

Emergence of *S. Lugdunensis* in Burn Wound Infection of Hospitalized Patients

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ABSTRACT

Introduction: Although Coagulase Negative Staphylococci (CoNS) were previously considered to be harmless bacteria, some species have recently been shown to be potential pathogens in humans. One of these species, which has emerged in nosocomial infections, is *Staphylococcus lugdunensis*. Given the importance of recognizing new infections in hospital settings and their prevention, the present study aimed to investigate the presence of *S. lugdunensis* in patients with burn injuries.

Materials and Methods: In this study, 124 CoNS isolates were evaluated in the patients admitted in a burn injury center in the southwest of Iran during January 2016-May 2017. The detected *S. lugdunensis* isolates were assessed in terms of drug susceptibility pattern, β -lactamase production, *mecA*-mediated oxacillin resistance, and inducible clindamycin resistance. The applied methods included disk diffusion, penicillin minimal inhibitory concentration, cefoxitin broth microdilution, and erythromycin/clindamycin disk diffusion, respectively.

Results: Among the CoNS samples, 25 cases (20.2%) were *S. lugdunensis*. In the confirmed isolates, *mecA*-mediated oxacillin resistance was detected in 21 cases (84%), and 18 isolates (72.0%) produced β -lactamase. In addition, 23 isolates (88.5%) showed inducible clindamycin resistance. In the antibiogram pattern, more than 70% of the methicillin-resistant isolates were also resistant to chloramphenicol, trimethoprim/sulfamethoxazole, gentamicin, azithromycin, and ceftazidime.

Conclusion: According to the results, *S. lugdunensis* was the cause of a new infection emerging in the studied burn injury center. Considering the resistance of the isolates against the most routine antibiotics, vancomycin is suggested as an alternative. Due to the prevalence of *S. lugdunensis* in different hospital wards, it is strongly recommended that CoNS isolates be evaluated for the detection of this bacterium.

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Introduction

Although the *Staphylococcus* family is among the largest groups of bacteria, only few species have significant interactions with humans. According to the first reports on these bacteria in 1884, the most frequent species of staphylococci (e.g., *Staphylococcus aureus*) could cause staph infections, especially in healthcare centers (1). Most of the species belonging to this family of bacteria, which are known as Coagulase-Negative Staphylococci (CoNS), were previously considered to be harmless with low pathogenicity in humans and other mammals. However, recent findings have denoted

that several species of CoNS are increasing steadily, some of which have been recognized as potential pathogens (2).

Among the CoNS species, *Staphylococcus lugdunensis* is an emerging pathogen implicated in the community and hospital-acquired infections (3).

This bacterium was first described in 1988 and is currently spreading, accounting for a major cause of significant invasive infections with a similar epidemiological pattern to *S. aureus* (4, 5). Furthermore, various reports have stated that *S.*

lugdunensis is capable of producing diseases similar to those caused by *S. aureus* (6).

Although *S. lugdunensis* is part of the normal flora of the human skin and is present in the perineum and inguinal area together with other coagulase-negative staphylococci, it could be virulent and cause severe infections.

Among the CoNS isolates, *S. lugdunensis* is comparatively more aggressive and has been reported to cause abscesses, surgical site infections, prosthetic joint infections, and septicemia (5-7).

In addition, *S. lugdunensis* has the potency to produce an arsenal of virulence factors, such as hemolysins, esterases, lipases, DNase, and adhesins (8).

According to the epidemiologic profile and increased frequency of *S. lugdunensis* in the normal flora of the human body, special attention must be paid to detecting the pathogenicity of this bacterium among the other CoNS species due to its capability to produce diseases similar to those caused by *S. aureus*, as well as the inadequate follow-up of the associated infections in some conditions, such as burn injuries.

In such cases, possible infections might be neglected due to the lack of detection and laboratory testing.

