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ORIGINAL ARTICLE

Bacterial Profile and Antibiotics Susceptibility Pattern of Pleural Effusion Isolates from Sohag University Hospital

[1]Ashraf Khodeary, [2]Tamer Mohamed*, [3]Khaled F. Alkhayat, [1]Sherif A. Sayed
[1]Clinical Pathology Department, Faculty of Medicine, Sohag University, Egypt
[2]Medical Microbiology and Immunology Department, Faculty of Medicine, Sohag University
[3]Chest Department, Faculty of Medicine, Sohag University, Egypt

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Corresponding Author:
Tamer Mohamed Mahmoud
Medical Microbiology and Immunology Department, Faculty of Medicine, Sohag University
Tel.: 01115420226
Tamermmm2000@yahoo.com

ABSTRACT

Background: *Diagnosis of pleural effusion (PE) infection and the isolated organisms reported a significant resistance to the commonly used antibiotics, and the choice of appropriate antibiotics for the management should be guided by microbiological results.* ***Objectives:*** *This study aimed to detect the bacterial profile and antibiotics susceptibility pattern of PE isolates and the prevalence of methicillin-resistant Staph. aureus.* ***Methodology:*** *Plural fluid samples from 121 patients with PE infection were collected for microbiological identification and antibiotic sensitivity testing by Vitek 2 system, Staphylococcal aureus isolates were tested for MecA gene by RT-PCR.* ***Results:*** *47% of PE samples exhibited positive culture, the most encountered organisms were Staph. aureus (24.2%), Klebsiella pneumoniae (16.1%), Staph aureus had the highest sensitivity to Vancomycin and Linezolid (100%) and (40%) of Staph aureus had mecA gene.* ***Conclusion:*** *Bacteria isolated from PE exhibited increasing levels of antibiotic resistance that should be reflected in the choice of empirical antimicrobial treatment.*

INTRODUCTION

Pleural effusion (PE) is one of the most encountered conditions in the medical settings with a significant burden on patients' morbidity and mortality; more than one million persons are affected by PE in the United States each year [1]. This condition is defined as the excessive aggregation of transudate or exudate within the pleural cavity due to pathological conditions [2]. Pleural effusion can occur secondarily to a wide range of causes, the most common of which are congestive heart failure, malignancy, infection, and inflammatory disorders [3].

Patients with PE can present with multiple clinical findings, according to primary causes and amount of fluids; the presentation differs from asymptomatic viral pleuritis to severe dyspnea in case of decompensated heart failure or malignancy [4]. Besides, patients with pleuritis may present with localized pain that is either increasing or decreasing [5].

Pleural effusion can significantly increase the risk of mortality in affected patients; previous reports showed that patients with non-malignant pleural effusion have two-timed increase in the risk of mortality in the first year after the event [6]. Therefore, proper evaluation and prompt management represent the cornerstone for the management of patients with PE. The evaluation of PE is usually based on imaging modalities, thoracocentesis, and/or aspiration with cytological and microbiological examinations [7].

Alongside the management of underlying etiology, the management of PE infection involves chest tube drainage with antibiotics administration; thoracoscopy-based or surgical interventions may be needed in patients not responding to treatment [1]. The choice of antibiotics should be guided by cultural and microbiological results, whenever feasible. Recent guidelines recommend empirical antibiotics for patients with PE and lack of positive culture results [8]. According to these guidelines, the choice of antibiotic regimens should be based on the setting of infection (community versus hospital-acquired) and the pattern of bacterial infections in the local setting [9]. Nonetheless, previous reports indicated that a significant proportion of patients with PE exhibited resistance to commonly used antibiotics [10]. Besides, it is known that the causative organisms in patients with PE are subjected to geographic variations [11].

Unfortunately, data are scarce regarding the bacterial profile and resistance pattern among Egyptian patients with pleural effusion.

