The Emergence of Linezolid-resistant Staphylococcus Epidermidis in the COVID-19 Hospitalized Intubated Patients in North Khorasan, Iran

Amir Azimian (amir_azimian2003@yahoo.com)
North Khorasan University of Medical Sciences
Mahsa Khosrojerdi
North Khorasan University of Medical Sciences
Hamed GhasemZadeh-Moghadam
North Khorasan University of Medical Sciences
Hasan NamdarAhmad-Abad
North Khorasan University of Medical Sciences
Seyed Ahmad Hashemi
North Khorasan University of Medical Sciences

Research

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Abstract

Background

In the COVID-19 pandemic from 2019 to date, we confront secondary bacterial and viral infections in SARS-CoV2 infected patients, especially hospitalized patients. Coagulase-negative staphylococci, are commensals of the human body and can lead to infections in immunocompromised patients. The antimicrobial resistance is increasingly reported in coagulase-negative staphylococci, especially in *Staphylococcus epidermidis*. One of the most critical problems is resistance to linezolid in *S. epidermidis*, observed in Europe since 2014. The aim of this study was to evaluation of bacterial Co-infections and determination of antimicrobial resistance pattern of co-infection isolated strains in North Khorasan, Iran, in the last six-month period.

Methods

After microbiological evaluation of pulmonary samples of hospitalized intubated patients with signs of bacterial pneumonia, we found co-infection in 11 of 185 patients with *S. epidermidis, S. aureus,* and *Acinetobacter baumani,* respectively. Interestingly seven of nine *S. epidermidis* isolates were linezolid resistant. For identification of the isolates at the species level, we used phenotypic methods and also the Polymerase Chain Reaction (PCR) for *the atlE* gene. Selected isolates were characterized by determining their antimicrobial resistance patterns and using molecular methods including SCCmec typing, detection of *ica, mecA, vanA,* and *cfr* genes.

Results

All isolates were resistant to methicillin, and Seven isolates were resistant to linezolid. It should be noted that all nine isolates were positive for *the ica* gene. Nine of 11 isolated have belonged to the SCCmec I, and two belonged to the SCCmec IV. It should be noted that all patients had the underlying disease and six patients died.

Conclusion

The increasing linezolid resistance in bacterial strains becomes a real threat for patients, and monitoring such infections combined with surveillance and infection prevention programs is very important to decrease the number of linezolid-resistant staphylococcal strains.

Introduction

Microbial co-infections are one of the significant causes of increasing mortality and morbidity among patients with respiratory tract viral infections such as COVID-19. Its rate is variable between 2–65%. Respiratory viruses such as Influenza virus, Adenovirus, Respiratory Syncytial Virus, Parainfluenza Virus, Human metapneumovirus, and Bocavirus are the most common viruses that account for viral co-infections[1, 2] and between bacteria, staphylococcus, and streptococcus spp. and some gram-negative bacteria account for these co-infections [3, 4]. Coagulase-negative staphylococci (CoNS) colonize mucosal membranes and human skin, so, at the most times, they were categorized as inoffensive commensals [5], however, to date, they are increasingly isolated as related agents to hospital-acquired infections (HAIs), such as bloodstream, urinary tract and pulmonary infections [6]. Among CoNS related to humans, *Staphylococcus epidermidis* is the most frequent species [6]. Antimicrobial resistance in *S. epidermidis* increasingly reported from Europe [7–10]. Linezolid, a member of oxazolidynones, has been added to the treatment regimen of various skin and pulmonary communities and Hospital related gram-positive infections Since 2000 [11], however unfortunately, shortly after the entrance into the clinical use, the first case of linezolid-resistant *Staphylococcus aureus* was reported in the United States in 2001 [12], after that Linezolid resistance in *S. epidermidis* (LRSE) is increasingly reported in many countries, such as France, Germany, Ireland, Italy, Greece, and Portugal [7]. Resistance to linezolid can be acquired by mutations during prolonged linezolid therapy or horizontal gene transfer [13–16]. The G-T mutation in the 23S rRNA gene is the primary determinant of the linezolid resistance. It should be noted that other mutations in the *rpl* gene family are frequently observed in LRSE [17–20].

