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GENOTYPE AND PHENOTYPE IDENTIFICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: NOVEL DISCOVERIES FROM PNEUMONIA CASE SERIES

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ABSTRACT

Background: *Staphylococcus spp. identification results showed a difference between phenotypic and genotypic methods.* ***Objectives:*** *To observe the phenotype and genotype of MRSA in patients with pneumonia.* ***Materials and methods:*** *We reviewed 10 patients with methicillin-resistant Staphylococcus aureus (MRSA) isolated in lower respiratory tract samples. Samples were investigated with the VITEK system and real-time PCR methods with the MRSA Quant Real-TM kit (Sacace™ Biotechnologies, Italy).* ***Results:*** *The results recognized 10 cases of MRSA infections from culture. Still, from these samples, real-time PCR detected 4 MRSA, 5 MRCoNS (methicillin-resistant Coagulase-negative Staphylococci), 1 MRCoNS or MRCoNS, and MRSA. Interestingly, levels of MICs were different between the MRSA and MRCoNS genotypes.* ***Conclusion:*** *Our results showed that the MRSA phenotype method was incompatible with the genotype method. The existence of MRCoNS in these cases can be evidence that MRCoNS in lower respiratory tract samples should also be considered causative agents in pneumonia.*

Keywords: *Methicillin-resistant Staphylococcus aureus, Coagulase-negative Staphylococci, real-time PCR.*

I. INTRODUCTION

Staphylococcus spp. are the predominant causative organisms associated with infections in both humans and animals [1], [2]. *Staphylococcus aureus* is a leading cause of bacteremia, infective endocarditis, osteoarticular infections, skin and soft tissue infections, pleuropulmonary infections, pneumonia, and device-related infections [1]. Coagulase-negative *Staphylococci* (CoNS) are typical opportunistic pathogens and represent one of the major causes of nosocomial infections [3–5]. The isolation and identification of *Staphylococcus* species present several challenges. Rapid and accurate methods for identifying methicillin-resistant *Staphylococcus aureus* (MRSA) are often expensive [6]. Manual or automated phenotypic methods can be unreliable, as they rely on the expression of metabolic activity and morphological characteristics [7].

This study aims to observe the difference between the genotype and phenotype of MRSA in lower respiratory tract samples and to gain a deeper understanding of the role of *Staphylococcus spp.* in pneumonia

II. MATERIALS AND METHODS

2.1. Materials

This study was conducted at Cho Ray Hospital from 2021 to 2023. We observed the phenotype and genotype of MRSA infection from patients with pneumonia. A definite diagnosis of MRSA pneumonia was based on the isolation results of MRSA in sputum culture.

- **Inclusion criteria:** Patients with pneumonia who had lower respiratory tract specimens collected for both culture and real-time PCR analysis.

- **Exclusion criteria:** Patients without available lower respiratory tract specimens.

2.2. Methods

- **Study design:** A cross-sectional descriptive study.

Lower respiratory tract specimens, including sputum, bronchial aspirates, and bronchoalveolar lavage fluid, were collected. Specimen quality was assessed in the laboratory; samples that were salivary or watery were excluded. A sputum sample was considered of acceptable quality if it contained more than 25 polymorphonuclear cells and fewer than 10 squamous epithelial cells per low-power field (10x objective) [8].

All specimens were processed in a biosafety level 2 clinical microbiology laboratory following protocols approved by the Ministry of Health. Bacterial cultures were performed and phenotypic identification was carried out using standard bench methods. Antimicrobial susceptibility testing was conducted using the VITEK-2 automated system. For molecular analysis, automated DNA extraction, PCR amplification, and real-time hybridization were performed using the MRSA Quant Real-TM kit (Sacace™ Biotechnologies, Italy).

III. RESULTS

All sputum samples collected during the study period demonstrated bacterial loads exceeding 10^5 CFU/mL by culture and 10^5 copies/mL by real-time PCR. The results revealed discrepancies between phenotypic and genotypic methods. Although all 10 patients were confirmed to have MRSA infection by sputum culture, real-time PCR detected the ***mecA*** gene in all samples but identified different Staphylococcal species: 4 MRSA, 5 MRCoNS (methicillin-resistant coagulase-negative Staphylococci), and 1 case with either MRCoNS alone or a combination of MRCoNS and MRSA. (Table 1). In the antibiogram, all samples were resistant to cefoxitin, benzylpenicillin, oxacillin, and erythromycin but were sensitive to vancomycin, daptomycin, and linezolid (Table 2).

Table 1. Minimum Inhibitory Concentration (MIC) values (µg/mL) of daptomycin, linezolid, teicoplanin, and vancomycin in 10 clinical isolates identified by real-time PCR

Case	PCR result	Daptomycin (µg/mL)	Linezolide (µg/mL)	Teicoplanin (µg/mL)	Vancomycin (µg/mL)
1	MRSA	1	0.5	16	0.5
2	MRSA	2	0.5	16	0.5
3	MRSA	1	0.5	16	0.5
4	MRSA	1	0.5	16	0.5
5	MRCoNS	2	1.0	1	0.5
6	MRCoNS	1	0.5	1	0,5
7	MRCoNS	1	0.5	16	0.5
8	MRCoNS	2	0.5	16	0.5
9	MRCoNS	2	0.5	1	0.5

Case	PCR result	Daptomycin (µg/mL)	Linezolide (µg/mL)	Teicoplanin (µg/mL)	Vancomycin (µg/mL)
10	MRCoNS/ MRCoNS and MRSA	2	0.5	16	0.5

Table 1 showed that MRCoNS genotypes had higher MICs for daptomycin. There was no significant difference in MIC levels of vancomycin and linezolid between genotype types.

