The molecular analysis of antibiotic resistance and identification of the aerobic bacteria isolated from pleural fluids obtained from patients

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Abstract. – OBJECTIVE: Pleural effusion is a common clinical condition due to various etiological causes. Infectious pleural effusion can be seen in 20-40% of patients. In this study, follow-up of patients admitted to our hospital and diagnosed with pleural effusion are reported. It was aimed to investigate the prevalence of bacteria isolated from patients with pleural effusion and their antibiotic resistance profiles.

MATERIALS AND METHODS: The pleural fluids obtained from the patients during surgical operations were analyzed microbiologically. Conventional culture, Vitek 2, 16S rRNA, and single Polymerase Chain Reaction (sPCR) were used for microbiological analysis.

RESULTS: Twenty-two (12.2%) bacteria were isolated from 180 patients. The most prominent of them were 16 (8.8%) Klebsiella pneumoniae strains. As for the antibiotic sensitivity, gram-negative bacteria showed the highest sensitivity to colistin, while Gram-positive bacteria showed sensitivity to different antibiotics. In 16S rRNA PCR, 22 samples were found to be positive. In the analysis of antibiotic resistance genes, the OXA-48 gene was determined as the highest.

CONCLUSIONS: In our region, it is essential to perform a microbiological analysis of the sample in patients with pleural effusion. It was thought that revealing both the phenotype and genotype of the antibiotic resistance of the patients was important in terms of treatment. In hospital surveillance, it was considered important to reveal and record the resistance gene profiles of the patients.

Key Words: Pleural effusion, OXA-48, NDM-1, MecA, Antibiotic resistance.

Introduction

The definition of pleural effusion can be given as the pathological accumulation of fluid in the pleural space, and it is a globally prevalent condition. It is estimated that annually around 400,000 to 500,000 people suffer from this medical condition in Germany. It may vary from viral pleuritis to pleuritis secondary, as well as to congestive heart failure and cancer-induced pleural fusion. In addition, each year, approximately half of the four million patients suffering from pneumonia develop a parapneumonic effusion that bacteria can infect secondarily. The one-year mortality of patients with non-malignant pleural effusion ranges from 25% to 57%.

Success in treating a pleural effusion is associated with elucidating its underlying pathology. The identification of the organisms causing this condition is vital for administering the appropriate antimicrobial treatment. Inappropriate utilization and delayed initiation of antibiotic therapy contribute to morbidity. The sensitivity of conventional bacteria has reduced due to the increased complexity of causative agents and the use of empirical antibiotics. Furthermore, the pleural fluid culture becomes difficult to analyze with the use of broad-spectrum antibiotics, which can inhibit the growth of bacteria. A maximum yield of 60% can be attained using inoculation in standard bottles containing blood cultures. Gram staining process may assist in determining the underlying pathogen. The microbiological identification determination of the pathogenic organisms in a non-purulent parapneumonic effusion is successful in generally 25% of cases. The application of 16S-rRNA gene Polymerase Chain Reaction (PCR) has been reported to increase the sensitivity in comparison to the conventional culture analysis techniques.

With recent advancements in scientific processes, various molecular techniques are being applied for the identification of the precise pathogen in diagnostic microbiology. Polymerase Chain Reaction (PCR) based tests have demonstrated the means of providing a microbial detection rate. In a Chinese study, a gram probe...
real-time PCR system containing the 16S rRNA gene was described, which permitted the simultaneous detection and differentiation of clinically relevant gram-positive and gram-negative bacteria directly from the pleural fluid samples. Broad range 16S rRNA gene Polymerase Chain Reaction (PCR) has frequently been utilized in detecting and identifying all the bacterial pathogens in primary sterile clinical specimens mainly when a bacterial infection is suspected, but when cultures are found to be negative. PCR using broad range 16S RNA gene primers have been utilized in investigating the etiology of pleural empyema and offers a detection rate of up to 85% in a single assay, allowing rapid administration of targeted antibiotic therapy. Multiplex PCR methods in matrices (blood, sputum, throat, or nasopharyngeal swabs) have been helpful in the diagnosis of various respiratory tract infections as well as in the reduction of financial costs.

