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EVALUATION OF THE BIOFIRE FILMARRAY PNEUMONIA PANEL FOR LOWER RESPIRATORY TRACT SPECIMENS IN TERTIARY CARE MEDICAL CENTRE

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Abstract

Lower respiratory tract infections are an important cause of morbidity and mortality. Identification of infectious etiology is very important for specific treatment. In addition to routine methods in diagnosis, polymerase chain reaction-based tests have been used. By identifying pathogenic organisms and specific antibiotic resistance markers earlier than routine methods, these tests have the potential to accurately detect agent and reduce the duration of antibiotic therapy. The aim of this study is to show that the use of multiplex polymerase chain reaction-based method may be useful in early diagnosis and specific treatment.

In this study, 283 lower respiratory tract samples sent to Ondokuz Mayıs University Faculty of Medicine Hospital Molecular Microbiology Laboratory between 01/08/2021–01/04/2023 were retrospectively analyzed. Lower respiratory tract samples were studied with BioFire® FilmArray Pneumonia Panel plus (BioMérieux, France). The lower respiratory tract samples of the patients sent at the same time were planted on 5% sheep blood agar, chocolate and EMB agar and incubated at 36 °C for 24 h. Identification of bacteria was performed by Vitek MS. Antibiotic sensitivities were studied in Vitek2 Compact automated systems.

Two hundred and eighty-three patient samples were included in the study; 156 (55%) of the samples were bronchoalveolar lavage samples and 127 (45%)

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are sputum samples. When panel and culture results were compared, compatible results were found in 70%. In 88 (61%) of the patients with no growth in culture, no agent was detected as a result of the panel or only viral agents were detected. MecA/C and MREJ resistance genes were detected in 26% of 46 patients with *S. aureus* detected in the pneumonia panel. CTX-M resistance gene was detected in a total of 28 samples in the pneumonia panel. The OXA-48 carbapenem resistance gene was detected in 14 samples.

In our study, it has been shown that Multiplex-PCR has an important place in detecting atypical bacterial and viral factors that do not grow in routine culture in lower respiratory tract infections. In addition, by identifying the resistance genes in the detected bacterial agents, it will contribute to the correct initiation of empirical treatment and to prevent unnecessary antibiotic use in lower respiratory tract diseases where viral factors play a role.

Key words: pneumonia, multiplex PCR, respiratory pathogen

Introduction. Lower respiratory tract infections (LRTIs), especially hospital-acquired pneumonia (HAP), community-acquired pneumonia (CAP) and ventilator-associated pneumonia (VAP), are an important cause of morbidity and mortality [1]. Bacterial, viral or fungal agents may cause these infections depending on the patient's exposure and clinical risk factors. Especially among bacterial agents, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacteriaceae* members are frequently detected and are generally multidrug resistant [2]. Identification of the infectious etiology has been associated with a significant reduction in mortality and morbidity as it enables effective treatment [3]. Patients with viral pneumonia can be treated differently from patients with bacterial infections, but due to the similarities in clinical presentation, it is not possible to distinguish viral infections from bacterial infections without laboratory diagnostic tests [4]. Molecular diagnostic tests, including polymerase chain reaction-based (PCR) tests, can identify pathogenic organisms or antibiotic resistance indicators in less time than routine methods, reducing the duration of empirical antibiotic therapy [5]. Since the respiratory tract is not sterile, it is very difficult to distinguish between colonization and disease-causing pathogens [6]. Recent advances in molecular diagnostic methods have led to an increase in diagnostic panels targeting atypical bacterial agents and viruses [7]. In addition to epidemiologic profiles of respiratory pathogens, rapid molecular diagnostic tools with antimicrobial susceptibility models need to be developed [8]. The aim of this study was to show that the use of PCR techniques in addition to conventional methods in pneumonia cases may be useful in early diagnosis. In addition, it was aimed to prevent unnecessary or incorrect antibiotic use by differentiating bacterial from viral agents with PCR techniques and to contribute to the decrease in the development of resistance to these antibiotics.

