High prevalence and expression of antiseptic resistance genes among infectious t037/ST239 methicillin-resistant Staphylococcus aureus (MRSA) strains in North Khorasan Province, Iran

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A B S T R A C T

Objective(s): Staphylococcus aureus is an important infectious agent and the majority of methicillin-resistant S. aureus (MRSA) infections are of nosocomial origin. To define the level and distribution of antiseptic resistance among infectious S. aureus strains we studied MRSA and methicillin-susceptible S. aureus (MSSA) isolates collected from different infection sites in an assortment of patients.

Materials and Methods: S. aureus isolates were investigated for in vitro susceptibility to antiseptic agents and detection of qacA/B, smr, vanA, and mecA genes.

Results: Among the S. aureus isolates we studied, 25 and 41 were MRSA and MSSA, respectively. The mean of minimum inhibitory concentrations (MICs) for benzethonium chloride (BTC) among MRSA was statistically significantly higher than for MSSA (26 µg/ml versus 11.7 µg/ml, P=0.003) while there was no significant difference between MRSA and MSSA for benzalkonium chloride (BKC) and chlorhexidine digluconate (CHG). The qacA/B genes were carried in 68% of the MRSA and 58.2% of MSSA (P=0.601), while smr was carried in 39% of MRSA and 29.3% of MSSA strains (P=1.000). In 15 out of 25 cases, MRSA ST239 with spa types t037, t030, and t7688 was isolated from the infection site with 86.6% of them carrying a resistance gene (qacA/B or qacA/B + smr).

Conclusion: The frequent presence of antiseptic resistance genes and a consequently elevated MIC against antiseptics among ST239 MRSA emphasizes the importance of mandatorily monitoring MRSA for effective infection control.

Introduction

Staphylococcus aureus is an important infectious agent, and the majority of methicillin-resistant S. aureus (MRSA) related infections are of nosocomial origin. MRSA is well known to be resistant to beta-lactam antibiotics and other antibiotics as well (1). Also, vancomycin resistance among S. aureus is becoming an increasingly important issue (2) reported from different parts of the world (3, 4), including Iran (5). Despite the selective use of antibiotics and additional prevention strategies, MRSA-related nosocomial infections are still a major problem. Disinfection and decolonization may fail in the hospital setting because of possible resistance to antibiotics and antiseptic agents that may lead to increased severity of staphylococcal infections and extension of associated clinical and epidemiological problems. Infection control management strategies including decontamination of colonized body sites using antiseptic agents such as chlorhexidine and quaternary ammonium compounds have been shown to reduce the risk of invasive nosocomial infections (6, 7).

Damaging the phospholipid bilayer and cytoplasmic membrane disruption are anti-bacterial characteristics of chlorhexidine and quaternary ammonium compounds. Resistance to these compounds is primarily mediated by multidrug efflux pumps encoded by two families of PCR non-differentiable genes (qacA, qacB and qacC, qacD, and ebr) known as the qacA/B and smr families, respectively (8). These plasmid-located, transferable gene families confer high and low-level resistance to antiseptics, respectively (9, 10).

In the current study, we define the level and distribution of antiseptic resistance among infectious S. aureus strains. We studied the MRSA and MSSA isolates collected from different infection sites of hospitalized patients in Imam Hassan Hospital of Bojnurd, the main referral hospital center in the North Khorasan Province in Iran.

Materials and Methods

Study samples

The current study used the S. aureus isolates collected

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from infected hospitalized patients in Imam Hassan Hospital, the main tertiary care referral hospital in North Khorasan Province, Iran. The study duration was from January to December 2020.

All isolates were identified to the species level in the hospital laboratory and were reconfirmed in the microbiology laboratory at the Faculty of Medicine by standard Gram staining, catalase testing, mannitol testing, and tube coagulase assays. All isolates were further confirmed as S. aureus by spa442 PCR (11). The S. aureus isolates were confirmed as MRSA by oxacillin and cefoxitin susceptibility testing according to Clinical and Laboratory Standard Institute (CLSI) guidelines (12) and meca PCR (13) (sequence of primers and used programs deposited in the supplementary information file).