Carbapenemases are considered as a diversified group of β-lactamases as part of the Ambler classes A, B, and D of inactivated carbapenemases. The clinically common plasmid encoded KPC enzymes belong to Class A. The KPC enzymes mainly consist of Klebsiella spp. However, it can also be found in other gram-negative bacteria as well. After its initial detection in the USA in 1996, the KPC manufacturers have become widespread in a global manner within the span of a few years. The Class B carbapenemases include the plasmid gene-encoded NDM-1 carbapenemase. EDTA inhibits these Metallo-β-lactamases (MBL), but not by clavulanic acid. After it was first detected for inhibiting these Metallo-β-lactamases (MBL), but not gene-encoded NDM-1 carbapenemase. EDTA in the Class B carbapenemases include the plasmid gene-encoded KPC enzymes. Oxacillinase test, and gram stain were conducted. The colony morphology of the cultures was assessed. One hundred eighty patients were classified according to age and gender. Pleural fluid from the patients was taken into sterile syringes during the surgical operations and these were stored and transported using a cold chain system. In the postoperative period, pleural fluids were immediately transferred under a cold chain to the Pharmaceutical Microbiology Research Laboratory at the Van Yüzüncü Yıl University, Faculty of Pharmacy. The pleural fluids were inoculated on 5% sheep blood agar (Acumedia, USA), McConkey agar (Oxoid, Hampshire, UK), and Eosin Methylene Blue (EMB, Oxoid, UK) agar. The cultures were incubated at 37°C for a duration of five days. The colony morphology of the cultures was assessed. Several biochemical tests, such as catalase test, oxidase test, and gram stain were conducted. The Vitek 2 Compact (Biomerieux, St. Louis, MO, USA) device was utilized to identify the bacteria and to evaluate the antibiogram tests. Bacteria identified as Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus were stocked at -20°C.

**Materials and Methods**

**Bacteria Isolation, Identification, and Antibiogram Tests**

One hundred eighty patients diagnosed with pleural effusion at Van Yüzüncü Yıl University Research and Training Hospital were evaluated. One hundred eighty patients were classified according to age and gender. Pleural fluid from the patients was taken into sterile syringes during the surgical operations and these were stored and transported using a cold chain system. In the postoperative period, pleural fluids were immediately transferred under a cold chain to the Pharmaceutical Microbiology Research Laboratory at the Van Yüzüncü Yıl University, Faculty of Pharmacy. The pleural fluids were inoculated on 5% sheep blood agar (Acumedia, USA), McConkey agar (Oxoid, Hampshire, UK), and Eosin Methylene Blue (EMB, Oxoid, UK) agar. The cultures were incubated at 37°C for a duration of five days. The colony morphology of the cultures was assessed. Several biochemical tests, such as catalase test, oxidase test, and gram stain were conducted. The Vitek 2 Compact (Biomerieux, St. Louis, MO, USA) device was utilized to identify the bacteria and to evaluate the antibiogram tests. Bacteria identified as Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus were stocked at -20°C.

**16s rRNA Extraction and Amplification**

Approximately 1000 µl of pleural fluid was centrifuged at 14,000 rpm for a duration of 10 minutes. DNAs from the pellet were extracted using the G-SpinTM Total DNA Extraction kit (Intron-Bio, Gyeonggi-do, Korea) according to the manufacturer’s recommendations. Next, the primer fD1 5′-AGA GTT TGA TCC TGG CTC AG-3′ and rP2 5′-ACG GCT ACC GTG TTG TTA CGA CTT-3′ were used, yielding an amplicon of approximately 1500 base pairs. 0.25 µM of each primer, 0.2 µM deoxyribonucleotide triphosphate (dNTP) (Roche Diagnostics GmbH, Penzberg, Germany), 1.5 µM
MgCl₂, 5 µL of 10 X Taq buffer, 1 unit of Taq as the final solution containing 50 µl of nuclease-free H₂O for PCR DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany) was calculated as 5 µL of purified DNA. The cycle conditions were respectively: 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, with a final elongation at 72°C for 10 minutes. Amplicon products were run on a 1.5% agarose gel for 1 hour at 100 V in a Thermo EC300XL2 electrophoresis device. The visualization of the amplicons was achieved by utilizing the Bio-Print-ST4 equipment (Vilber Lourmat, Collégien, France).

