before thoracocentesis, likely decreasing the yield of the cultures [12].

The paucity (absence) of anaerobes in our series is notable and could be an incriminating factor in the low culture positivity rate. The incidence of anaerobic isolates depends both on the care with which they are searched for and on the type of population studied. Inadequate methods to collect and transport the pleural specimens to the laboratory and technical difficulty of growing anaerobes could also have contributed to our missing exclusively anaerobic pathogens [15-16].

Our study highlights the emergence of aerobic gram negative bacteria as the predominant pathogens in empyema. Out of the 87 pyogenic isolates, 77(88.5%) were aerobic GNB. A similar high rate of isolation ofGNB from pleural fluid cultures was reported in India by Jain S et al**.** [17] (88.5%), Mohanty et al [9] (86.4%), Ramana B V et al [14] (95%).

After the discovery and widespread use of antibiotics in the 1940s, Staph aureus succeeded S. pneumoniae and S.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 GNB and anaerobes have increased markedly. These observations are in consensus with the reports of various workers who have emphasized the emergence of GNB as the predominant pathogen [18-19].

The isolation of aerobic GNB or multiple pathogens from pleural fluid is associated with a poor prognosis and indicates a more aggressive antimicrobial chemotherapy in contrast to the empyema caused by Gram positive pathogens [19].

More than one isolate was seen in 5 patients (5.74%) mostly due to a combination of isolates from the Enterobacteriaceae group and Pseudomonas aeruginosa. A polymicrobial etiolgy of empyema thoracis is also well documented [20]. A similar combination was also reported by Jain S et al. [2]. The most frequent isolate in our study population was *Pseudomonas aeruginosa* (n=87, 45% of the total pyogenic isolates) a finding in agreement with many Indian study [10,21].

Gram negative enteric bacteria (42.36%) were the next most common; the once predominant Gram positive organisms were conspicuous by their absence. This is comparable with earlier reports where streptococcus was rarely if ever seen as a cause of empyema [20].

Table 6. Percentage of Bacteria (In Total cases)

Bacteria	Number	% of Total
Pseudomonas aeruginosa	40	8%
Klebsiella pneumonaie	21	4.20%
E.coli	8	1.60%
Acinetobacter spp.	4	0.80%
Proteus spp.	3	0.60%
Providencia	1	0.20%
Staphylococcus aureus	10	2%
No growth	413	82.60%
Total	**500**	**100%**

Total positive culture found in 17.4%.Among them; mostcommon Bacteria isolatedwasPseudomonas aeruginosain40(45%) Patients, thiswasfollowedbyKlebsiella pneumonaie in 21(24%),Staphylococcus aureusin10(11.49%),Acinetobacter spp.in4(4.59%), Proteus spp.in3(3.44%)andProvidencia in1(1.14%)

Table 7. Antibiotic sensitivity pattern for gram negative bacteria

Antibiotic sensitivity pattern of Gram negative isolates		Klebsiella (21)	E.coli (8)	Acinetobacter spp.(4)	Proteus spp.(3)	Providencia (1)	Pseudomonas aeruginosa (40)
Penicillin	Ampicillin(AMP)	0	0	0	0	0	-
	Piperacillin(PI)	19	13	25	66.6	0	93.3
	Ampicillin-sulbactam(A/S)	72	75	75	100	100	-
	Piperacillin-tazobactam(PIT)	-	-	-	-	-	100
3rd Genaration Cephalosporin	Ceftazidime(CAZ)	67	63	50	100	100	93
	Ceftazidime-clavulanic acid(CAC)	76	75	50	100	100	-
	Cefoperazone(CPZ)	-	-	-	-	-	90
4th Genaration Cephalosporin	Cefepime(CPM)	76	75	75	100	100	100
Fluroquinolone	Levofloxacin(LV)	90	75	75	100	100	86
Aminoglycosides	Amikacin(AK)	88	88	100	66.6	100	93.7
	Gentamicin(GEN)	88	75	75	66.6	100	83
Tetracyclines	Tetracycline(TE)	67	63	50	66.6	100	-
Monobactam	Aztreonam(AT)	-	-	-	-	-	93.3
Carbapenam	Imepenam(IPM)	100	100	100	100	100	-
	Cotrimaxole(COT)	57	63	50	33.3	100	-
	Chloramphenicol(C)	81	63	-	66.6	100	
	Polymyxin B(PB)	-	-	-			100