All isolates were then stored at -30°C in Trypticase Soy Broth (TSB), supplemented with 20% glycerol (HiMedia, India). Chromosomal DNA was extracted using a commercial DNA extraction kit (Poyagene Azma, Iran) according to the manufacturer’s instructions. Purified DNA was used for molecular investigations.

**Antiseptic susceptibility testing**

Susceptibility to quaternary ammonium compounds such as benzenthionium chloride (BTC), benzalkonium chloride (BKC), and biguanide compounds such as chlorhexidine digluconate (CHG; Sigma-Aldrich, Steinheim, Germany) was determined using the Mueller–Hinton broth microdilution method (BMD) (8). A concentration of 1.25 to 1250 µg/ml of antiseptic was used for measuring the MICs.

**Detection of genes encoding antibiotic resistance, antiseptic resistance, and regulators**

All isolates were screened for the presence of qacA/B, smr (qacC, qacD, and ebr), and vanA genes as described before (5, 8). Multiplex PCR for detection and characterization of the staphylococcal cassette chromosome mec (SCCmec) and accessory gene regulator (agr) typing were performed by protocols described before (13-15) (sequence of primers and used programs deposited in the supplementary information file).

**Results**

Table 1. Patients’ demographic data and Staphylococcus aureus infection sites

| S. aureus | Male | Female | Mean Age | Ward | Site of infection |
|----------|------|--------|----------|------|------------------|
| MSSA | 19 (46.5) | 22 (53.5) | 49 (4.8-85) | 12 (29.3) | BC (n=14), wound (n=12), urine (n=6), and others (n=7) |
| MRSA | 17 (6) | 24 (21) | 35 (38-48) | 11 (29.3) | WC (n=12), blood (n=14), and others (n=15) |
| Total | 36 (57.6) | 46 (24) | 48 (4.8-85) | 23 (29.3) | ICU (n=9), INT (n=8), and others (n=15) |

ICU: intensive care unit, INT: internal medicine, Heart: cardiology, EMRG: emergency, SW: special ward, INF: infectious diseases, PEDI: pediatric, LC: tracheal aspirate culture, BC: blood culture, UC: urine culture, WC: wound culture

Table 2. MIC of antiseptic compounds among MSSA and MRSA strains

| MIC | BTC | BKC | CHG |
|-----|-----|-----|-----|
| S. aureus | Mean (range) µg/ml | P | Mean (range) µg/ml | P | Mean (range) µg/ml |
| MSSA | 12.5 (2.5-39) | 0.003 | 20.6 (2.5-39) | 0.003 | 22.2 (2.5-625) |
| MRSA | 26 (5-78) | 0.200 | 35.5 (5-155) | 0.200 | 274 (78-625) |
| Total | 26.5 (2.5-625) | 0.460 | 35.5 (5-2625) | 0.460 | 259 (39-625) |

MSSA: methicillin-sensitive Staphylococcus aureus, MRSA: methicillin-resistant Staphylococcus aureus, BTC: benzenthionium chloride, BKC: benzalkonium chloride, CHG: chlorhexidine digluconate, MIC: minimum inhibitory concentration, P: independent samples t-test
MSSA: methicillin-sensitive Staphylococcus aureus, MRSA: methicillin-resistant Staphylococcus aureus; *: Some strains had two genes simultaneously, NT: Not typeable, smr: identical to qacC, qacD, and ebr, qacA/B: identical to qac A and qacB

MICs for BKC (P=0.200, 35.5 µg/ml versus 20.6 µg/ml) and CHG (P=0.460, 272 µg/ml versus 222 µg/ml) among MRSA and MSSA were observed (Table 2 and the supplementary data file).

