



## Bacteriological Profile of Pleural Fluid in Empyema Thoracis

### KEYWORDS

empyema, bacteria, antibiogram

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**ABSTRACT** **INTRODUCTION-** *In para pneumonic effusion, empyema is the common problem during natural course. Studies of pleural fluid samples using standard culture and molecular techniques have demonstrated that it is associated with increased prevalence of bacteria. METHODOLOGY-100 clinically diagnosed cases of empyema of age  $\geq 15$  years were recruited. Pleural fluid samples was processed by conventional methods. Preparation of media, reagents, Gram staining, identification of culture isolates and antibiotic sensitivity tests were carried out following standard laboratory methods. RESULT- The prevalence of empyema was most common in age group of 26 to 45 year (48%) with ratio between male and female of 2.57:1. Streptococcus pneumoniae was the commonest bacteria i.e. in 19% cases, followed by Staphylococcus aureus isolated in 16 % cases, klebsiella in 9% cases, bacteroids in 9% cases, S.millieri in 8% cases, Pseudomonas aeruginosa in 8% cases, E.coli in 8% cases, hemophilus influenzae in 4% cases, peptostreptococcus in 4 % cases, S.pyogenus in 3% cases, and fusobacterium in 2% cases. Gram positives were most sensitive to cefoxitin (45%) followed by ceftarolin (42%) and vancomycin (41%). Gram negative isolates were most sensitive to meropenem (29%) followed by imepenem (29%) and cefepime (24%). 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.*

### INTRODUCTION

Pleural effusions are a common finding in patients with pneumonia. More than 40% of patients with bacterial pneumonia develop parapneumonic effusions. Some patients develop a more fibrinous reaction, with the presence of frank pus in the most severe cases referred to as an empyema or empyema thoracis. Bacterial pneumonia is the cause for thoracic empyema in 70% of cases<sup>1</sup>. Empyema thoracis is a complication of previous surgery, which accounts for almost 30% of cases. Chest trauma may also be complicated by infection of the pleural space. In the absence of trauma or surgery, the infecting organism may spread from blood or other organs into the pleural space.

*S pneumoniae* and *Staphylococcus aureus* account for approximately 70% of aerobic Gram-positive cultures in cases of empyema. Presently, aerobic organisms are isolated slightly more frequently than anaerobic organisms (2), . *Klebsiella*, *Pseudomonas*, and *Haemophilus* species are the three most commonly isolated aerobic Gram-negative organisms. *Bacteroides* and *Peptostreptococcus* species are the two most commonly isolated anaerobic organisms<sup>3, 4</sup>. Currently, empyema thoracis is most often associated with aspiration pneumonia with mixed bacterial flora containing aerobic and anaerobic bacteria. The usual organism isolated in empyema thoracis complicating previous surgery is *S aureus*<sup>5</sup>.

### METHODOLOGY

100 clinically diagnosed cases of empyema of age between 15-75 years were recruited from indoor of department of TB Chest of Gandhi Medical College and hamidia

hospital, Bhopal. The exclusion criteria were patients < 15 years of age, Patients with diagnosis of tubercular empyema, sterile culture, immunosuppressive disorders including DM, HIV and pts on treatment of oral steroids were excluded.

Aseptically aspirated pleural fluid samples were transported immediately to Microbiology laboratory for further processing by conventional methods.

Appropriate pleural fluid sample was inoculated on 5% Sheep Blood agar, Chocolate agar and MacConkey's agar. These inoculated plates were then incubated for a period of 18- 24 hours after which they were examined for evidence of 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, Vogesproskauer test, Citrate utilisation test, Urease test, Triple sugar iron agar, Nitrate reduction test, Hugh-Leifson's oxidation-fermentation test, coagulase production (for Staphylococci), Optochin Sensitivity (for *Streptococcus pneumoniae*) were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and inositols 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 using Mueller Hinton agar and antibiotic discs, as described by Clinical Laboratory Standard Institute (CLSI) guidelines.

Antibiogram was read, that is zones of inhibition were measured and sensitivities to various antibiotics were determined using CLSI guidelines, for each antibiotic.

**RESULTS**

Individual bacterial isolates and their sensitive pattern to various antibiotics were also recorded in all hundred (100) patients.