With this background in mind and considering the scarce data on *S. lugdunensis* in burn wound infections, the present study aimed to investigate *S. lugdunensis* infections in the patients admitted in Amir-al-Momenin Burn Injury Center, affiliated to Shiraz University of Medical Sciences in Shiraz, Iran. Our findings could lay the groundwork for the assessment and management of the patients with *S. lugdunensis* burn wound infections by microbiologists and clinicians.

Materials and Methods

Specimen collection

This cross-sectional study was conducted during January 2016-May 2017. In total, 124 CoNS isolates (one sample per each patient) were obtained and identified from the patients with burn injuries admitted in Amir-al-Momenin Burn Injury Center in Shiraz, Iran. Samples were collected from the male and female patients with thermal burns and sustained deep burns (second degree and above).

The CoNS isolates were extracted from the obtained samples within 5-7 days after hospitalization. Exclusion criteria were impure samples or the CoNS isolates accompanied by other infectious agents. The samples meeting the inclusion criteria were transferred to the microbiology department of the Burn and Wound Healing Research Center in Shiraz within the shortest time after sampling.

Data of the patients were collected using questionnaires. Since the present study was performed on the bacterial samples of the patients as part of their treatment process, informed consent was not obtained individually, and we relied on the prior consent provided by the patients or their companions.

The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (code: 93-01-63-8100).

Sample analysis and identification

Prior to the identification of *S. lugdunensis* in the isolates, the collected samples were analyzed for gram-positive cocci and *Staphylococcus* spp. using standard microbiological methods. Gram-positive cocci isolates were tested for catalase activity, slide, and tube coagulase, and the screened CoNS isolates were selected for further experimentation. Initially, the detected isolates were assessed in terms of nitrate reduction, ornithine decarboxylase, and pyrrolidonyl arylamidase activities. The isolates with positive results for the mentioned tests and negative results for oxidase and alkaline phosphatase were considered to be *S. lugdunensis*. To confirm the detection of *S. lugdunensis*, the detected isolates were examined through molecular methods for the *fbl* gene, as proposed in another study in this regard (9).

Drug susceptibility test

The confirmed isolates for *S. lugdunensis* were evaluated in terms of β -lactamase production, methicillin resistance (oxacillin resistance), inducible clindamycin resistance, and resistance patterns against some other antibacterial agents. All the procedures in the present study were performed in accordance with the guidelines of the Clinical & Laboratory Standards Institute (CLSI) (10).

To detect β -lactamase production in the *S. lugdunensis* isolates, penicillin minimal inhibitory concentration (MIC) was used, and MICs of ≤ 0.12 $\mu\text{g/ml}$ were considered positive for β -lactamase. To detect methicillin resistance (oxacillin resistance) in the isolates, the studied bacteria were evaluated using cefoxitin and broth microdilution method, and MICs of >4 $\mu\text{g/ml}$ were considered positive for *mecA*-mediated oxacillin resistance.

Disk diffusion was applied to detect inducible clindamycin resistance in the isolates. In this method, 15 μg of erythromycin and 2 μg of clindamycin disks were used in accordance with the CLSI standards. The disks were placed on Muller-Hinton agar plates, which were inoculated with 0.5 McFarland of the studied bacteria at a distance of 15-26 millimeters. After incubation (18-20 hours) at the temperature of 37°C, the plates were evaluated for the inhibition zone in the space between the two disks. Based on the observations, the flatten zone of inhibition was adjacent to the erythromycin disk (D-zone), and the results were positive for inducible clindamycin resistance.

For the other antibacterial agents, the Kirby-Bauer disk diffusion test was carried out in accordance with the CLSI guidelines (10). To do so, we used the commercially available disks of MAST-UK. A suspension of 0.5 McFarland was prepared from each isolate in the nutrient broth and swabbed onto the Muller-Hinton agar dispensed in a plate (10 cm). In this

stage, we used the drug disks specified with tetracycline, trimethoprim/sulfamethoxazole, vancomycin, oxacillin, gentamicin, ciprofloxacin, and cotrimoxazole.