Among the S. aureus isolates, 69.7% harbored at least one resistance gene while no significant difference between MRSA and MSSA was observed (72% MRSA versus 68.3% MSSA, P=0.7899) (Table 3). The incidence of the qacA/B genes among MRSA (68%) was higher than MSSA (58.2%) but again there was no statistically significant difference (P=0.6016). No difference in the distribution of the smr gene between MRSA and MSSA was observed either (P=1.000). The qacA/B genes presented among n=41/66 isolates (62.1%) in comparison with the smr gene that was detected among n=30/66 of S. aureus isolates (30.3%) (Table 3). No vanA gene responsible for vancomycin resistance and phenotypic resistance to vancomycin was detected.

The most common agr type among MSSA (n=33/41, 80.5%) and MRSA (23/25, 92%) was agr I. Among agr I type isolates, the qacA/B gene was detected in 34.7% (8/23) of MRSA and 63.6% (21/33) of MSSA isolates with no significant difference (P=0.2449). Among the MRSA, SCCmec III (11/25, 44%) and SCCmec IV (7/25, 28%) were the most common types (Table 3).

Among the MRSA strains, the mean MICs against three antiseptic reagents were higher in t037, t030, and t7688 (n=15) ST239 strains (Table 4). The majority of t037/ST239 (n=11/12) and the only t7688/ST239 strains were isolated from tracheal aspirate cultures among ICU ward patients. Wound cultures (n=4/8) and urine cultures (n=2/8) were the common clinical sources for t230/ST45 MRSA strains from the patients in different wards (Table 4 and the supplementary information file).

Among MRSA strains, the majority of t037, t030, and t7688/ST239 strains (86.6%) were qacA/B gene-positive, and five t037/ST239 strains were positive for qac and smr simultaneously (Table 4). Only one t037/ST239 harbored no antiseptic resistance gene at all. The prevalence of resistance genes among other MRSA strains was 40% and 30% for qacA/B and smr, respectively (Table 4).

A review of the SCCmec types encountered in our study is presented in Table 4. The majority of MRSA strains were typed as SCCmecIII (n=11) when 9 of them were t037/ST239 strains (Table 4). The only t7688/ST239 MRSA strain was SCCmecXI, and it harbored the qacA/B gene as well (Table 4).

Spa typing of MSSA strains revealed more genetic diversity in comparison with Spa typing of MRSA. The distribution of resistance genes among MSSA strains was more diverse than among MRSA (supplementary information file).

**Discussion**

*S. aureus*, especially MRSA, is a well-known source of nosocomial infection in Iran (17). Staphylococcal infections are occurring from both endogenous and exogenous sources. Even though S. aureus preferentially colonizes the anterior nares, which may lead to endogenous infection, such colonized patients remain at risk of exogenous infections as well (18). Antiseptic resistance among MRSA has been reported in Iran before (19), but we here present the first study that compared the actual MIC values in combination with documented presence of antiseptic resistance genes in strains isolated from infections in Iran.

The highest measured MICs for antiseptic agents (625 µg/ml) that we documented among S. aureus strains included in the present study are still lower than the recommended concentration of use (2000, 1000, and 5000 µg/ml for BKC, BTC, and CHG, respectively) (19). t037, t030, and t7688 (ST239/CC8) strains were reported as successful MRSA and MSSA strains in the hospital setting of Iran (17, 20-

**Table 3. Distribution of resistance and regulatory genes among MSSA and MRSA strains**

| S. aureus | Resistance genotypes | agr resistance | SCCmec |
|----------|----------------------|---------------|--------|
| Genes    | pAqB     | smr | Sum* | I | II | III | IV | NT | I | II | III | IV | XI |
| MSSA     | 24 (58.1) | 12 (28.9) | 28 (66.3) | 33 (78.5) | 1 (2.4) | 4 (9.8) | 0 | 3 (7.5) | - | - | - | - |
| MRSA     | 17 (68)  | 8 (32)  | 18 (72)  | 23 (92)  | 0 | 2 (8)  | 0 | 0 | 4 (16) | 2 (8) | 11 (44) | 7 (28) | 1 (4) |
| Total    | 41 (62.1) | 20 (30.3) | 46 (69.7) | 56 (84.8) | 1 (1.5) | 5 (9.1) | 0 | 3 (4.5) | 4 (16) | 2 (8) | 11 (44) | 7 (28) | 1 (4) |