**AGE DISTRIBUTION**

**Table 1: Age wise distribution of EMPYEMA cases**

| Age (in years) | Number | Percentage (%) |
|----------------|--------|----------------|
| 15 – 25        | 16     | 16             |
| 26 – 35        | 32     | 32             |
| 36 – 45        | 20     | 20             |
| 46 – 55        | 16     | 16             |
| 56-65          | 12     | 12             |
| 66-75          | 4      | 4              |
| Total          | 100    | 100            |

In this study the prevalence of empyema in patients aged between 15 and 25 were 16 (16 %), between 26 and 35 were 32 (32%), between 36-45 were 20(20%) between 46-55 were 16 (16%), between 56-65 were 12(12%) and lastly between 66 and 75 were 4 (4%). (Table-1)

**SEX DISTRIBUTION**

**Table 2 : Sex wise distribution of eMPYEMA cases**

| Sex     | Number | Percentage (%) |
|---------|--------|----------------|
| Males   | 72     | 72             |
| Females | 28     | 28             |
| Total   | 100    | 100            |

It is evident from that out of 100 patients admitted 72 (72%) were males and 28 (28%) were females. The ratio between male and female is 2.57:1. (Table-2)

**BACTERIOLOGICAL PROFILE:**

Out of 100, monomicrobial isolates were 90 (90%), poly microbial isolates were 10 (10%).

**Table 3: bacterial strains in PLEURAL FLUID samples**

| S.No | Gram positive organisms  | Number | Percentage (%) |
|------|--------------------------|--------|----------------|
| 1    | S. pneumoniae            | 19     | 19             |
| 2    | S. aureus                | 16     | 16             |
| 3    | S.milleri                | 8      | 8              |
| 4    | S.pyogenus               | 3      | 3              |
|      | Total GP                 | 46     | 46             |
|      | Gram negative organisms  |        |                |
| 5    | Klebsiela                | 9      | 9              |
| 6    | E.coli                   | 8      | 8              |
| 7    | Pseudomonas              | 8      | 8              |
| 8    | Hemophilus               | 4      | 4              |
|      | Total GN                 | 29     | 29             |
|      | Anaerobic organism       |        |                |
| 9    | Bacteroids               | 9      | 9              |
| 10   | Paptostreptococcus       | 4      | 4              |
| 11   | fusobacterium            | 2      | 2              |
|      | Total anaerobic organism | 15     | 15             |
|      | Total                    | 90     | 90             |

Out of ninety (90) pathogenic bacteria, *S. pneumoniae* was the commonest bacteria, isolated in 19 cases, followed by *Staphylococcus aureus* isolated in 16 cases, klebsiela in 9 cases, bacteroids in 9 cases, S.milleri in 8 cases, *Pseudomonas aeruginosa* in 8 cases, E.coli in 8 cases, hemophilus in 4 cases, peptostreptococcus in 4 cases, S.pyogenus in 3 cases, and fusobacterium in 2 cases.

**Table 4 :bacterial strains in EMPYEMA samples (poly microbial)**

| Isolates                               | No. of cases |
|--|--------------|
| S. pneumoniae + pseudomonas aeruginosa | 4            |
| S. pneumoniae + Staphylococcus aureus. | 2            |
| E.coli + S. aureus                     | 2            |
| S. pneumoniae + E.coli                 | 2            |
| Total                                  | 10           |

Out of 100 positive pus cultures, ten (10) samples showed more than one isolates (Table 4).

**ANTIBIOTIC SENSITIVITY PATTERNS OF THE ISOLATES:**

**Table 5: ANTIMICROBIAL susceptibility pattern of isolated Gram negative**

| Antibiotics             | Kleb-siella n =9 | pseu-domon- asn=8 | E.colin = 8 | He-mo-phillus n=4 | Total n =29 | Per-cent-age(%) |
|-------------------------|------------------|-------------------|-------------|-------------------|-------------|-----------------|
| Ampicillin              | 4                | 3                 | 1           | 0                 | 8           | 8               |
| Ampicillin sulbactam    | 6                | 3                 | 3           | 1                 | 13          | 13              |
| Aztreonam               | 7                | 6                 | 6           | 2                 | 21          | 21              |
| gentamicin              | 7                | 6                 | 6           | 2                 | 21          | 21              |
| Amikacin                | 8                | 6                 | 7           | 3                 | 24          | 24              |
| Amoxyclave              | 5                | 3                 | 1           | 1                 | 10          | 10              |
| Cefuroxime              | 6                | NR                | 6           | 3                 | 15          | 15              |
| Ceftriaxone             | 8                | NR                | 7           | 3                 | 18          | 18              |
| Cefotaxime              | 8                | NR                | 7           | 3                 | 18          | 18              |
| Cefoxitin               | 7                | NR                | 6           | 3                 | 16          | 16              |
| Ciprofloxacin           | 7                | 7                 | 5           | 1                 | 20          | 20              |
| Imipenem                | 9                | 8                 | 8           | 4                 | 29          | 29              |
| Cotrimoxazole           | 3                | 7                 | 4           | 0                 | 14          | 14              |
| Piperacillin tazobactam | 7                | 8                 | 4           | 3                 | 22          | 22              |
| Ceftazidim              | 7                | 8                 | 5           | 2                 | 22          | 22              |
| Meropenem               | 9                | 8                 | 8           | 4                 | 29          | 29              |
| Cefepime                | 7                | 8                 | 6           | 3                 | 24          | 24              |
| Piperacillin            | 5                | 7                 | 2           | 2                 | 16          | 16              |
| Levofloxacin            | 8                | 7                 | 7           | 2                 | 23          | 23              |
| Tobramycin              | 7                | 8                 | 6           | 2                 | 23          | 23              |
| Ticarillin              | NR               | 8                 | NR          | 2                 | 10          | 10              |
| Colistin                | NR               | 7                 | NR          | 3                 | 10          | 10              |

