The Prevalence of antimicrobial resistance in *Staphylococcus aureus* and coagulase-negative Staphylococci strains isolated from inpatients

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Research note

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Abstract

Objectives: Globally nosocomial infection is a significant problem. Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin resistant coagulase negative staphylococci (MRCoNS) are major causes of nosocomial infections. Aim of this study was to determine the prevalence and antimicrobial susceptibility of MRSA and MRCoNS in the southwest of Iran. This cross-sectional study was conducted on 221 non-duplicated staphylococci isolates collected from teaching hospital in Shiraz. The prevalence of MRSA and MRCoNS in clinical samples was identified with conventional microbiological tests. After identification, all of the isolates were subjected to antimicrobial susceptibility test and PCR to identify the presence of *femA*, *mecA* and *pvl* genes.

Results: 70 (41.7 %) MRSA among 168 *S. aureus*, and 26 (15.48%) MRCoNS among 53 CoNS were examined. All of the isolates were susceptible to Trimethoprim/Sulfamethoxazole (100%). Chloramphenicol (65%) and Gentamicin (23%) were the other most active antibacterial agents against MRSA and MRCoNS. The frequency of *pvl* gene among *S. aureus* strains was 3.57%. There is need for developing the antibiotic policy and limiting the use of powerful antibiotics.

Introduction

Staphylococcus aureus is a remarkably successful pathogen in the healthcare and community setting(1). It is capable of persistence as a pathogen, because of its frequent resistance to multiple antimicrobial agents and its virulence determinants such as enzymes and toxins (2). In recent decades, methicillin resistant *S. aureus* (MRSA) has been a major agent of health care- and community-associated infections (3). Coagulase-negative staphylococci (CoNS) are part of the human normal flora; especially, the skin is also associated with severe infections such as bacteremia and septicemia, (4). CoNSs consist of a variety of *Staphylococcus* spp. and are usually resistant to most ß-lactams antibiotics including methicillin (5) also has a major role in clinical infections (6). The main mechanism of resistance to methicillin in staphylococci is the expression of *mecA* gene coding penicillin binding protein 2a (PBP2a), a transpeptidase with low affinity for β-lactams which confers resistance to methicillin and other β-lactam antibiotics (8). *mecA* is carried on a mobile genetic element called the Staphylococcal Chromosome Cassette *mec* (SCC*mec*) (9). So far, 12 SCC*mec* types described that HA-MRSA strains carry SCC*mec* types I, II and III, while CA-MRSA strains have SCC*mec* types IV and V (10). In recent years, treatment of MRSA has been one of the major problems in hospitals because these bacteria are multiply resistant and are susceptible only to glycopeptide antibiotics such as vancomycin and investigational drugs (11). Panton-Valentine Leukocidin (PVL or *lukS*/*F*) is a bi-component pore-forming toxin causing tissue necrosis and lysis of phagocytic leukocytes that is associated with deep-seated abscess, multiple lesions and multiple antibiotic resistances (12). PVL is produced in the majority of community associated MRSA isolates and rarely is present in hospital isolates (13). Epidemiological data suggest that high virulence of community-acquired MRSA is associated with *PVL* genes but antibiotic resistance among *PVL* negative MRSA isolates was found to be higher than positive isolates. (14). In our hospitals, the incidence rate of MRSA has been increased in clinical isolates and the prevalence of MRCoNs is also

rising. Although many studies have been done on prevalence and antibiotic susceptibility of *Staphylococcus*, many of these studies have focused only on MRSA, not on MRCoNS which are similarly important. Therefore, this study aimed to determine the prevalence and antimicrobial susceptibility of MRSA and MRCoNS in a major teaching hospital in Shiraz, southwest of Iran.

Methods

Study design and identification of the isolates

This cross-sectional study was performed from March 2016 to March 2017 in Nemazee teaching hospitals in Shiraz. A total of 221 isolates were collected from different clinical specimens such as the blood, urine, stool and sputum with initial diagnosis of *S. aureus*. Bacterial isolates were identified by conventional microbiological procedures including: Gram stain, colony characteristics and conventional biochemical tests, namely catalase and coagulase (slide and tube) reaction; growth on mannitol salt agar.