Materials and methods. In this study, 283 lower respiratory tract samples sent to Ondokuz Mayıs University Faculty of Medicine Hospital Molecular Micro-

biology Laboratory between 01/08/2021–01/04/2023 were retrospectively evaluated. Cultures of sputum or bronchoalveolar lavage samples of these patients were also included in the study. Lower respiratory tract samples sent to our laboratory were analyzed with BioFire® FilmArray Pneumonia Panel plus (BFPP, BioMérieux) (BioMérieux, France) according to the manufacturer's recommendations. This is a multiplex PCR assay that can simultaneously detect 27 pneumonia pathogens and seven antibiotic resistance genes. Panel lists 15 typical bacteria (*Acinetobacter calcoaceticus/baumannii complex*, *Escherichia coli*, *Enterobacter cloacae complex*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Proteus spp.*, *S. aureus*, *Serratia marcescens*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*), three atypical bacteria (*Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*), seven viruses (*Coronavirus*, *Rhinovirus/Enterovirus*, *Metapneumovirus*, *Influenza A*, *Parainfluenza*, respiratory syncytial virus (RSV)) and seven antimicrobial resistance genes (mecA/C and MREJ causing methicillin resistance, carbapenemases (KPC, NDM, OXA-48, VIM, IMP) and extended spectrum beta lactamase (ESBL-CTX-M). Simultaneously sent lower respiratory tract samples of the patients were inoculated on 5% sheep blood agar, chocolate agar and EMB agar for culture and incubated at 36 °C for 24 h. Bacteria were identified by Vitek MS (Biomeriux, France). Antibiotic susceptibilities were studied on Vitek2 Compakt (Biomeriux, France) automated systems. Multiplex PCR was performed with BioFire ® FilmArray Pneumonia Panel plus (BioMérieux, France) for confirmation in four isolates that were carbapenemase detected but phenotypically carbapenem susceptible. PCR was performed in an automatic thermal cycler (Eppendorf, Hamburg, Germany) for the detection of resistance genes. The presence of KPC, NDM, OXA-48, VIM, IMP genes was investigated using specific primers.

Results. The study included 283 patient samples from which the respiratory tract panel was studied. Of the samples obtained from these patients, 156 (55%) were bronchoalveolar lavage (BAL) samples and 127 (45%) were sputum samples. Of the patients included in the study, 178 (63%) were male and 105 (37%) were female. Of the 283 patients studied with the pneumonia panel, at least one respiratory agent was detected in 194 (69%) and no agent was detected in 89 samples (31%). The most common bacterial agents detected in the samples of the patients investigated by the panel were *S. aureus*, *P. aeruginosa*, *A. calcoaceticus/baumannii complex*, *K. pneumoniae* and *E. coli*. Pneumonia panel and culture results for bacteria are given in Fig. 1.

Regarding viral agents, *Rhinovirus/Enterovirus* was detected in 36 (13%) of the samples, *Parainfluenza virus* in 15 (5.3%), *Coronavirus* in 14 (5%), *Influenza A* in 11 (3.9%), *Metapneumovirus* in 10 (3.5%), RSV in 10 (3.5%) and *Adenovirus* in 4 (1.4%). Lower respiratory tract cultures were requested simultaneously in 236 of the 283 samples investigated in the pneumonia panel. Forty-seven sam-

