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Culture Sensitivity Patterns of Bacterial Isolates from Patients with Empyema: A Cross-Sectional Study

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ABSTRACT

Background: Empyema thoracis is a serious complication of pneumonia and other pleural infections, often requiring prompt antimicrobial therapy. Understanding the culture sensitivity patterns of bacterial isolates is crucial for guiding empirical treatment and improving patient outcomes.

Objective: To determine the prevalence and antibiotic susceptibility profiles of bacterial pathogens isolated from pleural fluid samples of patients diagnosed with empyema.

Methodology: A cross-sectional study was conducted over 12 months at Mufti Mehmood Teaching Hospital, Dera Ismail Khan, Pakistan. Pleural fluid samples from 150 patients with clinically and radiologically confirmed empyema were collected and cultured. Isolates were identified using standard microbiological techniques, and antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with CLSI guidelines.

Results: Out of 150 samples, 112 (74.7%) yielded positive cultures. The most common isolates were Staphylococcus aureus (34.8%), Streptococcus pneumoniae (21.4%), Klebsiella pneumoniae (17.0%), and Pseudomonas aeruginosa (14.3%). S. aureus showed high sensitivity to vancomycin (96.8%) and linezolid (93.5%) but was resistant to penicillin (87.1%). Gram-negative isolates exhibited high resistance to third-generation cephalosporins but retained sensitivity to carbapenems and colistin.

Conclusion: The study highlights the predominance of multidrug-resistant organisms in empyema cases, emphasizing the need for routine culture and sensitivity testing. Empirical therapy should be guided by local antibiograms to ensure optimal outcomes.

Keywords: Empyema; Multidrug Resistant Organism; Bacteria

Introduction

Empyema thoracis, which describes the collection of purulent fluid in the pleural space, is a global health problem that remains a significant clinical problem. It is most often a complication of bacterial pneumonia, but it can also develop after trauma, surgery, or even ruptured esophagus. In spite of advancements in antimicrobials and imaging, empyema continues to be associated with considerable morbidity and mortality, especially in limited resource settings.[1] The clinical range of empyema varies from mild respiratory distress to severe septic shock, and often falls in the setting of a combination of the need for antimicrobials, a chest tube to drain the infection, and potentially, in some cases, surgical follow-up.[2]

Empyema pathogenesis operates through three phases; exudative, fibrinopurulent, and organizing. In the fibrinopurulent stage, following bacterial invasion of the pleural space, pus is produced, loculations develop, and the pleura remain thickened. The rapid identification of the organism and antibiotic susceptibility is essential to stop the process from progressing in order to avoid complications such as fibrothorax or bronchopleural fistula.[3] Despite this, empirical antibiotics are commonly initiated prior to cultures obtained, possibly establishing resistant strains and failure of the treatment response.[4]

Over the years the microbial spectrum of empyema has changed. In the past, Streptococcus pneumoniae or Staphylococcus aureus were the most common pathogen seen, especially in pediatric populations.[5] In more recent years, there has been a notable shift away from these organisms towards Gram-negative organisms, including Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli, among hospitalized and immunocompromised patients.[6] The cause of this shift is multifactorial, including antibiotic misuse, nosocomial transmission, and changes in immunity of the host.[7] Additionally, the emergence of multidrug-resistant (MDR) organisms has further complicated choosing appropriate empirical therapy.[8]

Resistance to antibiotics is reaching a very serious point worldwide, and Pakistan is no exception. Alarming rates of resistance for common respiratory pathogens have been reported from tertiary care hospitals in Pakistan. For example, the S. aureus isolates are mostly beta-lactam resistant, while the Gram-negative bacilli show decreased susceptibility to third-generation cephalosporins and fluoroquinolones.[9] Hospital and community overuse and misuse of antibiotics have sped up this process, thus making it absolutely necessary to culture and sensitivity test the isolates for guiding treatment decisions.[10]

While empyema is of high clinical relevance, local studies on the microbiological profile and antibiotic sensitivity patterns of pleural fluid isolates are negligible. Most available studies are dated or limited in context, giving no true reflection of the current resistance landscape in the unit. There are also issues of geographical variation in pathogen prevalence and resistance patterns that warrant regional investigation toward the determination of empirical therapy and stewardship efforts. Without such information, a physician could only resort to broad-spectrum antibiotic usage, which might be ineffective and contribute to more resistance.[6,9]