The current study aimed to detect the bacterial profile and antibiotics resistance pattern of pleural fluid infection in a tertiary center in Egypt. Also we aimed to detect the prevalence of MRSA organism among bacterial isolates. The results of our study would provide data that aid the clinicians during the choice of empirical antibiotics for pleural effusion patients in Egypt.

METHODOLOGY

Ethical considerations

The protocol of the study was approved by the Ethical Committee of Sohag University Hospital, Egypt. The study objectives and procedures were explained for all eligible patients, or their legal guardians and informed consents were included. We confirm that none of the study's procedures violated the main principles of the Declaration of Helsinki [12].

Study design and patients

This cross-sectional study was conducted in the Central Research Laboratory in collaboration with Chest and Cardiothoracic Departments of Sohag University Hospital. The recruitment period lasted from May 2018 to April 2019, we recruited 121 patients with suspected PE infection and 10 patients with massive PE secondary to decompensated heart failure without symptoms or signs of infection who performed therapeutic thoracentesis as a control group, tuberculous cases and patients with difficulties obtaining their pleural fluid samples for the microbiological examination were excluded.

The clinical data were collected from the patient medical records, 4 ml of venous blood were obtained from each patient and distributed equally into two tubes of anticoagulants; EDTA tube was used to perform a complete blood count (CBC) using the Celtak hematology analyzer (*Nihon Kohden, Japan*) and Sodium citrate tube was used for erythrocyte sedimentation rate (ESR) measurement using the Westergren tube method.

Furthermore, 20 ml of pleural fluid were aspirated for a complete examination, including the physical and chemical characterization, white blood cells (WBCs) count, and other microbiological examinations. Each pleural fluid sample was inoculated in two bottles of BACT/Alert blood culture (*Biomerieux, France*) for aerobic and anaerobic cultures, Gram stain was performed from positive bottles and then sub-cultured on blood, chocolate and MacConkey agar plates, the growing colonies were used for bacterial identification and detection of antibiotics sensitivity by the automated Vitek 2 system (*Biomerieux, France*). Besides, ZN-stained films were performed for the detection of acid-fast bacilli to be excluded.

Staph aureus isolates were examined for the mecA gene for detection of methicillin-resistant *Staph aureus* (MRSA) Prevalence, DNA extraction and MecA gene detection by real-time PCR were done using Genesig®PLEX kit (*Primerdesign™ Ltd*) and StepOne real-time PCR system according to the protocol described by *Geha et al*[13].

Statistical Analysis

The statistical software SPSS, version 24.0 (*IBM Corp., Armonk, NY, USA*) was used for data processing and statistical analysis; variables were summarized by frequency and percentages.

RESULTS

One hundred and twenty one patients with PE were included with ages varied from 32-72 years with a mean of 54±14 years. Most patients were males (64.5%), Concerning the laboratory parameters, the mean hemoglobin, and WBCs count were 11.4 ± 2.1 g/dL and 15.7 ± 7.2cell /mm3, respectively, the mean ESR at first hour was 44 ±21 mm /hour. (Table 1)

Table 1: Demographic and Laboratory Parameters of the study population

Variables		
Age (Years)		54±14
Gender	Males	78 (64.5%)
	Females	43 (35.5%)
Hb Conc (gm/dl)		11.4±2.1
WBCs count (× 109/l)		15.7±7.2
Platelet count (× 103/µl)		188±42
ESR 1st hour (mm/hr)		44±21

A total of 57(47%) PE samples exhibited positive culture from which 31(50%) samples showed gram-positive aerobic bacteria, 27(43.5%) were gram-negative bacteria and 4(6.5%) were anaerobic bacteria, the most commonly encountered organisms were *Staph aureus* (n=15, 24.2%), followed by *Klebsiella pneumonia*e (n=10, 16.1%), *Pseudomonas aeruginosa* and *Pneumococi spp* (n =8, 12.9%), *and Escherichia coli* (n =6, 9.7%). None of the control group PE samples showed positive growth on the BACT/Alert blood culture bottles. (Table 2, Figure 1)