|              |    |   |    |   |   |   |
|--------------|----|---|----|---|---|---|
| Polymyxin-B  | NR | 6 | NR | 2 | 8 | 8 |
| Gatifloxacin | NR | 6 | NR | 1 | 7 | 7 |
| Netlimicin   | NR | 7 | NR | 1 | 8 | 8 |

NR: Not Recommended by CLSI; hence not tested

*Klebsiella pneumoniae* was sensitive to meropenem, Imepenem, cefotaxime, amikacin, ceftriaxone, levofloxacin, cefepime and aztreonam. It was resistant to cotrimoxazole, ampicillin, amoxycylav, piperacillin. *Pseudomonas aeruginosa* was mainly sensitive to imipenem, meropenem, cefipime, colistine, tobramycin, ticarcillin, ceftazidime, cefepime. It was resistant to ampicillin, ampicillin-sulbactam, amoxycylave. *Escherichia coli* were sensitive to meropenem, imipenem, amikacin, ceftriaxone, cefotaxime, levofloxacin. They were resistant to ampicillin and amoxiclav. *Hemophilus* was sensitive to imipenem, meropenem, cefepime, it was resistant to ampicillin, cotrimoxazole.

**Table 6: Antimicrobial susceptibility pattern of isolated Gram positive**

| Antibiotics               | S.pneumonea | S.aureus | s. milleri | s. pyogenus | Total | Percentage (%) |
|---------------------------|-------------|----------|------------|-------------|-------|----------------|
|                           | n = 19      | n = 16   | n=8        | n = 3       | 46    | 46             |
| Penicillin                | 3           | 4        | 0          | 0           | 7     | 7              |
| Cefoxitin                 | 16          | 14       | 3          | 2           | 45    | 45             |
| Erythromycin              | 17          | 9        | 4          | 0           | 30    | 30             |
| Clindamycin               | 16          | 10       | 2          | 2           | 30    | 30             |
| Linezolid                 | 15          | 15       | 5          | 3           | 38    | 38             |
| Cotrimoxazole             | 16          | 4        | 3          | 2           | 25    | 25             |
| Vancomycin                | 18          | 15       | 6          | 2           | 41    | 41             |
| Ciprofloxacin             | NR          | 12       | 3          | 0           | 15    | 15             |
| Gentamycin                | NR          | 12       | 2          | 3           | 17    | 17             |
| Amikacin                  | NR          | 10       | 2          | 2           | 14    | 14             |
| Novobiocin                | NR          | 14       | 5          | 2           | 21    | 21             |
| Ceftaroline               | 18          | 15       | 6          | 3           | 42    | 42             |
| Oxacillin                 | 15          | 15       | 5          | 2           | 37    | 37             |
| Levofloxacin              | 3           | 10       | 4          | 2           | 19    | 19             |
| Tetracycline              | 0           | NR       | 0          | 0           | 0     | 0              |
| Chloramphenicol           | 4           | 11       | 1          | 2           | 18    | 18             |
| Quinpristin-dalfopristine | 14          | 9        | 3          | 2           | 28    | 28             |

NR : Not Recommended by CLSI; hence not tested

*Streptococcus pneumoniae* the commonest isolated organism was sensitive to vancomycin, ceftarolin, erythromycin, cotrimoxazole, clindamycin, cefoxitin and resistant to tetracycline, chloramphenicol, levofloxacin, penicillin. *S.aureus* was sensitive to linezolid, vancomycin, ceftarolin, oxacillin, it was resistant to penicillin, cotrimoxazole, erythromycin. *S.milleri* was sensitive to vancomycin, ceftarolin, linezolid, novobiocin, oxacillin, it was resistant to penicillin, tetracycline, chloramphenicol, clindamycin, gentamycin, amikacin. *S.pyogenus* was sensitive to linezolid, gentamycin, ceftarolin, it was resistant to penicillin, erythromycin, ciprofloxacin, tetracycline.