Table 8. Antibiotic sensitivity patterns for gram positive Bacteria:

Antibiotic disc	Staphylococcus aureus (10)
Chloramphenicol	90
Cotrimaxole(10 mcg)	70
Cefoxitin	90
Clindamycin	50
Erythromycin	40
Gentamycin	90
Levofloxacin	60
Linezoid	100
Penicillin G	0
Rifampicin	100
Tetracyclin	90
Vancomycin	100

The antimicrobial resistance among the respiratory pathogens is a major barrier that might interfere with an effective treatment. This study depicts the antimicrobial susceptibility patterns among the gram negative and gram positive, respiratory pathogens which were isolated during the study, as has been shown in above Table. In present study Gram positive organisms showed 100% susceptibility to vancomycin, linezolid, Rifampicin and followed by their susceptibility against tetracycline, gentamycin. There are similar reports from other investigators [22-23].

The resistance among the respiratory pathogens especially Pseudomonas aeruginosa to the agents that have traditionally been recommended as the first line therapy, is on the rise. Like, our study showed Pseudomonas aeruginosa has maximum sensitive to cefepime, ceftazidime, Piperacillin and Piperacillin-tazobactum, present study is in concordance with results found by Ahemad M S et al. [24] and contrast to Nidhi Goel et al. [25]. In our study amikacin and Levofloxacin had shown greater activity against Gram negative bacilli similar to study conducted by Bajpai et al. [26] and contrast to Ahmed et al. [24].

Pairs et al. [27] first time reported an increase in ADA level in TB pleural effusion; other studies have also confirmed such an increase in TB pericardial effusions, peritoneum, and central nervous system (CNS) [28-29]. The main reason for the increased ADA levels in tubercular effusion is the movement of T lymphocytes toward the area. Increase in ADA level is the result of a tropical inflammatory reaction caused by monocytes and macrophages [30]. When alveolar macrophages are infected by mycobacterium, this enzyme could be found in fluid during active disease. Jamenez D at el**.** [31] in their study stated that the ADA level in non-tubercular lymphocytic pleural effusion seldom exceeds the cut-off set for tuberculous effusions.

Strankinga W.F [33] investigated 10 patients with tuberculosis pleurisy and 76 patients with pleural effusions of other etiology. The ADA activity in the tuberculous patients was significantly higher than in the other groups while the exception of those with empyema. Specificity 87% and sensitivity 100% of this test for tuberculosis are high when a reference limit of more than 53 U/L is taken.

Two studies, one by Gakis in 1998 and the other by Klockars in 1991, have shown that serum ADA levels increased in pneumonia patients [27,33]. Fernandez and coworkers in 2003 compared serum ADA levels between TB patients and patients with other pulmonary infections and found no significant difference between the 2 groups [29].

Tian RX et al and Aoe K et al studies showed higher serum ADA level in Pulmonary TB patients than in normal individuals. However, ADA was not found to be a suitable marker for differentiating between pulmonary TB and other pulmonary infections [34-35]. In our study, ADA was Positive in 232 cases, Out of 232 positive samples of ADA, 18 samples were (7.7%) also positive for bacterial culture growth.

ADA assay should not be considered as an alternative to biopsy and culture, but it has very important role in screening test and supportive test to guide further diagnostic procedures and management of an exudative pleural effusion in country like India where tuberculosis is more prevalent.

5. CONCLUSION

Pleural space infection continues to be prevalent in our country particularly in the lower socioeconomic strata due to the delay in seeking medical care, inappropriate antibiotics and dosages and duration of antibiotic treatment. Total positive culture found in 17.4%. Among them; most common microbiological organism isolated was *Pseudomonas aeruginosa* in 45%. Patients, this was followed by *Klebsiella pneumonaie* in 24%, *Staphylococcus aureus* in 11.49%, Acinetobacter spp.in 4.59%, Proteus spp.in 3.44% and Providencia in 1.14%. ADA Positive in 232 cases(46.40%). All gram positive bacteria isolate were 100% sensitivity to Rifampicin, Vancomycin, Linezolid. Indiscriminate use of antibiotics might have increased the overgrowth of multiresistant organisms, there on leading to chronicity and morbidity of empyema. Empyema fluid is diagnostic for pathogens if appropriate handling and early cultures but in the present scenario with prior antibiotic treatment, the fluid is sterile most of the times.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Ethical approval was taken from the institutional ethical committee and written informed consent was taken from all the participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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