Table 2: The distribution of culture results and causative organisms of PE

Variables		No (%)
PE Samples (No. 121)		
Positive Culture		57 (47)
Negative Culture		64 (53)
Polymicobial Culture		5 (8.8)
Isolated organisms (No. 62)		
Gram Positive Aerobes	*Staphylococcus aureus*	15 (24.2)
	Pneumococci	8 (12.9)
	Streptococcus viridians	5 (8.1)
	Streptococcus pyogenes	2 (3.2)
	CoNS	1 (1.6)
	Total	**31 (50)**
Gram Negative Aerobes	*Klebsiella pneumoniae*	10 (16.1)
	Pseudomonas aeruginosa	8 (12.9)
	Escherichia coli	6 (9.7)
	Acinetobacter spp.,	1 (1.6)
	Proteus mirabilis	1 (1.6)
	Serratia marcescens	1 (1.6)
	Total	**27 (43.5)**
Anaerobes		**4 (6.5)**

Abbreviations: CoNS: Coagulase negative staphylococcus, Data are expressed as number (%)

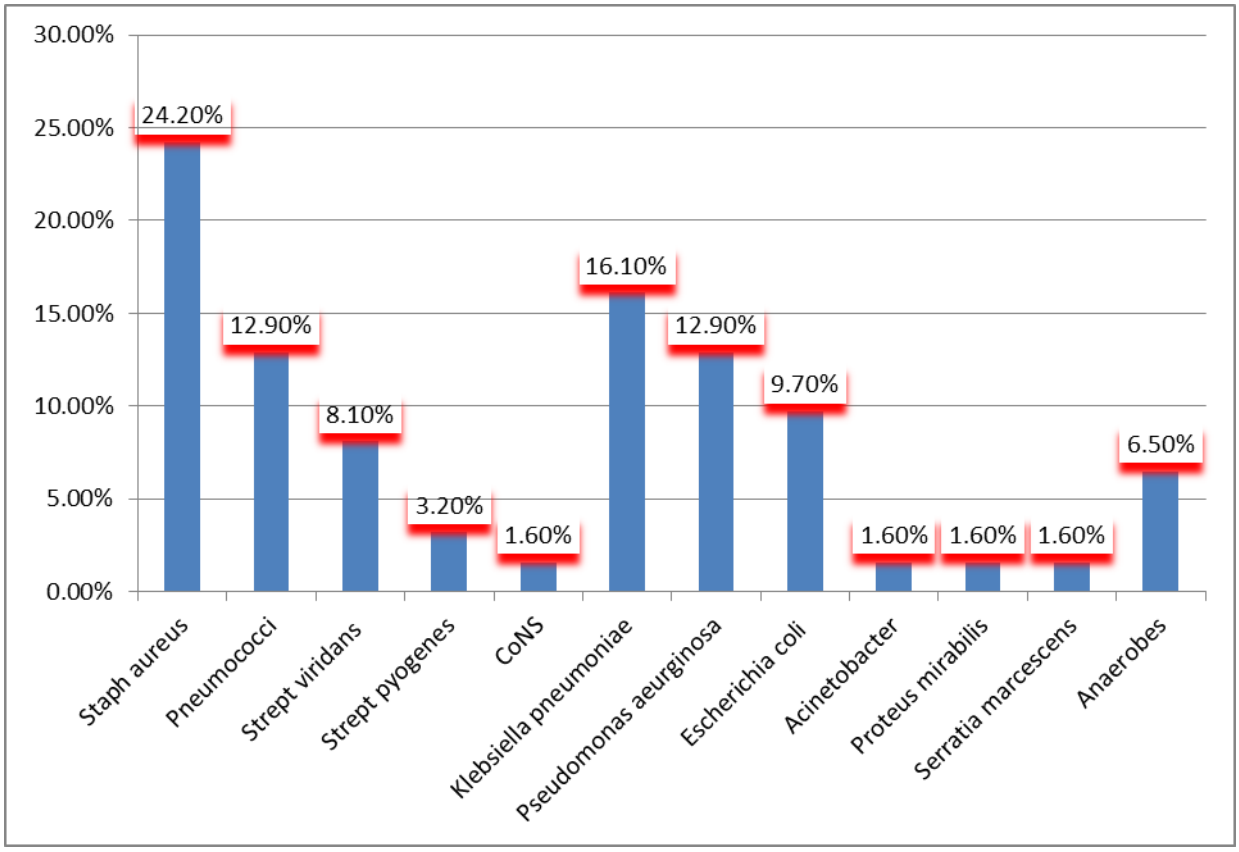

Fig. 1: Distribution of culture results and causative organisms of PE.

Polymicrobial results was found in 5 samples from the 57 positive cultures representing (8.8 %), 3 of them were mixed anaerobes with *Streptococcus viridians* and two were mixed Gram positive and negative aerobes

Out of the 15 *Staph aureus* isolated, mecA gene was detected by real-time PCR in 6 isolates donating 40 % MRSA prevalence rate (Figure 2).

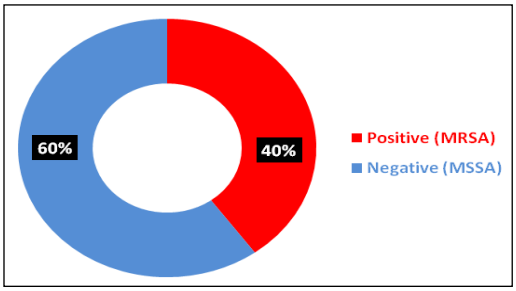

Fig. 2: Frequency of *Staphylococcus aureus* with positive MecA gene (MRSA).