Antibiotic susceptibility testing

Disk diffusion method (Kirby-bauer) was performed for MRSA and MRCoNS isolates against Gentamicin (10µg), Clindamycin (2µg), Ciprofloxacin (5µg), Erythromycin (15µg), Tetracycline (30µg), Chloramphenicol (30µg), Terimethoprim-sulfamethoxazole (1.25/23.75µg), and Tobramycin (10µg), according to the CLSI guideline (16). Methicillin resistance for *Staphylococcus* spp. isolates was primarily detected based on resistance to cefoxitin (30 µg) disk (Rosco, Denmark) by CLSI recommended disk diffusion method; also, zone diameter ≤ 21mm was estimated cefoxitin resistant(16). Based on CLSI guidelines, *S. aureus* ATCC 25923 was used as positive control for disk diffusion method.

DNA extraction and polymerase chain reaction (PCR) assay

Whole DNAs of methicillin resistant staphylococci were extracted, using boiling method Extracted DNA was checked through spectrophotometer and it was acceptable through standard guidelines and used as PCR templates. Detection of the presence of the *mecA, femA* and *pvl* genes was confirmed by PCR assay with specific primers (13, 17). PCR amplifications were performed on a T100™ thermal cycler (Bio- Rad, Hercules, CA, USA). The cycling condition was set up as follows: initial denaturation at 96 °C for 3 min; followed by 35 cycles of 30 s at 96°C, annealing for 1 min at 55°C and 2 min at 72°C; and an extension for 10 min at 72°C. PCR products were loaded into the wells of agarose gel (1.5%) carefully and electrophoresed at 75 V for 90 min. Staining was performed with safe stain load dye (CinnaGen Co., Iran) and then observed under the UV trans-illuminator. *S. aureus* ATCC 25923 is used as the positive control.

Statistical analysis

Statistical descriptive analyses of the parameters were conducted using SPSS for Windows statistical software (version 22).

Results

221 staphylococci clinical isolates containing 168 (76%) *S. aureus* and 53 (24%) CoNS were included in the study. Among 221 isolates of staphylococci, 96 (43.4%) methicillin-resistant isolates consisting of 70 (31.7 %) MRSA and 26 (11.8%) MRCoNS were examined. Among 221 isolates, 99 (44.8%) and 122 (55.2%) were from male and female patients, respectively. Among MRCoNS isolates, the frequency of *S. epidermidis* and *S. saprophyticus* was 76.9% and 23.1%, respectively. Also, in both male and female patients, the frequency of *S. epidermidis* (75.5%) was more than *S. saprophyticus* (15.24). the isolates were collected from different wards of hospitals; MRSA and MRCoNS were isolated more commonly from internal 19 (27.1%) and clinical treatment 10 (38.5%) wards. The frequency of MRSA and MRCoNS in different wards is shown in Table 1.

Table 1: Frequency of MRSA and MRCoNS isolates in different parts of the hospital

Ward	MRSA No. (%)	MRCoNS No. (%)
ICU	18 (25)	4 (15)
Surgery	10 (14)	5 (19)
Acute treatment	8 (11)	2 (7)
Pediatric internal	5 (7)	3 (11)
Clinical treatment	3 (4)	10 (38)
NICU[a]	7 (10)	1 (3)
Internal	19 (27)	1 (3)
Total	70	26

[a] Neonatal intensive care unit

Bloodstream was the most common site of bacterial isolation of MRSA and MRCoNS (40%), as presented in Table 2.

Table 2: Frequency of MRSA and MRCoNS isolates based on the isolation site

Infection source	MRSA No. (%)	MRCoNS No. (%)
Nose	3	2
Wound	10	3
Urine	11	1
Blood	23	17
Sputum	5	0
ETT[a]	4	0
Throat	2	0
Auxillary	2	0
Abdominal	3	1
Pleural	2	0
Eye discharge	3	1
Joint	2	0
CSF[b]	0	1

[a]: Endotracheal tube

[b]: Cerebrospinal fluid

All of the isolates were susceptible to Trimethoprim/Sulfamethoxazole (100%). Chloramphenicol (65%) and Gentamicin (23%) were the other most active antibacterial agents against MRSA and MRCoNS. Intermediate results were also reported as resistant. The antibiotic susceptibility pattern of MRSA and MRCoNS is shown in Table 3.