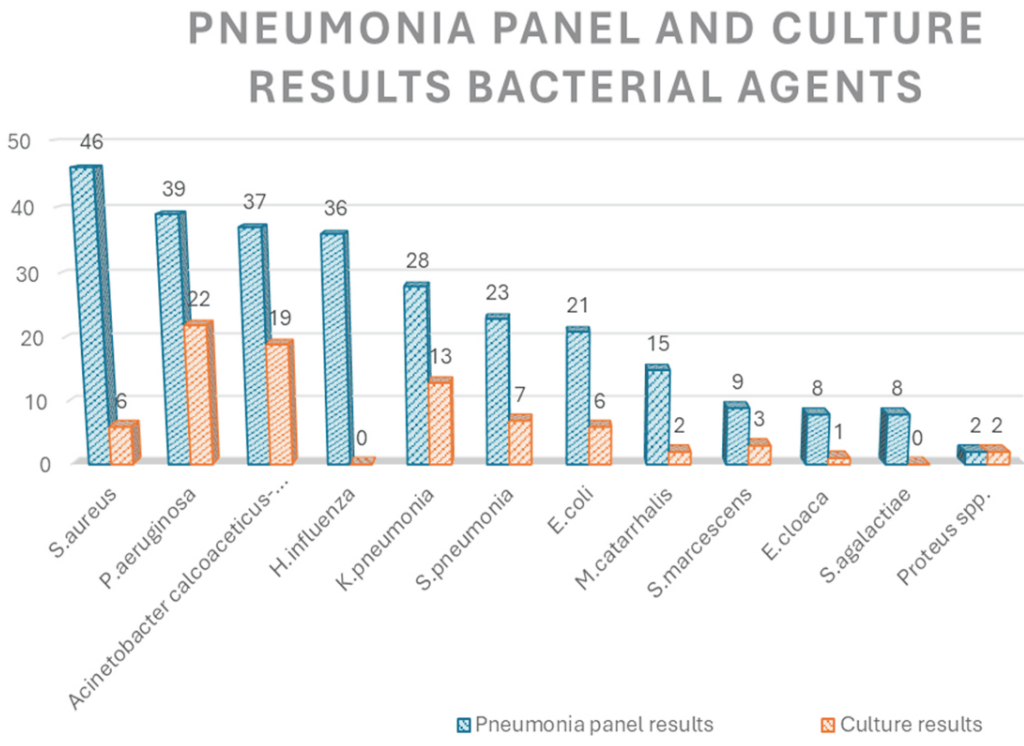

Fig. 1. Pneumonia panel and culture results bacterial agents

ples were not cultured at the same time. Therefore, 236 samples were evaluated together with culture. When samples with panel and culture results were compared, compatible results were found in 70%. In 61% (88) of the patients who did not grow in culture, no causative agent or only viral agents were detected as a result of the panel. In 4 of the samples, there was growth in culture. Three of these bacteria were not included in the panel (*Achromobacter denitrificans*, *Corynebacterium striatum*, *Stenotrophomonas maltophilia*) and one was included in the panel (*A. baumannii*). Accordingly, compatible results were obtained in 27 of 28 samples in which only viral agents were detected. In the pneumonia panel, mecA/C and MREJ resistance genes were detected in 12 (26%) of 46 patients with *S. aureus*. *S. aureus* grew in one of the samples in which the resistance gene was detected and was identified as methicillin-resistant *S. aureus* (MRSA) according to the antibiotic susceptibility result. Of the 34 samples in which *S. aureus* was detected in the pneumonia panel and no mecA/C and MREJ resistance genes were detected, 5 grew *S. aureus* in culture. According to the antibiogram results, 4 were identified as methicillin-sensitive *S. aureus* (MSSA) and 1 as MRSA. In the pneumonia panel, CTX-M resistance gene was detected in a total of 28 samples. *K. pneumoniae* was detected in 14 (50%) of the samples with CTX-M resistance gene. *K. pneumoniae* grew in culture in 7 (50%) of the samples in which *K. pneu-*

moniae was detected and 6 (86%) of them were ESBL positive according to the antibiogram result. Of the 28 samples in which CTX-M resistance gene was detected, 12 (43%) were identified as *E. coli* by panel and 6 (50%) of them grew *E. coli* in culture. Of these, 4 (67%) were ESBL positive according to the antibiogram results. OXA-48 carbapenem resistance gene was detected in a total of 14 samples with the pneumonia panel. *K. pneumoniae* was grown in 6 (43%) of the samples in which OXA-48 gene was detected. Three (50%) of the *K. pneumoniae* isolates were resistant to carbapenems. *E. coli* was grown in 1 (7%) of the samples with OXA-48 resistance gene and was found carbapenem sensitive according to the antibiogram result. *K. pneumoniae*, *A. calcoaceticus/baumannii complex*, as well as OXA-48, NDM and KPC resistance genes were detected in the culture of one sample. No carbapenemase gene was detected in the isolates reported as OXA-48 positive but found to be carbapenem susceptible by multiplex PCR.