**Bacterial Genomic DNA Extraction and Amplification**

The DNA extraction of bacteria was performed in the Pharmaceutical Microbiology Laboratory located at the Faculty of Pharmacy of Van Yüzüncü Yıl University. The bacteria stocked at -20°C were thawed at room temperature. Bacteria were cultivated in Trypton Soy Agara (Acumedia, San Bernardino, CA, USA) and incubated at 37°C for 24 hours. Subsequently, DNAs of multidrug-resistant *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *S. aureus* strains were obtained using the G-Spin™ Total DNA Extraction kit (IntronBio, Korea) protocol. The DNA samples of bacteria were stored at -20°C.

The DNA amplification of the gram-negative bacteria was conducted in the study by Poirel et al.[21] by using 5 µl template DNA as a 50 µl final solution for PCR, with 200 µM each of deoxynucleotide triphosphate (Life Technologies, Carlsbad, CA, USA), 1.5 U Taq DNA polymerase (ABM, Canada), buffer (20 mM Tris-HCL, 50 mM KCL) and 3 mM MgCL₂ (BioTools, South San Francisco, CA, USA) as calculated in the study. PCR conditions were set as 10 min at 94°C, 30 sec at 94°C, 40 sec at 52°C, 50 sec at 72°C, 5 min at 72°C, 40 cycles. The amplicon products of bacteria were run on a 1.5% agarose gel for 1 hour at 100 Volts in a Thermo EC300XL2 electrophoresis device. The visualization was achieved through the use of the Bio-Print-ST4 (Vilber Lourmat, Collégien, France) device. The DNA amplification of isolated and identified bacteria was conducted by utilizing the reference primers given in Table I. The identification of antibiotic resistance for each bacterium was confirmed by the use of a PCR test.

The DNA amplification of gram-positive bacteria was carried out in the study by Sahebnasagh et al.[22] 5 µl template DNA as 50 µl final solution for PCR, 200 µM each deoxynucleotide triphosphate (Life Technologies, Carlsbad, CA, USA), 1.5 U Taq DNA polymerase (ABM, Canada), buffer (20 mM Tris-HCL, 50 mM KCL) and 3 mM MgCl₂ (BioTools, South San Francisco, CA, USA) is set. PCR conditions for the mecA gene; 2 min at 94°C, 20 sec at 94°C, 45 sec at 54°C, 60 sec at 72°C, 5 min at 72°C, 40 cycles. The Amplicon products of bacteria were run on a 1.5% agarose gel for 1 hour at 100 Volts in a Thermo EC300XL2 electrophoresis device. The visualizations of the amplicons were achieved by utilizing the Bio-Print-ST4 (Vilber Lourmat, Collégien, France) equipment. The DNA amplification of the isolated and identified bacterial DNA amplification was conducted by utilizing the reference primer given in Table I. The identification of antibiotic resistance identification of each bacteria strain.

**Ethics Committee Approval**

The on-patient-invasive-application part of the study was conducted as per the approval of the Clinical Research Ethics Committee of Van Yüzüncü Yıl University.

**Table I.** Reference oligonucleotide sequences utilized.

<table>
<thead>
<tr>
<th>Gene Area</th>
<th>Primer Sequence</th>
<th>Base Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaOXA-48</td>
<td>F: 5′-GCYTGTTAAAGGATGAACAC-3′&lt;br&gt;R: 5′-CAITAGGATCAACCCACCG-3′</td>
<td>438bp</td>
<td>Poirel et al[21] (2011)</td>
</tr>
<tr>
<td>blaIMP</td>
<td>F: 5′-GGAATAGACCAGCTCTAATTC-3′&lt;br&gt;R: 5′-GGTTTTGGATCAACCGCCA-3′</td>
<td>232bp</td>
<td>Poirel et al[21] (2011)</td>
</tr>
<tr>
<td>blaNDM-1</td>
<td>F: 5′-GGGTTTGGCAATTCTGTTTCTC-3′&lt;br&gt;R: 5′-CGGAATGGTTCCTACGATC-3′</td>
<td>621bp</td>
<td>Poirel et al[21] (2011)</td>
</tr>
<tr>
<td>blaVIM-1</td>
<td>F: 5′-GATGTTTTGTCGACAT-3′&lt;br&gt;R: 5′-CGAATGCAGCACCAG-3′</td>
<td>390bp</td>
<td>Poirel et al[21] (2011)</td>
</tr>
<tr>
<td>blaKPC-2</td>
<td>F: 5′-CTCCTGTTTCTGCTTCTG-3′&lt;br&gt;R: 5′-CTGTCATCTGTGTTAAGG-3′</td>
<td>798bp</td>
<td>Poirel et al[21] (2011)</td>
</tr>
<tr>
<td>mecA</td>
<td>F: 5′-CAAACTCACCATGTTGCTGCAT-3′&lt;br&gt;R: 5′-GTAGGAATGACTGAAGTCCGATA-3′</td>
<td>310bp</td>
<td>Sahebnasagh et al[22] (2014)</td>
</tr>
</tbody>
</table>
Yüzüncü Yıl University, Faculty of Medicine, dated 20 November 2019, and numbered 2019/08.