Table3: Pattern of susceptibility and antibiotic resistance in the collected isolates

	MRCoNS		MRSA	
	R	S	R	S
Tetracycline	16(61.4%)	10(38.4%)	53(65.7%)	24(34.2%)
Chloramphenicol[a]	22(92.2%)	2(7.6%)	72(92.8%)	5(7.1%)
Erythromycin	21(77.6%)	5(19.2%)	69(81.3%)	13(18.5%)
Tobramycin	20(76.8%)	6(23%)	62(77.1%)	16(22.8%)
Gentamicin	23(88.4%)	3(11.5%)	72(85.6%)	10(14.2%)
Cefoxitin	26(100%)	0	70(100%)	0
Clindamycin	16(61.5%)	10(38.4%)	38(47.5%)	36(51.4%)
Ciprofloxacin	21(80.7%)	5(19.2%)	55(71.3%)	20(28.5%)
SXT[b]	0	26(100%)	0	70(100%)

[a.] Chloramphenicol is in systemic use anymore

[b] Trimethoprim /Sulfamethoxazole

PCR results also confirmed the presence of *pvl*, *femA* and *mecA* genes. The prevalence of Panton–Valentine Leukocidin (PVL) producing MRSA isolates by detection of *pvl* gene was 3.57% (6 isolates).

Discussion

Many strains of *Staphylococcus aureus* have been colonized on the surface of humans bodies, and some of these bacteria are pathogenic (18). The prevalence of MRSA is now increasing worldwide and this bacterium has become a major concern. Different Staphylococcus species have the ability to transmit the genes vertically. Today, coagulase-negative staphylococci (CoNS) are among the main human pathogens (19). Horizontal translocation of genes, such as *SCC mec,* occurs easily between MRCoNS and different CoNS strains receiving these genes. Presence of diverse antibiotic resistance genes found in CoNS makes them one of the most important infectious pathogens in the hospital (20, 21). MRCoNS are one of the first causes of bloodstream infections and catheter-related infections that cause high mortality despite antibiotic use and supportive treatments (22). In our study, the rate of CoNS was estimated 53 (24%) in the statistical population of Shiraz teaching hospitals. In a study conducted by Soumya et al. on 173 isolates, 152 (88%) isolates were identified as CoNS (23), whereas in a study in Nepal, about 24% of the isolates were CoNS(24). In a study carried out by Morgenstern et al. in the United States in 2016, they examined the frequency of MRCoNS and MRSA in carriers (through the nose), which estimated the

frequency of MRCoNS at 21.4% and the frequency of MRSA isolates at 2%. This study suggests that the high frequency of MRCoNS is possible even in developed communities(25). In our study, the frequency of MRSA and MRCoNS was evaluated among the patients, showing that the prevalence of MRSA was 31.7% and that of MRCoNS was 11.8%. In comparison to the study performed in the United States, the frequency of MRSA was more. In this study, Cefoxitin disc can be considered as a very effective method for detecting the presence of *mecA* gene although it was found that PCR was an easy, fast and inexpensive method for detecting the presence of this gene. Kim et al. in China used the *16s rRNA* gene PCR to identify different CoNS strains(26) and, as in this study, demonstrated the importance of rapid detection of CoNS. Among the samples examined in this study, 6 (3.57%) had *pvl* gene. The occurrence of *pvl* gene in previous studies has been reported from 2 to 35% (26, 27). In comparison, the obtained results for *pvl* gene in the present study were near the lower limit obtained from similar studies. Chantratita in Thailand estimated this frequency at 16% in hospital patients(27). Kong in Taiwan reported the prevalence of this gene in about 13%(28). Odhiano estimated the prevalence of *pvl* in a hospital in Tehran to be 20%(29). Motamedi reported this prevalence in Ahwaz to be around 3%(30).

In conclusion, the results of the current study showed that MRSA and MRCoNS were more commonly isolated from the patients hospitalized in internal and clinical treatment wards. Bloodstream was the most common sites of MRSA and MRCoNS isolation and trimethoprim /Sulfamethoxazole and Gentamicin has been the most active antimicrobial agents against MRSA and MRCoNS. Regular follow-up of antibiotic resistance profiles of hospital pathogens, especially *Staphylococci,* and developing hospital antibiotic policy accordingly, will be useful for prescribing appropriate antibiotics by clinicians.