Table 2. Antimicrobial susceptibility of methicillin-resistant Staphylococcal isolates by case

Case	1	2	3	4	5	6	7	8	9	10
Cefoxitin	+	+	+	+	+	+	+	+	+	+
β-lactams (Benzylpenicillin, Oxacillin)	R/R	R/R	R/R	R/R	R/R	R/R	R/R	R/R	R/R	R/R
Aminoglycoside (Gentamicin)	I	R	R	S	R	R	I	I	R	R
Fluoroquinolone (Ciprofloxacin)	R	R	S	S	R	R	R	R	R	R
MLSB (D-test)	-	-	-	-	-	-	-	-	-	-
Macrolide (Erythromycin)	R	R	R	R	R	R	R	R	R	R
Clindamycin	S	S	S	S	S	S	S	S	S	S
Daptomycin	S	S	S	S	S	S	S	S	S	S
Vancomycin	S	S	S	S	S	S	S	S	S	S
Linezolid	S	S	S	S	S	S	S	S	S	S
Teicoplanin	R	R	R	R	S	S	R	R	S	R

Table 2 presents the antibiotic resistance patterns. Samples that were phenotypically MRSA but genotypically MRCoNS showed higher resistance to ciprofloxacin and gentamicin. In contrast, samples that were both phenotypically and genotypically MRSA exhibited higher resistance to teicoplanin.

IV. DISCUSSION

Staphylococcus spp. identification differed between phenotypic and genotypic methods. Our findings (Table 1) are consistent with those of Ashraf A, who reported 16 *S. aureus* isolates; however, only 10 cases showed the *S. aureus* genotype, while 6 cases revealed CoNS [9]. In such cases, relying solely on manual identification methods led to the misidentification of CoNS as *S. aureus*, whereas PCR was able to accurately identify and distinguish both typical and atypical *S. aureus* from other *Staphylococcus* species [10]. Therefore, PCR is considered the gold standard for determining *Staphylococcus* species. However, the accuracy of PCR-based identification may vary across studies depending on the target genes and primer design of the diagnostic kits used [10].

CoNS are also significant pathogens in nosocomial pneumonia. Although they are among the most common constituents of normal skin flora, CoNS are increasingly recognized as causative agents of clinically significant infections, including bacteremia and endocarditis [2], [3]. Recent studies have highlighted the role of CoNS as pathogens in nosocomial pneumonia [11], [12]. In June 2020, Michał Michalik *et al.* demonstrated that CoNS were frequently responsible for laryngological diseases [11]. In April 2021, Ricarda Michels *et al.* suggested that CoNS are causative pathogens rather than mere contaminants.

Furthermore, infections caused by CoNS often require second-line antimicrobial therapy [12]. Notably, in January 2025, the CDC updated the definitions of ventilator-associated and non-ventilator-associated pneumonia to include CoNS species as organisms that can meet the diagnostic criteria for pneumonia [5].

Several studies have shown that *Staphylococcus spp.* is increasingly resistant to antibiotics; however, these strains generally remain susceptible to vancomycin and linezolid [13]. Our findings indicated no significant difference between phenotypic and genotypic methods in detecting oxacillin resistance in *Staphylococcus spp.* [2]. Additionally, *Staphylococcus spp.* isolates tended to show resistance to erythromycin, gentamicin, and ciprofloxacin, while all clinical samples remained susceptible to vancomycin, daptomycin, and linezolid. These antibiotics may, therefore, be essential therapeutic options for *Staphylococcus* infections.

This study has several limitations. Our diagnostic kit was unable to determine specific CoNS subspecies, such as *Staphylococcus epidermidis* or *Staphylococcus haemolyticus*. Additionally, the prevalence of MRSA in sputum samples from patients with hospital-acquired pneumonia was relatively low, ranging from 5.17% to 13% [14,15], and some patients did not produce sputum or provided insufficient quantity for both culture and PCR testing. As a result, only 10 suitable samples were included to compare genotypic and phenotypic characteristics and assess antimicrobial susceptibility.

Despite these limitations, our findings underscore the discrepancies between phenotypic and genotypic identification of MRSA. Moreover, they provide evidence supporting the consideration of CoNS as potential pathogens in sputum samples from patients with pneumonia. The study also offers insight into the antibiotic resistance patterns of *Staphylococcus spp.* based on genotypic profiles.

V. CONCLUSION

Staphylococcus spp. phenotype results showed incompatible with genotype. PCR would be more effective to identify and distinguish CoNS and *S. aureus*. CoNS should be considered causative agents rather than contaminants in pneumonia**.**

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