Results

Bacterial Isolation, Identification, and Antibiogram Test Results

Twenty-two bacteria were isolated from pleural fluid samples of 180 (90 female and 90 male) patients hospitalized at the intensive care units. Ten (11.1%) and 12 (13.3%) bacteria were isolated respectively. The distribution of isolated bacteria were 1 (0.5%) Staphylococcus aureus, 16 (8.8%) Klebsiella pneumoniae, 4 (2.2%) E. coli and 1 (0.5%) P. aeruginosa. The distribution of multi-drug resistant bacteria according to patient gender and age is given in Table II. K. pneumoniae, E. coli, P. aeruginosa, and S. aureus strains were mainly isolated from male and female patients over 50 years of age. There was no difference in the microbial exposure rates of the pleural fluids in both female and male patients. However, K. pneumoniae invasion was observed only in male patients, while different types of bacteria caused invasion in female patients.

In this study, isolated bacteria were tested for antibiotic resistance (Table II). Fifty percent of the identified K. pneumoniae strains were tested as multi-drug resistant isolates (8; 4.4%). The E. coli (2; 1.1%) strain showed a multi-drug resistance phenotype, P. aeruginosa (1; 0.5%) strain had a multi-drug resistance profile. It was concluded that pleural effusion caused by multi-drug resistant K. pneumoniae, P. aeruginosa, and E. coli could be best treated with colistin. S. aureus (MRSA) strain isolated from a female (72) patient was tested sensitive to different antibiotics.

16s rDNA Extraction and Amplification Results

As a result of the analysis performed with 16s rDNA targeted PCR, 22 (12.2%) positive and 158 (87.8%) negative samples were detected. It was interpreted that all samples with positive PCR had growth in conventional culture.

Bacterial Genomic DNA Extraction and Amplification Results

Eleven bacteria isolated and identified from 180 patients were molecularly analyzed for they showed phenotypically multi-drug resistance. According to the phenotypic antibiotic resistance of gram-negative bacteria, eight K. pneumoniae, one E. coli, and one P. aeruginosa strains showing carbapenem and expanded β-lactam resistance were included in the study, and their bla OXA-48, \( \text{bla KPC-2} \), \( \text{bla VIM-1} \), \( \text{bla IMP} \) and \( \text{bla NDM-1} \) gene carrier status were evaluated. It was found that eight K. pneumoniae isolates from gram-negative bacteria were carriers of \( \text{bla OXA-48} \). It was observed that only one E. coli isolate carrier of the \( \text{bla NDM-1} \) gene. Gram-negative bacteria causing pleural effusion did not carry the \( \text{bla IMP} \), \( \text{bla KPC-2} \), and \( \text{bla VIM-1} \) genes.

Discussion

Pleural infection often occurs following the translocation of bacteria from the infected lung to the pleural cavity in patients with pneumoniae. Pleural infections due to community-acquired bacteria may differ from nosocomial infections in mortality. In addition, approximately 50% of patients with community-acquired pneumoniae may develop parapneumonic fusion, and 10% of these effusions may become infected. The drainage of infected pleural fluid and administration of appropriate antibiotics affect the success in managing pleural infection. Misdiagnosis, delay in drainage, chest tube malposition, and inappropriate antibiotic use play an essential role in the treatment failure. The low sensitivity (40-60%) of cultures makes it challenging to have culture results at diagnosis and to have microbiological guidance in most cases. Therefore, it is essential to define the bacteriology of pleural infection, which also shows significant geographical differences with the source of the infection (community or hospital origin).