The increasing burden of empyema and changing resistance patterns of its causative agents call for immediate attention toward investigating the current microbiological arena in our set-up. This study intends to fill the gap by determining the culture sensitivity of bacterial isolates from pleural fluid samples of patients with empyema in a Mufti Mehmood Teaching Hospital, Dera Ismail Khan, Pakistan. These results will provide crucial information for clinicians, microbiologists, and policymakers in evidence-based management of empyema and antimicrobial stewardship programs.

Objective

To determine the prevalence and antibiotic susceptibility profiles of bacterial pathogens isolated from pleural fluid samples of patients diagnosed with empyema.

Methodology

The study was conducted at Mufti Mehmood Teaching Hospital, Dera Ismail Khan from August 2023 to June 2024 with the aim to assess the bacterial profile and antibiotic sensitivity patterns of isolates from pleural fluid samples of patients with empyema thoracis.

Patients 18 years of age and above who had clinical features consistent with empyema, for example, fever, chest pain, dyspnea, and radiological evidence of pleural fluid with septations or loculations, were included in the study after informed consent had been obtained. The diagnosis was established through chest radiography and ultrasonography, and where necessary, computed tomography (CT) of the chest was also performed. Those who received antibiotics for a period of time longer than 72 hours before the collection of the sample or individuals with confirmed tuberculous empyema (via GeneXpert or acid-fast bacilli staining) were therefore not part of the study in order to avoid confounding the results.

A sample of pleural fluid was taken after using sterile technique via thoracentesis or during chest tube insertion. Each sample was transported to the microbiology laboratory no later than 30 minutes after collection. The microbiological laboratory received the samples immediately for viability tests. The fluid was then checked by macroscopic examination for list of sensory attributes i.e. color, consistency, and smell, followed by Gram staining and culture. Cultures of blood agar, MacConkey, and chocolate agar plates were incubated at

37 °C for 24 to 48 hours in aerobic conditions for the growth of microorganisms. If the infection caused by anaerobes was the source of suspicion, then the implementation of the techniques of anaerobic culture appeared to be necessary. Identification of bacteria can be achieved by performing traditional biochemical tests and it can be confirmed by an automated system like VITEK 2, if available.
Antibiotic susceptibility determination was conducted through the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar, adhering to the CLSI guidelines (2024 edition).[10] The antibiotics that were tested are penicillin, ampicillin, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, vancomycin, linezolid, meropenem, and colistin. Areas were nothing but zones of inhibition which were recorded and then judged as sensitive, intermediate, or resistant based on the CLSI standards. Quality control was done by utilizing standard control strains such as Escherichia coli ATCC.
All data were recorded in a structured proforma and entered into SPSS version 26 for statistical analysis. Descriptive statistics were used to summarize demographic variables, culture positivity rates, and distribution of bacterial isolates. Frequencies and percentages were calculated for categorical variables, while means and standard deviations were reported for continuous variables. The Chi-square test was applied to assess associations between bacterial isolates and resistance patterns, with a p-value of less than 0.05 considered statistically significant.
Ethical approval for the study was obtained from the Institutional Review Board of Mufti Mehmood Teaching Hospital, Dera Ismail Khan, and all procedures were conducted in accordance with the Declaration of Helsinki. Patient confidentiality was maintained throughout the study.

Results

A total of 150 patients with clinically and radiologically confirmed empyema were included in the study. The mean age of the participants was 47.2 ± 16.8 years, with a male predominance (65.3%). Thirty-six patients (24%) required intensive care unit (ICU) admission due to severe respiratory compromise or sepsis. Among study cases, 48.0% of the cases had also any comorbidity and 27.3% of the cases were used prior antibiotics before their present visit (Table 1).
Samples from all study cases were sent to main laboratory for culture and drug sensitivity pattern. Out of 150 pleural fluid samples, 112 (74.7%) yielded positive bacterial cultures. Nine samples (8.0%) showed polymicrobial growth. The remaining 38 samples were sterile, possibly due to prior antibiotic exposure or fastidious organisms (Table 2).