The patterns of antibiotic sensitivity of the most common bacteria isolated from PE samples showed that *Staphylococcus aureus* species had the highest sensitivity for Vancomycin and Linezolid (100%), followed by Oxacillin (60%) and Cefazolin (47%). Also *Klebsiella pneumoniae* had the highest sensitivity for Tigecycline (100%) followed by Meropenem(90%), Levofloxacin and Amikacin (80%). *Pseudomonas aeurginosa* had the highest sensitivity for Tigecycline and Meropenem (100%) followed by Ceftazidime (87%), Piperacillin/Tazobactam, Cefepime, Ciprofloxacin, Levofloxacin and Amikacin (75%). *Pneumococci* had the highest sensitivity for Vancomycin and Ceftriaxone (100%) followed by Cefotaxime (87%) and Amoxycillin/Clavulanic acid (75%) (Table 3 and Figures 3, 4)

Table 3: Antibiotics susceptibility pattern of the most commonly isolated bacteria from PE

Organisms (No)	Antibiotics	Susceptible No (%)
***Staphylococcus aureus* (15)**	Penicillin	0 (0)
	Oxacillin	9 (60)
	Vancomycin	15 (100)
	Linezolid	15 (100)
	Cefazolin	7 (47)
	Clindamycin	6 (40)
***Klebsiella pneumoniae.*, (10)**	Cefazolin	4 (40)
	Cefoxitin	5 (50)
	Ceftazidime	6 (60)
	Ceftriaxone	6 (60)
	Cefepime	7 (70)
	Meropenem	9 (90)
	Ciprofloxacin	7 (70)
	Levofloxacin	8 (80)
	Tigecycline	10 (100)
	Amikacin	8 (80)
	Gentamycin	5 (50)
***Pseudomonas aeurginosa.*, (8)**	Piperacillin/Tazobactam	6 (75)
	Cefazolin	2 (25)
	Cefoxitin	2 (25)
	Ceftazidime	7 (87)
	Ceftriaxone	5 (62)
	Cefepime	6 (75)
	Meropenem	8 (100)
	Ciprofloxacin	6 (75)
	Levofloxacin	6 (75)
	Tigecycline	8 (100)
	Amikacin	6 (75)
	Gentamycin	4 (50)
***Pneumococci* (8)**	Penicillin	5 (62)
	Ampicillin	5 (62)
	Amoxicillin/clavulanic	6 (75)
	Vancomycin	8 (100)
	Ceftriaxone	8 (100)
	Cefotaxim	7 (87)
	Clindamycin	5 (62)
	Erythomycin	5 (62)