Among the gram-positive cocci with a high clinical isolation rate, Staphylococcus aureus causes skin and soft tissue infections, as well as lung infections, by producing mainly three types of toxins (Superantigens, Pore-forming toxins, Exfoliative toxins) to lyse host cells, and thus, lead to a weakened response. The virulence factors (toxins or biofilms) of gram-negative bacteria such as Escherichia Coli, Acinetobacter baumanii, and K. pneumoniae may escape bacterial adhesion, invasion, and the host’s immune defense, destroying the host cells or tissues. Hence, infections such as lung infections, circulatory system infections, abdominal infections, as well as urinary tract infections may develop due to these factors. The geographical location of pleural infection was closely related to the common patho-
genic bacteria, and *S. aureus* was the most dominant species in subtropical regions\(^{32}\). In the study by Chen et al\(^ {41}\), *S. aureus* was detected in all the pleural effusion samples and *hospital-acquired-S. aureus strains* were determined to be the more common species. In contrast, *Streptococcus pneumoniae* was the most common pathogen among 261 community-acquired pleural effusion patients in Spain\(^ {33}\).

In one study, it was reported that streptococci (55%), staphylococci (17.1%), gram negatives (10.1%), and anaerobes (8.2%) constituted the bacterial profile of patients with community-acquired pleural effusion\(^ {34}\). With the increasing prevalence of gram-negative bacteria, particularly *Klebsiella* spp., among the community-acquired pleural fusion patients in Taiwan, it was emphasized that it is important to consider regional differences in the bacteriology of the disease when deciding on the selection of the right antibiotic\(^ {26}\). In a study conducted in Romania, *P. aeruginosa* (29.31%) was the most isolated bacteria in patients with pleural fusion\(^ {35}\). In our study, twenty-two bacteria were isolated from the pleural fluid samples of 180 (90 female and 90 male) patients hospitalized at the intensive care units. Ten (11.1%) and 12 (13.3%) bacteria were isolated respectively. The distribution of the isolated bacteria was 1 (0.5%) *Staphylococcus aureus*, 16 (8.8%) *Klebsiella pneumoniae*, 4 (2.2%) *E. coli*, and 1 (0.5%) *P. aeruginosa*.

<table>
<thead>
<tr>
<th>No</th>
<th>Gender</th>
<th>Age</th>
<th>Bacteria</th>
<th>Genes</th>
<th>Antibiotic Sensitivity (MIC, S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>72</td>
<td><em>S. aureus</em></td>
<td>MecA</td>
<td>Gentamicin, Ciprofloxacin, Levofloxacin, Moxifloxacin, Linezolid, Q/D, Vancomycin, Tigecycline, Nitrofurantoin</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>84</td>
<td><em>K. pneumoniae</em></td>
<td>OXA-48</td>
<td>Colistin, SXT</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>75</td>
<td><em>K. pneumoniae</em></td>
<td>OXA-48</td>
<td>Tigecycline, Colistin</td>
</tr>
</tbody>
</table>
| 4  | Female | 61  | *P. aeruginosa* | -     | Amikacin,
Gentamicin, Colistin, Tigecycline |
| 5  | Female | 53  | *E. coli*     | NDM-1 | Gentamicin, Colistin, Tigecycline, SXT |
| 6  | Male   | 62  | *K. pneumoniae* | OXA-48 | Colistin, SXT |
| 7  | Male   | 60  | *K. pneumoniae* | OXA-48 | Colistin, SXT |
| 8  | Male   | 69  | *K. pneumoniae* | OXA-48 | Amikacin, Gentamicin, Colistin, Tigecycline, SXT |
| 9  | Male   | 54  | *K. pneumoniae* | OXA-48 | Amikacin, Gentamicin, Colistin |
| 10 | Male   | 69  | *K. pneumoniae* | OXA-48 | Piperacillin/Tazobactam, Cefoxitin, Amikacin, Ciprofloxacin, Tigecycline, Colistin |
| 11 | Male   | 77  | *K. pneumoniae* | OXA-48 | Colistin, SXT |
The distribution of multi-drug resistant bacteria according to patient gender and age has been provided in Table II. *K. pneumoniae* was the top isolated and identified bacteria from patients with pleural effusion. Although the male patients had mainly *K. pneumoniae*, different strains were found to cause infection in female patients. In addition, the susceptibility to infection was high in both male and female patients over 50 years of age. It was predicted that geographical difference, gender, and age might be effective in the presence and distribution of the causative agent in pleural effusion infections.