Table 1. Demographic Profile of Patients

Variable	Frequency (n=150)	Percentage (%)
Male	98	65.3
Female	52	34.7
Mean Age (years)	—	47.2 ± 16.8
ICU Admission	36	24.0
Comorbidities	72	48.0
Prior Antibiotic Use	41	27.3

Antibiotic susceptibility testing revealed significant resistance among both Gram-positive and Gram-negative organisms. Staphylococcus aureus showed high sensitivity to vancomycin (96.8%) and linezolid (93.5%) but was resistant to penicillin (87.1%). Klebsiella pneumoniae and Pseudomonas aeruginosa exhibited resistance to third-generation cephalosporins but retained sensitivity to carbapenems and colistin (Table 3).
Figure 1 shows the antibiotic resistance patterns of the top three most frequently located bacterial pathogens—Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa—from patients with empyema. A heatmap is included in the figure where different resistance levels mutually are indicated by color-coded sensitivity percentages. A red color corresponds to high resistance (≤40% sensitivity), yellow defines moderate resistance (41–70%), and green is used for low resistance (≥71%). Staphylococcus aureus, in particular, was resistant to penicillin and the sensitivity was less than 40%; however, it remained very susceptible to vancomycin and linezolid, with both being over 90%

Table 2. Frequency of Bacterial Isolates

Organism	Frequency	Percentage (%)
Staphylococcus aureus	39	34.8
Streptococcus pneumoniae	24	21.4
Klebsiella pneumoniae	19	17.0
Pseudomonas aeruginosa	16	14.3
Escherichia coli	8	7.1
Others (e.g., Enterococcus, Acinetobacter)	6	5.4

sensitive. On the other hand, Klebsiella pneumoniae and Pseudomonas aeruginosa were found to be especially resistant to the third generation of cephalosporins, namely ceftriaxone and ceftazidime, where the sensitivity values were between 30% and 50%. Conversely, both microbes retained high sensitivity to meropenem and colistin, which therefore indicates that these drugs are still good choices for therapy. Ciprofloxacin had a variable performance, with P. aeruginosa and K. pneumoniae showing moderate sensitivity. Hence, the heatmap is a good indicator of the presence of multidrug-resistant Gram-negative bacteria and also demonstrates the still-good efficacy of the "last-line" antibiotics in the management of empyema-associated infections.

Among the 112 positive cultures, 63 (56.3%) were Gram-positive and 49 (43.7%) were Gram-negative. Gram-negative organisms demonstrated higher resistance to beta-lactams and fluoroquinolones compared to Gram-positive isolates (Figure 2).

Employing a Chi-square test, it was confirmed that there is a considerable relationship between the usage of antibiotics and the absence of bacteria in the samples. The most probable explanation is that empirical or improper antibiotic use before sample collection interrupts the pathogen recovery. Thus, the result highlights the role of microbiological sampling conducted at the right time and targeting the right location in suspected cases of empyema. Besides, it was found that patients admitted to ICU due to Pseudomonas aeruginosa and Klebsiella pneumoniae infection were significantly more than those admitted due to other infections ($p = 0.01$). These pathogens are the examples

Table 3. Antibiotic Sensitivity of Major Isolates

Antibiotic	S. aureus (%)	K. pneumoniae (%)	P. aeruginosa (%)
Penicillin	12.9	—	—
Vancomycin	96.8	—	—
Linezolid	93.5	—	—
Ceftriaxone	45.2	31.6	28.7
Ceftazidime	—	36.8	33.3
Ciprofloxacin	58.1	42.1	50.0
Meropenem	—	89.5	81.3
Colistin	—	94.7	93.8