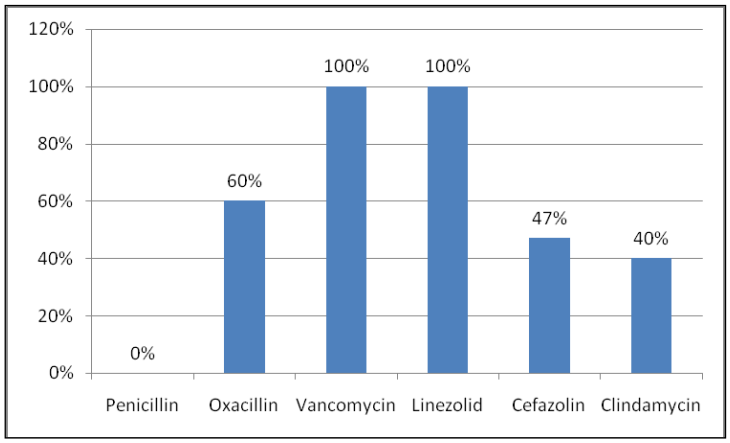

Fig. 3: Antibiotics susceptibility pattern of *Staphylococcus aureus* isolated from PE.

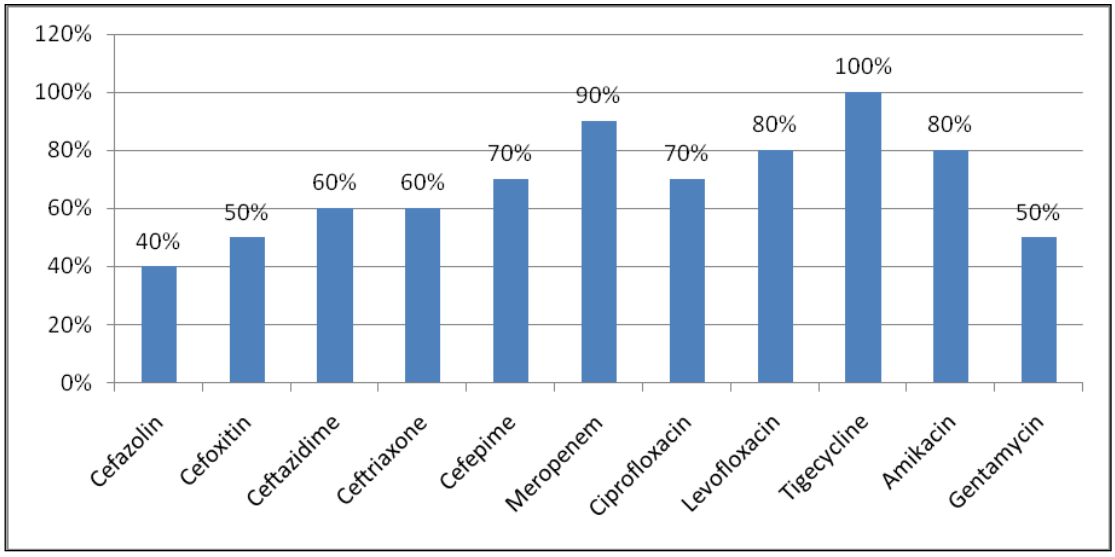

Fig. 4: Antibiotics susceptibility pattern of *Klebsiella pneumoniae* isolated from PE.