Results

According to the laboratory screening results, 124 CoNS isolates were detected in the samples collected from the patients with burn injuries. With regard to the identification of the bacterial species, the biochemical and molecular tests confirmed the presence of *S. lugdunensis* in 25 samples (20.2%). The breakdown results in each hospital ward are shown in Table 1.

Table1: Frequency of CoNS and *S. lugdunensis* and Methicillin Resistance Rate of *S. lugdunensis* Isolates in Various Wards

	Frequency of CoNS N (%)	Frequency of <i>S. lugdunensis</i> N (%)	Prevalence rate of <i>S. lugdunensis</i> in CoNS N (%)	Methicillin Resistance rate of <i>S. lugdunensis</i> N (%)
ICU	21 (16.9)	7 (28.0)	7 (33.3)	4 (16.0)
Surgery	8 (6.5)	2 (8.0)	2 (25.0)	2 (8.0)
Pediatric	18 (14.5)	2 (8.0)	2 (11.1)	2 (8.0)
Internal Medicine	22 (17.7)	5 (20.0)	5 (22.7)	5 (20.0)
Female	43 (34.7)	4 (16.0)	4 (9.3)	4 (16.0)
Men	12 (9.7)	5 (20.0)	5 (41.7)	4 (16.0)
Total	124 (100)	25 (100)	25 (20.2)	21 (84.0)

In terms of the demographic data, 11 (42.3%) and 14 patients (53.8%) were male and female, respectively, with the mean age of 30.6±21.2 years (age range: 1-70 years). The patients infected with the CoNS isolates were admitted in different wards of the hospital, while most of the bacterial isolates were collected from the intensive care unit (26.9%), male ward (19.2%), and internal medicine ward (19.2%), respectively. On the other hand, the lowest frequency of *S. lugdunensis* infections was observed in the patients admitted in the surgery and pediatric wards (n=2; 7.7%).

With respect to β-lactamase production, 18 *S. lugdunensis* isolates (72.0%) had the ability to produce β-lactamase. The prevalence of the resistant groups in different wards is depicted in Figure 1.

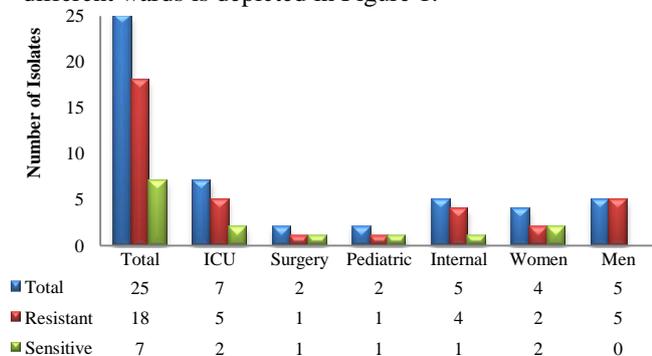


Figure1: Frequency of β-Lactamase in *S. lugdunensis* Isolates Based on Wards

The results of the study regarding the detection of inducible clindamycin resistance in the isolates indicated that 23 isolates (88.5%) were resistant to clindamycin. The findings in separate wards are illustrated in Figure 2.

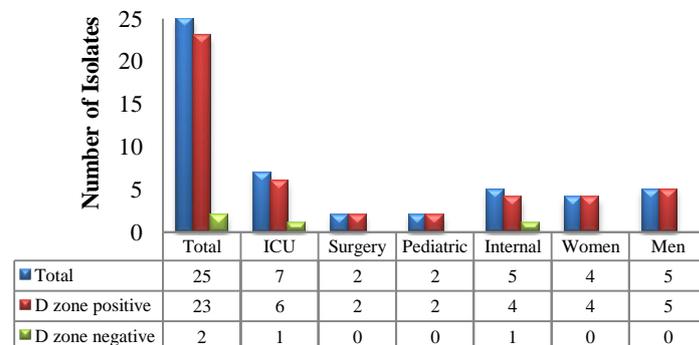


Figure2: Inducible Clindamycin Resistance Pattern in *S. lugdunensis* in Various Wards