Limitations

The present study had some limitations. First, this was a single-center study; therefore, generalization of the results to other regions requires further investigations. Second, it was a retrospective study with a relatively small sample size. More studies with a larger sample size, and evaluation of other virulence factors are also suggested.

Abbreviations

MRSA: methicillin resistant *Staphylococcus aureus*, CoNS: coagulase negative staphylococci; MRCNS: methicillin-resistant coagulase-negative staphylococci; CLSI: Clinical and Laboratory Standards Institute.

Declarations

Authors' contributions

Mohammad Motamedifar, Parva Farmehr: conceived the study. Mohammad Motamedifar , Javad Fathi, Mahtab Hadadi: participated in the design of the study and performed the statistical analysis. Javad Fathi, Mahtab Hadadi: interpreted the data. Mohammad Motamedifar: Supervised data collectors.

Mohammad Motamedifar Drafting the article or revisiting it critically for important intellectual content. Mohammad Motamedifar, Parva Farmehr were project leaders and primary investigators of the study. All authors read and approved the final manuscript.

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Competing interests

We declare that we have no conflict of interest.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

There is no limit to the publication.

Ethics approval and consent to participate

This study was in accordance with the declaration of Helsinki and an ethical permission was sought from the institutional Ethics Committee of Shiraz University of Medical Sciences (Approval No. IR.SUMS.REC.1395.S247). However, because we only used leftovers from clinical specimens, the local ethics committee waived the need for informed consent.

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Figures

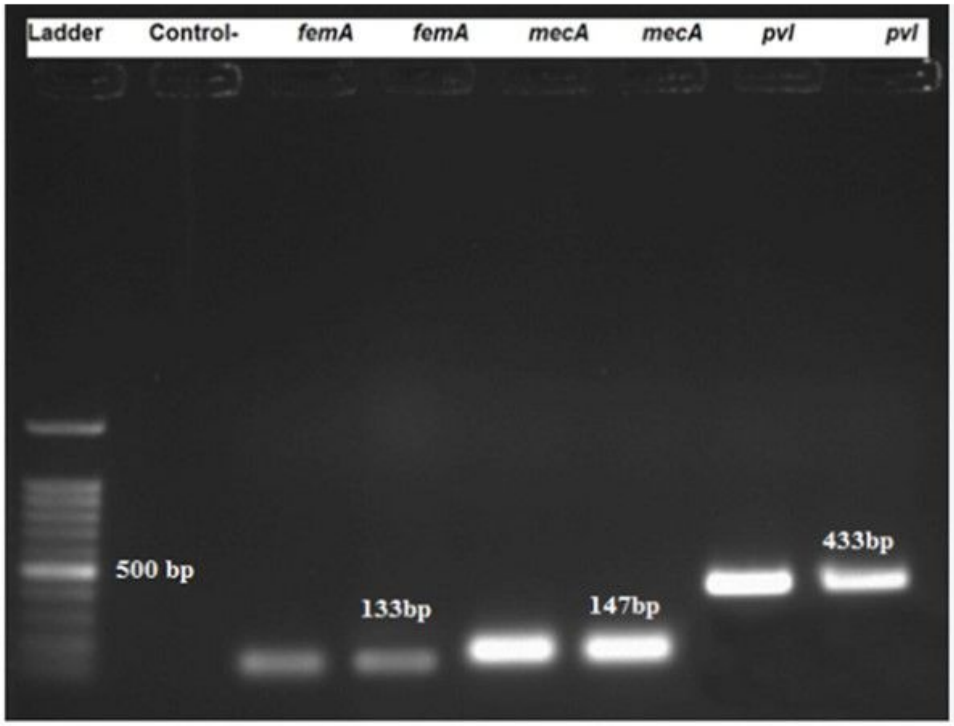

Figure 1

Agarose gel electrophoresis of PCR products for femA, mecA and pvl genes lane 1: DNA ladder, lane 2: negative control sample, lane 3 and 4: S. aureus positive isolate containing femA gene with 133 bp length and positive control, lanes 5 and 6: S. aureus positive isolate containing mecA gene with 147 bp length and positive control and lanes7and 8: S. aureus positive isolate containing pvlA gene with 433 bp length and positive control.