The *cfr* is the major transmissible gene that encodes ribosomal methyl transferase, conferring linezolid resistance in staphylococci. *optrA* and *poxtA* genes of ribosomal protection proteins also role in linezolid resistance [21–24]. Of these, only *cfr* has been reported in LRSE [25]. Resistance to linezolid and methicillin are commonly combined, therefore SCCmec is critical in these isolates [26, 27]. Regarding the importance of secondary viral and bacterial infections in an increase of mortality and hospitalization time of COVID-19 patients, we previously evaluated and reported viral co-infections in COVID-19 patients and this work evaluate bacterial co-infections in these patients. Here we report the emergence of LRSE in the nasal tract of COVID-19 hospitalized patients in North Khorasan, Iran.

Material And Methods

Sample collection.

In this study, we evaluated the Intensive Care Unit (ICU) admitted SARS-CoV-2 positive patients in the six months, between 2021 February 19 to 2021 August 23, in North Khorasan Hospitals. Inclusion criteria to our study were Hospitalization, SARS-Cov-2 positive reverse transcriptase Real Time PCR (rt-PCR) result, intubation, and mechanical ventilation for more than 48 hours in ICUs. One hundred eighty-five patients entered to study with these criteria. Of these, 11 patients had signs of bacterial pneumonia, including new persistent or progressive infiltration in chest radiography, body temperature more than 38 or less than 36 degrees of centigrade, WBC count more than 10^4 or less than 5×10^3 cells/ml, tracheal secretions, or decrease of O2 saturation. It should be noted that all 11 patients had a history of cough, sore throat, and distress in physical examination and neutropenia, elevated Estimated Sedimentation Rate (ESR), and C- Reactive Protein (CRP) in laboratory blood analysis.

Microbiological identification.

At the first step, respiratory samples were evaluated using conventional microbiological methods. We also evaluated the nasal swab culture of these patients. After primary culture and growth of gram-positive cocci in all samples, biochemical tests including catalase, coagulase, susceptibility to polymyxin B and novobiocin, acetoin production, ornithine decarboxylase test, PYR test, and fermentation of mannitol, glucose, sucrose, maltose, mannose, and trehalose were performed on all isolates [28, 29].

Antimicrobial Susceptibility Test.

The antibiogram test was performed using cefoxitin (CEF), penicillin (PEN), tetracycline (TET), erythromycin (ERY), levofloxacin (LEV), moxifloxacin (MOX), clindamycin (CLN), gentamicin (GEN), trimethoprim/ sulfamethoxazole (COT), minocycline (MIN), rifampicin (RIF), linezolid (LIN) and vancomycin (VAN) antimicrobial disks using Kirby Bauer method (MAST DISKSTM, UK) based on CLSI guidelines [30]. Methicillin resistance was initially identified using cefoxitin

disks (30 µg). Minimum Inhibitory Concentration (MIC) values for linezolid and vancomycin were determined using the E-test method (Biomeriux). We used the *Staphylococcus aureus* ATCC 29213 as a control.

Genomic DNA extraction.

Genomic DNAs of Coagulase Negative Staphylococci (*CoNS)* isolates were extracted using the Genet Bio Genomic DNA extraction Kit (KR-2000). According to the manufacturer's protocol for staphylococcus spp., we added lysostaphin at a final concentration of 20 µg/ml in the lysis buffer.

PCR detection of atlE, mecA, vanA, and cfr **genes.**

For identification of the *S. epidermidis* isolates at the species level, we used phenotypic methods and also the PCR for *the atlE* gene [31]. We also performed PCR to evaluate the presence of *ica*, *mecA, vanA, and cfr* genes as previously described [32–34].

SCC mec **typing.**

SCC*mec* typing was performed as previously described [35].

Statistical Analysis

The results were transferred to a Microsoft Excel spreadsheet for analysis. Statistical analysis was performed using the SPSS/16.0 software. Similarities or differences were evaluated using an ANOVA test. The P-values of$\leq$0.05 were considered statistically significant.

Results

Between 2021 February 19 to 2021 August 23, 185 Hospitalized intubated patients were evaluated in North Khorasan Province. Of these, 11 cases had bacterial pneumonia. In nine samples, *S. epidermidis* and the remaining two samples *S. aureus* and *Acinetobacter baumani* had been isolated. All *S. epidermidis* isolates were resistant to methicillin, and seven isolates were resistant to linezolid [Table 1]. The most frequent SCCmec types were I (77.8%) and IV (22.2%), respectively. It should be noted that all linezolid resistant isolates were

resistant to cefoxitin and belonged to SCCmec type I. All isolates were positive for the *ica* gene [Table 2]. The complete details of nine *S. epidermidis* positive patients are presented in Table 3.