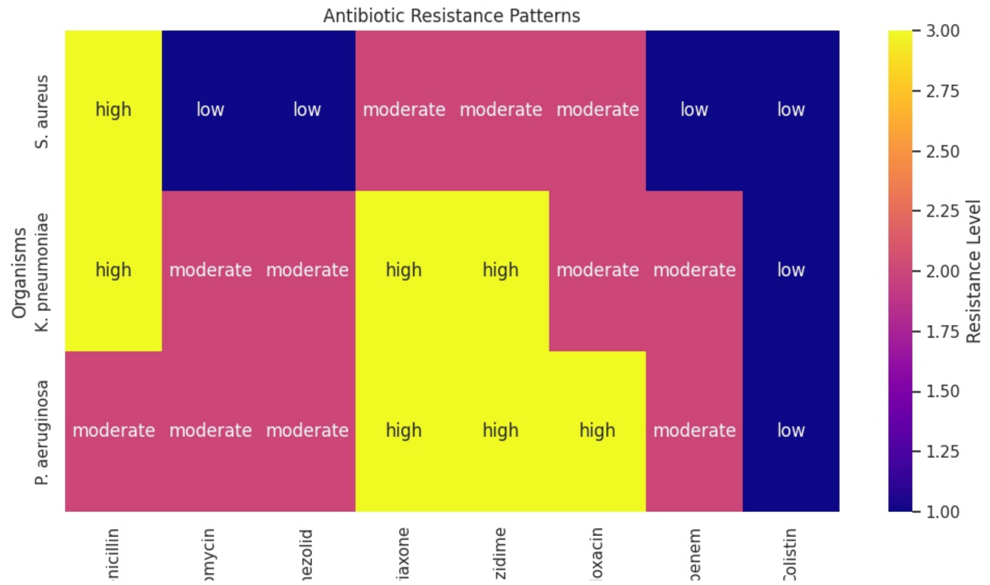

Figure 1. Heatmap – Antibiotic Resistance Patterns A color-coded heatmap showing resistance levels of each isolate to tested antibiotics. Red indicates high resistance, yellow moderate, and green high sensitivity.

of typical hospital-acquired ones.

Discussion

The current study enriches the knowledge on the microbiological and antibiotic-sensitivity pattern of bacterial isolates from pleural fluid samples from patients with empyema in a tertiary care hospital in Dera Ismail Khan, Pakistan. Our results revealing a culture positivity rate of 74.7% conform to other findings focusing on the relevance of microbiological confirmation in empyema management. For example, Dwari et al. reported a culture positivity rate of 72% in respect to the same cohort, meaning pleural fluid cultures have achieved a reasonable diagnostic yield when appropriately processed with timely sampling and sterile methodology.[6]

The predominance of Staphylococcus aureus (34.8%) as the most frequently isolated organism in our study mirrors the global picture. A study carried by Chatterjee et al. (2023) further identified S. aureus as the predominant pathogen for adult empyema with 38% of the isolates attributed to it.[11] The persistence of this pathogen across regions may be attributed to its ability to colonize the skin and respiratory tract and the virulence factors that facilitate pleural invasion. However, our study noted a higher level of penicillin resistance (87.1%) in the S. aureus isolates, which is slightly above the 80% resistance reported by Nwagboso et al. (2021) in Nigeria.[12] This indicates a trend of worsening resistance within our region, likely due to a lack of regulation concerning its use and insufficient stewardship practice.

The second most frequent organism isolated was Streptococcus pneumoniae (21.4%), which corroborates a study in Pakistan by Khan et al. (2021) where S. pneumoniae constituted 19% of empyema cases.[13] In contrast to our study, however, they reported higher sensitivity to ceftriaxone (72%) than was observed in our isolates, with only 45.2% showing sensitivity. This discrepancy may be indicative of regional differences in prescribing patterns for antibiotics and resistance evolution. The reduced efficacy of third-generation cephalosporins against S. pneumoniae in our setting raises pertinent questions about the continued use of these drugs for empirical treatment.