Discussion. The etiologic diagnosis of lower respiratory tract infections is a challenging part of microbiology due to the variety of potential pathogens and the non-sterile environment of the respiratory tract [6]. Current microbiologic techniques used to identify pathogens causative agents and resistance mechanisms are slow. PCR-based diagnostic methods such as the pneumonia panel offer the potential to rapidly identify organisms and resistance genes directly from a clinical sample without the need for culture [9]. This method has been approved by the FDA for the identification of approximately thirty respiratory targets within one hour [10]. The ability of multiplex PCR-based tests to detect viral agents, especially those that are difficult to diagnose, is crucial for optimal patient care [11]. In a study, it was shown that molecular tests may positively affect treatment decisions in the etiologic diagnosis of pneumonia [12]. In a similar study, it was reported that the pneumonia panel provided early information about the disease by detecting pathogens and resistance genes, gave compatible results in 70–90% for the most common bacteria causing pneumonia, and allowed a pathogen-directed antibiotic treatment [13]. In our study, we compared the results obtained by pneumonia panel and culture. We found that *S. aureus* was the most common causative agent. However, *S. aureus* was grown in a certain proportion of the cultures of these patients. In a study conducted on this subject, *S. aureus* was frequently detected in nasopharyngeal samples and it was stated that molecular test results should definitely be evaluated together with clinical findings considering the possibility of nasal colonization before the diagnosis of respiratory tract infection [14]. On the other hand, molecular detection of genetic markers associated with antibiotic resistance, such as carbapenemases, ESBL and mecA, has been associated with positive outcomes, including reduced time of antibiotic treatment, shorter intensive care unit stay and reduced mortality [15]. For carbapenemases and ESBLs, although there were results compatible with culture results especially for *E. coli* and *K. pneumoniae* in our study, incompatible results were also found when the resistance gene was detected and examined with culture and antibiogram.

According to the results of the panel, carbapenemase resistance genes were detected in isolates that were phenotypically susceptible and multiplex PCR was performed using specific primers and carbapenemase resistance genes were not detected. Although molecular detection of carbapenemase-encoding genes gives important results, carbapenem resistance in bacteria such as *Enterobacteriaceae*, *P. aeruginosa*, *Acinetobacter* may be accompanied by resistance mechanisms other than carbapenemases (e.g. efflux pumps or alteration in outer membrane porins). Since there are many resistance mechanisms, failure to detect the resistance gene does not necessarily indicate susceptibility to the relevant antibiotic. These problems are major shortcomings of all molecular diagnostic tests that target specific genetic markers to estimate phenotypic sensitivity [16]. Sparks et al. [17] found that PCR-based systems were highly sensitive in detecting viruses. In their study, Özer-Türk et al. [18] stated that rapid results obtained with molecular tests, high test sensitivity and the ability to detect microorganisms that cannot be detected by conventional methods help both the early initiation of appropriate antimicrobial treatment and cost savings. In addition, the identification of viral agents that cannot be detected in routine tests will reduce unnecessary or incorrect antibiotic use, side effect rate and resistance development. In most of the results we obtained with the panel, more than one bacterial agent could be detected in the same sample, but only one agent was grown in culture. Another result was that *S. Aureus*, *H. influenzae*, *S. pneumonia*, *M. catarrhalis*, *S. agalactiae*, *Rhinovirus/Enterovirus* were detected at a high rate with the panel, but the growth rates in culture were very low. Therefore, as we mentioned in the findings section, we think that molecular test results should be evaluated together with clinical findings and culture results, taking into account the possibility of nasal colonization in respiratory specimens before the diagnosis is made. Our evaluation shows that the pneumonia panel seems promising to improve the etiologic diagnosis of lower respiratory tract infections by offering a good agreement with standard methods. It has the potential to support clinical decision-making with a significantly faster turnaround time than routinely used methods.

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