Table 1
Results of antibiotic susceptibility test

PEN (n/%)	ERY (n/%)	CEF (n/%)	TET (n/%)	CLN (n/%)	LEV (n/%)	MOX (n/%)	COT (n/%)	GEN (n/%)	RIF (n/%)	MIN (n/%)	VAN (n/%)	LIN (n/%)
8(88.9)	5(55.5)	5(55.5)	4(44.4)	4(44.4)	5(55.5)	4(44.4)	4(44.4)	4(44.4)	5(55.5)	2(22.2)	0	7(77.7)

(PEN) penicillin, (ERY) erythromycin, (CEF) cefoxitin, (TET) tetracycline, (CLN) clindamycin, (LEV) levofloxacin, (MOX) moxifloxacin, (COT) trimethoprim/ sulfamethoxazole, (GEN) gentamicin, (RIF) rifampicin, (MIN) minocycline, (VAN) vancomycin, (LIN) linezolid.

Table 2
distribution of resistance and biofilm formation genes in *S. epidermidis* isolates

	mecA (n/%)	*vanA* (n/%)	*cfr* (n/%)	*ica* (n/%)
female	4 (44.5%)	0	2 (28.6%)	4 (44.5%)
male	5 (55.5%)	0	5 (71.4%)	5 (55.5%)
total	9	0	7	9

Table 3
complete details of *S. epidermidis* positive patients

patient	gender	Resistance gene	SCCmec type	*ica* gene	Blood culture	Nasal swab for *S. epidermidis*	Underlying disease	Survived/died
1	F	*Cfr, mecA*	*I*	+	Positive/ *S. epidermidis*	Positive	diabetes	died
2	F	*mecA*	*IV*	+	Positive/ *S. epidermidis*	Positive	Diabetes/ Hypertension	survived
3	M	*Cfr, mecA*	*I*	+	Negative	Positive	diabetes	died
4	M	*Cfr, mecA*	*I*	+	Positive/ *S. epidermidis*	Positive	diabetes	died
5	M	*Cfr, mecA*	*I*	+	Negative	Positive	diabetes	survived
6	F	*mecA*	*IV*	+	Negative	Positive	hypertension	survived
7	M	*mecA*, *cfr*	*I*	+	Positive/ *S. epidermidis*	Positive	diabetes	died
8	F	*Cfr, mecA*	*I*	+	Negative	Positive	Heart disease	died
9	M	*Cfr, mecA*	*I*	+	Positive/ *S. epidermidis*	Positive	diabetes	died

Conclusion

The COVID-19 pandemic started in 2019 in Wuhan, China. To date, this disease has spread in many countries and has resulted in millions of cases of infection and deaths around the world. The SARS-CoV-2 virus has numerous mutations and has caused several peaks of the disease around the world intermittently. To date, in addition to the COVID-19 disease, co-infections with various viruses, bacteria and fungal agents occurred, which leads to the difficulty of treatment of this disease and worsens the condition of patients, and ultimately increases their mortality. In the last work, we evaluated viral co-infections in COVID-19 patients [1], and in this work evaluated the bacterial co-infections in these patients. It should be noted that there are two types of bacterial infections associated with Covid-19; Concomitant infections with the viral infection and subsequent infection. Co-infection was reported in 3.5% of patients and secondary infection in 14.3% of patients with COVID-19 [36]. In the other study, the authors found that the highest prevalence of bacterial co-infection occurred in ICU submitted patients [37]. Despite the low prevalence of bacterial co-infections, more than 70% of patients had received broad-spectrum antibiotics such as cephalosporin and fluoroquinolones [36]. Bacterial infections are an important factor in

increased morbidity and mortality in viral respiratory infections and require precise and timely diagnosis and treatment. Sharifipour et al. reported that the most common bacteria in co-infections in COVID-19 patients were *Acinetobacter baumani* and *staphylococcus aureus* [38]. however, the most commonly found bacterial co-pathogens in other studies on respiratory viral infections were *Staphylococcus aureus* and *Streptococcus pneumoniae* [39, 40]. Unlike other studies, in the current study, we found *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Acinetobacter baumani* as the most frequent bacteria, respectively, in COVID-19 intubated patients, hospitalized in the Intensive Care Unit.