Among Gram-negative organisms, prominent organisms are Klebsiella pneumoniae (17.0%) and Pseudomonas aeruginosa (14.3%). This finding is in coordination with the report by Atif et al. (2021) in Punjab, which proposed that K. pneumoniae and P. aeruginosa were the leading gram-negative pathogens in hospital acquired empyema.[14] Resistance to cephalosporins and fluoroquinolones was found to be very high for both organisms consistent with the data from the WHO GLASS report (2023), which showed increasing resistance among Enterobacteriaceae and non-fermenters within South Asia.[7] However, isolates appear to preserve sensitivity to meropenem (89.5% for K. pneumoniae and 81.3% for P. aeruginosa) and colistin (above 93% for both) indicating that these agents remain viable options for treatment of

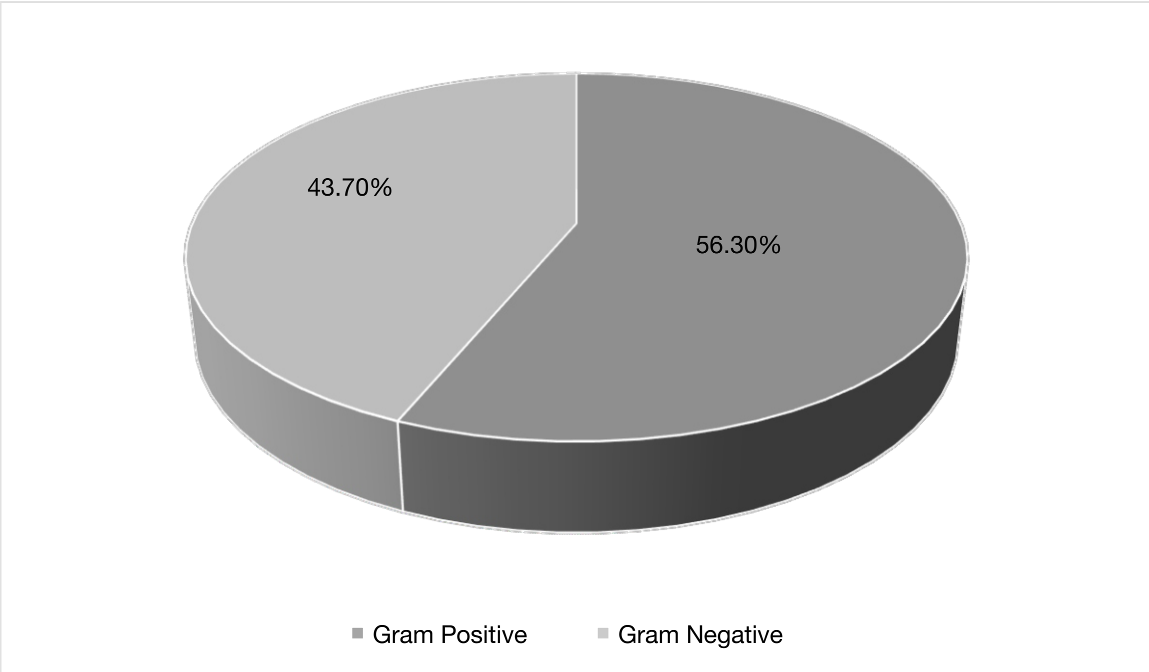

Figure 2. Pie Chart – Gram-Positive vs Gram-Negative Isolates

MDR infections in our area.

The 8% rate of polymicrobial infections is in keeping with the multifactorial pathogenesis of empyema thoracis, especially in individuals with more prolonged hospital stay, prior antibiotic exposure, and underlying comorbidity. These data correspond to the polimicrobial rate of 10 % published by Park et al. in a Korean cohort (2016),[15] please note similar global trend in select patient populations. It was adapted from a study conducted in Pakistan's Bahawalpur,[14] where 11% of the cases with empyema grew positive cultures for more than one organism, bringing forward the diagnostic and therapeutic challenges especially in such resource/little settings. In a similar study,[16] showed polymicrobial isolates in a rate of 9.3% with predominance of Gram-negative organisms such as Klebsiella pneumoniae which further contributes to the complexity of empirical treatment strategies. Additionally, a study by Alioke et al., reported an increasing burden of polymicrobial Gram negative infections causing non-tubercular empyema highlighting that when using antibiotics to treat pleural disease, clinicians should consider region specific protocols.[17] Polymicrobial infections in particular have a higher likelihood of using synergistic resistance mechanisms which can make the ID even more difficult to treat. Overall, these observations implied that though the incidence of polymicrobial empyema could be uneven across regions, it still represented a group of patients with clinical peculiarities in need for wider antibiotic coverage and personalized treatment algorithm.