DISCUSSION

While it is well established that the healthcare setting and geographical factors play a major role in determining the pattern of bacterial infections in patients with pleural effusion [11], little is known about the bacterial profile and antibiotic resistance patterns among Egyptian patients with PE. We aimed in our study to detect the bacterial profile and resistance pattern of pleural fluid infection in a tertiary center from Egypt. The results of our study may provide data that aid the clinicians during the choice of empirical antibiotics for PE patients in Egypt.

Pleural infection is a common finding in patients with pleural effusion, prompting the initiation of early and aggressive antimicrobial therapy. Thus, the identification of causative organisms is imperative to guide the antibiotic regimen choice and optimize patients' outcomes [14].

Previous reports showed that the bacteriology profile in pleural effusion is diverse and subject to wide geographical variation. It was shown previously that aerobic gram positive organisms accounted for the vast majority of infections in patients with pleural infection [15]. However, the situation appears to be changing in low and middle-income countries, with the emergency of gram negative bacteria as notable causative agents in a wide range of thoracic infections [16]. For example, a previous report from Egypt showed that, among patients with acute exacerbation of COPD, the most commonly encountered organisms were gram-negative bacilli [17], however, in the present study, we found that gram-positive organisms accounted for the vast majority of infections in patients with PE (50%), also (43.5%) were gram-negative and (6.5%) were anaerobic bacteria; with *Staph aureus, klebsiella spp, Pseudomonas spp* and *Pneumococi* accounted for the vast majority of the causative organisms. Our results are similar to the results from a review published by *Hassan et al* [21], who issued data collected from papers published between 2000 and 2018 and reported that; 50.4% of PE isolates were gram positive aerobic organisms, 37.5% were gram negative aerobic organisms and 12.1% were anaerobes.

Also, our findings run in line with a previous report from India in 2020 showing that nearly 63% of the isolates from patients with pleural infection were gram-positive bacteria [18]. On the contrary, another report from India showed that gram-negative Bacilli were present in two-thirds of the samples in patients with PE [16]; also a report from Romania reported that gram-negative Bacilli accounted for nearly half of isolates from patients with PE [19]. The exact cause of such discrepancy between our results and the above-mentioned reports is unclear. However, it may emphasize the influence of healthcare settings and geography on the distribution of causative organisms in patients with PE.

The positivity of microbiological cultures in our study was 47 % and this is in accordance with previous studies that showed positive culture results in 40- 60 % of PE patients [20].

We reported polymicrobial culture positive results in (8.8 %) of our PE samples and this is similar to the results that showed that the median percentage of polymicrobial results from the overall PE cultured samples was (12.9%) [21].

In our study, mecA gene was detected in 40 % of *Staphylococcus aureus* isolates; these results are near to the results reported by *Hassan et al* [21] who reported prevalence of MRSA in cultures from tropics (73%), sub-tropics (39%) and temperate (54%) regions , on Controversy to our results were less than those reported by *Anand et al* [22] who performed PCR for amplification of the mecA gene in Fifty strains of *S. aureus* and

showed that 32 isolates were mecA gene positive with 64% MRSA prevalence rate, and also less than those reported by *Marwa et al* [23] in 2019 (MRSA prevalence was (68%).

To our knowledge, our study is one of the first few reports that shed the light on the bacterial profile and pattern of resistance in patients with PE from Egypt. On the other hand, our study has certain limitations; the study was cross-sectional in nature without a pre-planned follow-up period to determine the patients' response to antibiotic regimens, or the correlation between the bacterial profile and patients' prognosis. The single-center nature of the present study is another limitation, besides our sample size was relatively small compared to other similar studies.

CONCLUSION

Egyptian patients with PE exhibited a bacterial profile like recent reports from other countries with *Staph aureus*, *Klebsiella spp*, *Pseudomonas spp* and *Pneumococci* accounted for the vast majority of the causative organisms. On the other hand, Egyptian patients exhibited increasing levels of resistance to different antibiotics. Also, a considerable proportion of the patients showed infection by MRSA, this emergency of resistant strains calls for national action as it limits available treatment options, compromises patients' outcomes, and increases the burden on the healthcare system.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted

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