| antibiotics     | Bacteroids n=9 | Peptostreptococcus n=4 | Fusobacterium n=2 | Total 15 | %  |
|-----------------|----------------|------------------------|-------------------|----------|----|
| Metronidazole   | 9              | 4                      | 2                 | 15       | 15 |
| Clindamycin     | 9              | 4                      | 2                 | 15       | 15 |
| Chloramphenicol | 6              | 2                      | 0                 | 8        | 8  |
| Penicillin      | 5              | 0                      | 0                 | 5        | 5  |
| cefoxitin       | 7              | 2                      | 1                 | 10       | 10 |

The commonest anaerobe bacteroids were sensitive to metronidazole, clindamycin, resistant to penicillin, chloramphenicol. *Peptostreptococcus* was sensitive to metronidazole, clindamycin, resistant to penicillin, fusobacterium was sensitive to metronidazole, clindamycin, it was resistant to chloramphenicol, penicillin, cefoxitin.

## DISCUSSION

In present study bacteriological spectrum was analysed in 100 empyema cases. It was observed that empyema was prevalent in 15-75 year age group. We have concluded here that empyema is higher in males 72 (72%) than females 28 (28%) with the ratio of 2.5:1 and the peak was in the range of 26-45 years. In a prospective study<sup>(9)</sup> of empyema thoracis on 40 patients with empyema thoracis, peak age was in the range of 21-40 years, the male-to-female ratio was 3.4:1. Our study has similar distribution of males and females with nearly same ratio.

In ninety (90) cases, *S. pneumoniae* was the commonest bacteria, isolated in 19 cases, followed by *Staphylococcus aureus* isolated in 16 cases, *Klebsiella* in 9 cases, bacteroids in 9 cases, *S.milleri* in 8 cases, *Pseudomonas aeruginosa* in 8 cases, *E.coli* in 8 cases, *Hemophilus* in 4 cases, *peptostreptococcus* in 4 cases, *S.pyogenus* in 3 cases, and *fusobacterium* in 2 cases. It was also observed that out of 100 cases 90% were single bacterial isolates and 10% were double bacterial isolates. In this study the prevalence of gram positive isolates was 46% as compared to 29% of gram negative. The prevalence of anaerobic organisms was 15% and mixed infection was noted in 10% cases. The microbiology of 197 patients whose pleural fluid was culture positive for bacteria was reviewed by Brook and Frazier<sup>(6)</sup>. In 64% of patients, only aerobic bacteria were isolated, whereas in 13% of patients, only anaerobic organisms were isolated and, in 23% of patients, both aerobic and anaerobic organisms were isolated. Alfragemet *al*<sup>(7)</sup> reviewed the microbiology of 82 patients treated for empyema at a respiratory unit in Spain and reported similar results. Out of their 76 patients with positive cultures, 62% had aerobic bacteria, whereas 16% had anaerobic bacteria, 17% had both aerobic and anaerobic organisms, and 5% *Mycobacterium tuberculosis* or fungi. In a analysis

of thirty-seven cases of pleural empyema done by Meyerovitch *al* revealed that *Streptococcus pneumoniae* was the most frequently isolated pathogen (41%), followed by *Staphylococcus aureus* (14%). Our study show similar pattern of aetiological agents.

In present study Gram positives were most sensitive cefoxitin (45%) followed by ceftarolin (42%) and vancomycin (41%). These were mostly resistant towards peniciline (7%), amikacin (14%) followed by ciprofloxacin (15 %). In present study it was analysed that gram negative isolates were most sensitive to meropenem(29%) followed by imepenem (29%), cefipime (24%). Resistance were noted towards getifloxacin (7%), netlimicin (8%), ampicillin (8%). In present study no MDR , XDR , PDR were found,

### Conclusion

Empyema a common complication of parapneumonic effusion, have a major impact on the quality of life of patients with the condition. Bacterial infection in empyema was seen more in the age group of 26-45 years. It was more common in males than females. *S.pneumoniae* (19%) was the commonest isolate followed by *Staphylococcus aureus* (16%), and *klebseila*(9%). Antimicrobials more effective against gram positive bacteria were cefoxitin , ceftarolin and vancomycin. Antimicrobials effective against gram negative bacteria were meropenem, imipenem and cefipime.

Pleural fluid culture is an excellent diagnostic tool to study the aetiology due to bacteria in empyema. Antibio gram helps in the correct treatment protocol for management of empyema. It also helps in screening resistant pathogens and better drug for treatment, thereby helping to decrease the mortality and morbidity in Empyema Thoracis.

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