The members of staphylococcacea are one of the most frequent colonizing bacteria in the skin and mucus membranes of the human body and act as opportunistic pathogens. Of these families, *Staphylococcus aureus* is the most pathogenic bacteria, and other members such as *Staphylococcus epidermidis* and *Staphylococcus saprophiticus* have the lower pathogenicity. *S. aureus* is one of the significant colonizing bacteria in the nasal tract and *s. epidermidis* is one of the major skin colonizing bacteria related to external medical devices in the human body, such as catheters, tubes, etc. These bacteria are resistant to many antibacterial agents. Linezolid is an effective treatment for multidrug-resistant gram-positive bacteria, and despite its general use for almost 20 years, it still exhibits excellent activity against staphylococci. Linezolid resistance among *S. epidermidis* remains uncommon worldwide, but the increasing resistance in European countries such as Greece, Spain, Portugal, Italy, France, and Ireland has been reported [41]. Linezolid resistance is rare in Iran Most previously found cases of resistance were related to *Staphylococcus aureus*, and there is no report of resistance to these antibiotics in *S. epidermidis* [42, 43]. The high linezolid resistance rate in our *S. epidermidis* isolates may be due to the exact origin of the strains. We need to perform MLST on our isolates to prove this. Another important finding in our study was the presence of biofilm formation-related gene (*ica*) in all isolates. *S. epidermidis* usually related to medical devices, and all of our patients were intubated and under mechanical ventilation. The presence of this bacteria in the nasal and pulmonary tract of patients may be related to these devices, and it seems that we need more attention to possible infections with biofilm-forming bacteria. However, to prove this relationship, sampling of the mentioned devices and molecular typing of possible isolated strains should be done. All isolates were resistant to methicillin, and our isolates belonged to SCCmec I and IV. Commonly in methicillin-resistant staphylococci, SCCmec types I- III are related to deep and severe infections, and IV-V are related to mild and superficial infections [44]. In our study, four of six dead patients had simultaneous septicemia, and isolates have belonged to SCCmec I while two SCCmec IV strains were isolated from survived patients. It may be related to the severity of the infection and its connection with the SCCmec type. The growth of *Staphylococcus epidermidis* in specimens is usually considered a contagion unless its role is clinically proven. In our study, the simultaneous presence of the *S. epidermidis* in the Nose, pulmonary tract, and bloodstream, and hematology and serology and clinical findings of patients deny the possibility of contamination. All of our patients had underlying diseases such as diabetes, hypertension, and heart disease. Five of six dead patients had diabetes. Diabetes can weaken the immune system and create the conditions for exacerbating the complications of Covid-19 and increasing the susceptibility to other microbes [45, 46]. To prove the relationship between underlying disease and risk of co-infection, we need to evaluate more patients. In conclusion, our findings show that we need more attention to bacterial co-infections especially, antibiotic-resistant bacteria. Due to the effect of COVID-19 and its related treatments, bacteria, including normal residents of skin and mucus membranes, can lead to co-infections. The emergence of antibiotic-resistant strains is a warning issue and should be considered in selecting experimental treatment regimens.

Abbreviations

PCR; Polymerase Chain Reaction.

LRSE; Linezolid resistance *Staphylococcus epidermidis.*

ICU; Intensive Care Unit.

rt-PCR; reverse transcriptase Real Time PCR.

ESR; Estimated Sedimentation Rate.

CRP; C- Reactive Protein.

MIC; Minimum Inhibitory Concentration.

CoNS; Coagulase Negative Staphylococci.

Declarations

- **Ethical Approval and Consent to participate**: Ethical clearance was granted by the North Khorasan University of Medical Sciences ethical board (IR.NKUMS.REC.1399.020).

- Consent for publication: The authors have agreed to publish the article.

- **Availability of supporting data**: If required by the journal, all information with details is ready to be provided.

- **Competing interests**: “The authors declare that they have no conflict of interests”.

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- **Authors' contributions**: Amir Azimian; conceptualized and designed the study, conducted the final revision of the manuscript, carried out analyses. Mahsa Khosrojerdi; collected data and interpret the clinical signs. Hamed Ghasemzadeh-Moghaddam; carried out analyses, Hasan Namdar-Ahmadabad; wrote the initial draft of the manuscript. Seyed Ahmad Hashemi; collected data.

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