The results of the study regarding the detection of inducible clindamycin resistance in the isolates indicated that 23 isolates (88.5%) were resistant to clindamycin. The findings in separate wards are illustrated in Figure 2. Other antibiotic resistance ratios of the isolates based on the methicillin resistance are presented in Table 2.

Table2: Antibiotic Resistance Ratios of Resistant and Susceptible *S. lugdunensis* Isolates to Methicillin

N=25	Methicillin-Resistant N=21 (84%)	Methicillin-Sensitive N=4 (16%)
Vancomycin (30 µg)	0	0
Chloramphenicol (30 µg)	19	4
Tetracycline (30 µg)	11	8
Ciprofloxacin (5 µg)	11	2
Trimethoprim/sulphamethoxazole (1.25/23.75 µg)	18	0
Clindamycin (2 µg)	12	2
Erythromycin (15 µg)	19	4
Gentamicin (10 µg)	20	2
Cefixime (5 µg)	14	1
Azithromycin (15 µg)	19	2
Ceftazidime (30 µg)	21	1

Discussion & Conclusion

CoNS are a group of bacteria, which are rarely diagnosed to the species level. However, several reliable studies in recent years have demonstrated that *S. lugdunensis* has potential pathogenicity in humans, and the diagnosis of this species could play a key role in the prospective detection of infectious diseases (11-13).

Evaluation of the infections in Amir-al-Momenin Burn Injury Center in the southwest of Iran has indicated that many samples, particularly wound and blood culture samples, are infected with CoNS, the majority of which are considered to be normal flora and are neglected in reports. Based on these data and

significant pathogenicity of *S. lugdunensis* among various CoNS isolates, findings of the current research showed that more than 20% of the reports on infections concern this group of bacteria. According to the present study, approximately 20% of the CoNS isolates constitute *S. lugdunensis* species. This finding could be regarded as a prognosis of an emerging infection that threatens the patients with burn injuries admitted in these healthcare centers. Although numerous have focused on CoNS, especially *S. lugdunensis*, the current research has been the first to investigate the presence of *S. lugdunensis* in burn wound infections. Considering that *S. lugdunensis* is a new infection emerging in patients with burn injuries, recognition of the phenotypic criteria of the bacteria could contribute to the initial screening of the species. According to the specifications in the current research and findings of the previous studies in this regard, this bacterium has better growth on Columbia Agar with 5% sheep blood, incubated for more than 48 hours with an *Eikenella*-like odor (14). Another feature of this bacterial species is colony pleomorphism and hemolysis on blood agar media. In general, these specifications could be beneficial in the screening for the *S. lugdunensis* species. Findings of the present study demonstrated that more than 70% of the *S. lugdunensis* isolates were methicillin-resistant and could produce β -lactamase. This is in line with the report by Koksal (Turkey) regarding methicillin resistance (15). Moreover, the antibiotic resistance ratio in our study was indicative of

no resistance to vancomycin, while the highest resistance (>70%) was observed against ceftazidime, gentamicin, and azithromycin.

In conclusion, the results of the present study confirmed that the CoNS genus is the origin of an emerging infection in the hospitalized patients with burn injuries.

These bacterial infections are extremely threatening, while the isolates were found to be resistant to many routine antibiotics used in the studied healthcare center. Therefore, screening and monitoring of nosocomial infections must be performed regularly based on surveys on possible emerging infections. Monitoring of antibiotic consumption and the resistance trends in nosocomial infections with effective preventive measures could prevent the emergence of new infections and multi-resistant infectious agents in hospitals and clinical settings.

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