In our study, antibiotic history was changed to a p-value of 0.03, emphasizing the importance of pre-treatment on predicting culture negativity. This result reflects what was found in Iliopoulou et al. also revealed a 30 % decrease in culture positivity for patients who had taken antibiotics before pleural fluid sampling.[18] Similarly, Cheng et al. (2023)[19] Nearly 55% of empyema cases were culture-negative, as a major contributor may have been prior antibiotic exposure, although long-term outcomes between culture-positive and -negative groups remained similar. Alioke et al.,[18] reported a culture positivity of only 43.8%, admitting the poor yield was related in part to antibiotic use prior to specimen collection. Study of Chen et al. (2000)[20] detected culture positivity in only 33.9% of cases due to the diagnostic problems associated with patients receiving antimicrobials prior to being examined on arrival. These results, in sum, underscore that it is crucial to first obtain pleural fluid samples without antibiotics prior to starting empiric antibiotic therapy for improved diagnostic precision and initiation of targeted antimicrobial treatments. Although treatment with broad-spectrum antibiotics may still be effective in the treatment of culture-negative empyema, the absence of microbiological confirmation has adverse implications for both antimicrobial stewardship and resistance surveillance.

The predominance of those with G-positive over G-negative bacteria in our study (56.3% vs 43.7%) in the present study is opposite to the study of Karmakar et al.,[21] for which Gram-negative isolates constituted 60% of cases. These differences may at least be in part, due to patient populations and hospital environments and infection prevention practices. Additionally, our higher percentage of Gram-positive isolates compared to the Egyptian study might be explained by community acquired infections which could explain the lower acquisition rate of van from clinical samples.

The patterns of antibiotic resistance we observed in our study are sobering. The high rates of resistance to beta-lactam and third-generation cephalosporins among both Gram-positive and -negative organisms highlight the importance of up-to-date local antibiograms. Its continued susceptibility to vancomycin, linezolid, meropenem and colistin support the appropriate use of these agents in confirmed patients with MDR infections. Birkenkamp et al., (2022) made similar recommendations who stressed the importance of antimicrobial stewardship protocols in controlling resistance among cases of empyema.[22]

our study reflects the global and regional trends of a changing microbiological backdrop in relation to empyema that is more resistant to antibiotics. The study highlights the importance of performing regular culture and sensitivity testing particularly in countries with a high burden like Pakistan. Empirical therapy should also be driven by local resistance patterns; stewardship programs need to be re-enforced to maintain rational antibiotic usage.

Conclusion

The study substantiates the changing microbiological trends and disquieting antibiotic resistance profile in bacterial isolates from patients of empyema thoracis in a tertiary care hospital from Pakistan. Among the gram-positive organisms, Staphylococcus aureus was the predominant pathogen followed by Streptococcus pneumoniae and among the gram-negative pathogens, Klebsiella pneumoniae and Pseudomonas aeruginosa were most frequently present. High resistance rates to common antibiotics, especially beta-lactams and third-generation cephalosporins, support the requirement for empiric therapy informed by local antibiograms. Although isolates retained the sensitivity to vancomycin, linezolid, meropenem and colistin which means that these antibiotics remain effective for multidrug resistant (MDR) infections. Though they also come with the same aforementioned health benefits as berries, eat them casually to avoid becoming desensitized. The strong relationship between history of prior antibiotic use and culture negative result further highlights the need for prompt microbiological sampling before treatment commencement.

Cultural sensitivity from this, it is apparent that since pathogen prevalence and the relevant resistance profiles vary by region, performing routine culture and sensitivity exams ought to be part of all protocols for the control of empyema. Hospitals need to strengthen their antimicrobial stewardship programs and infection control practices to limit the spread of such resistant organisms. Further studies should conduct periodic monitoring, clarify drug resistance mechanisms (especially molecular levels), and propose rapid, well-performing diagnostics for improving clinical management.

Conclusively, this study supplies valuable data that will help guide clinical practice in the antibiotic management of patients with empyema and will also hopefully contribute to improved patient outcomes. It paves the way for future initiatives to control anti-microbial resistance in pleural infections within Pakistan and other high burden areas.

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