The 16S rRNA PCR method has been proven to be reliable alternative to conventional culture methods. This process has been used to diagnose infections such as infective endocarditis, meningitis, keratitis, bacteremia, and brain abscess. It is more sensitive and specific at diagnosing infections than the conventional culture method. The negative aspects of this method are that it does not provide information about etiological agents, only one reaction is made, and it gives false results due to PCR contamination. In our study, 22 samples were found to be positive. The conventional cultures were also observed to be positive besides all these positive samples. False positives were not observed in our PCR results. Therefore, we concluded that the sensitivity and specificity of the 16S rRNA PCR method which we applied were 100%.

Microbial infections have increased in the last decade, and antibacterial medications are widely utilized as the first-line therapy in order to prevent bacterial infections. This has resulted in specific drugs and multi-drug resistance among various bacterial strains. Furthermore, multi-drug resistance may occur in gram-negative and gram-positive bacteria through hydrolyase production, efflux pump overexpression, or membrane pore protein mutation. Multi-drug resistance, also considered as a public health threat, has increased globally. The World Health Organization (WHO) published the first global antibiotic resistance report in 2014 based on the data from 114 countries worldwide and announced the problem of antibiotic resistance as a global concern. In 2020, WHO started an event themed “Unite to Protect Antimicrobial Medicines” to raise awareness of the drug resistance crisis among the public and healthcare professionals through the comprehensive promotion of the appropriate utilization of antimicrobial drugs. Antimicrobial resistance has significant outcomes for the physicians as well as the patients, as it increases the risk of treatment failure, prolongs hospital stay, and leads to extra costs related to healthcare. Multidrug-resistant gram-negative bacterial infections, which are the leading causes of nosocomial infections worldwide, are described as one of the greatest threats to global health. Therefore, it is crucial to monitor the distribution of clinical isolates and changes in drug resistance. Streptococci are the most commonly seen pathogens of community-acquired pleural infection, followed by anaerobes and staphylococci. Penicillin + β-lactamase inhibitor combination is selected for community-acquired pleural infections. It has been observed that the rate of drug-resistant gram-negative bacteria and staphylococci is higher in hospital-acquired pleural infections. Also, the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) may reach 25%. Therefore, vancomycin, linezolid, carbapenem, and third generation cephalosporins may be used appropriately in the upfront setting. In a study by Chubar et al., the prevalence of MRSA in patients with pleural fusion was reported to be 11.49%. Another study from Romania described that MRSA was isolated at 4.31% in patients with pleural fusion. Ciobotaro et al. reported that they isolated carbapenem-resistant *K. pneumoniae* from a patient with pleural fusion and had a positive KPC-3 profile. A Turkish study reported that carbapenem-resistant *E. coli* and *K. pneumoniae* were isolated from two patients with bilateral pleural effusion and the agents were OXA-48 positive. In our study, 1(0.5%) MRSA strain was isolated from a female patient with pleural effusion, and the mecA gene was found to be positive. In addition, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* strains showed carbapenem and extended-spectrum beta-lactam resistance. It was also observed that OXA-48 gene positivity was high in *K. pneumoniae* strains, while NDM-1 gene positivity was found in *E. coli* strains. Although the *P. aeruginosa* isolates showed multi-drug resistance, they did not contain the analyzed genes. While it was observed that bacteria and their genes could change antibiotic resistance, it was predicted that it would have a significant role in the success of the planning of the treatment protocols.

**Conclusions**

In conclusion, given the fact that male patients were exposed to bacterial disease higher than female patients, and that individuals with pleu-
ral effusion were exposed to Oxa-48 positive *K. pneumoniae* at a higher rate in our institution, it is vital to identify the bacterial profiles and antibiotic resistance characteristics of patients diagnosed with pleural effusion in a hospital.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Ethics Approval

This retrospective study was conducted in accordance with the ethical principles of the declaration of Helsinki and approved by Van Yüzüncü Yıl University Clinical Research Ethics Committee (20 November 2019, and numbered 2019/08).

### Informed Consent

With the approval of the ethics committee, patient consent forms were applied.

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### Authors’ Contribution

The study concept and design, data acquisition, data analysis and interpretation, and writing of manuscript were prepared by Ömer AKGÜL.

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### Data Availability

The data supporting this article are available from the corresponding author on reasonable request.

### References


The molecular analysis of antibiotic resistance and identification of the aerobic bacteria