**Table 4. Typing results, resistance genes distribution, and MIC against antiseptics among MSSA and MRSA strains**

| Spa type (No.)/CC | MLST ST/CC | SCCmecA | qac | BTC | BKC | CHG | mean MIC µg/ml (range) |
|-------------------|------------|---------|-----|-----|-----|-----|------------------------|
| t037/12(77),13(2)/37 | 239/18 | SCCmecC | 12 | 5 | 27.7| 43.7| 305| (78-825) |
| t030(1)/24 | 239/18 | SCCmecC | 17 | 0 | 39 | 155 |
| t7688/1/2 | 45/45 | IV(7), I | 2 | 0 | 21.3| 20.7| 214| (78-825) |
| t7341/1(2)/S | 3 | II | 2 | 352| 332| 468| (30-525) |
| t790(9),11184(7),701(4),17511(3) | 21 | 24 | 12 | 12 | 11.7| 20.6| 222| (39-525) |
| t7622(2)/1,706, NT(2) | 58.5% | 29.3% | | | | | |
| t176(20),1219(2), 5 | 259,30,22 | 3 | 0 | | | | |
26) and Asia (27). The elevated prevalence of antiseptic resistance genes and the concurrent increase in MIC against antiseptic agents among mentioned infectious strains poses a clinical dilemma. The possible use of a subinhibitory dose of disinfectants in the hospital setting may increase the chance of further enhancing those MICs. This could happen through dilution of cleansing liquids or other mechanisms such as up-regulation of efflux pumps (28). In consequence, it may also lead to a higher chance of exposing patients to these successful and potentially invasive MRSA isolates in hospitals.

In the current study, qacA/B genes, which provide resistance to a broader range of biocides than smr (29-31), were the predominant resistance genes as was reported before in the United States (32), European countries (33), Japan (34), Iran (19), China (35), and Hong Kong (36). The incidence of qacA/B reported here is higher than reported results from 11 Asian countries (38.5%) (37) including Korea (59%) (38). It is lower than what was reported before in Malaysia (83.3% and 69.4%) (39, 40). The detected prevalence of the qacA/B gene is almost the same as in 12 European countries (62.6%) (33).

This is the first report of t7688/ST239 strains from infections in our study region. t7688/ST239 was reported previously as a colonizing strain from Iran in Ridom spa server (https://spaserver.ridom.de/). t7688/ST239 was isolated before from the hands of staff nurses and the hospital environment (41), and it also revealed itself as a successful cause of infection which was isolated from different infection sites in hospitals in Iran (24-26). It seems that t7688/ST239 is one of the successful clones among ST239/CC8 MRSA strains in Iran. The presence of antiseptic resistance genes followed by elevated MICs against antiseptic agents verifies the importance of detailed monitoring of all MRSA strains, colonizers, or infections from different clones.

SCCmecIV was reported among t7688/ST239 MRSA strains in some Iranian studies before (25, 41), whereas the current study revealed for the first time the presence of SCCmecXI in this clone. We have seen no prior report of SCCmecXI among ST239/CC8 strains. The SCCmecXI was reported among CC130 strains from human and livestock sources from European countries including the United Kingdom and Denmark (42) (43). The majority of other reports involved different wild animals such as European otters and a European brown hare from Austria (44) as well as European hedgehogs from Sweden (45). Since then, zoonotic transmission (46) and its association with invasive disease (47) have been reported. There is no report for detecting the SCCmecXI gene among strains harboring the human-specific immune evasion cluster (IEC) (48). The presence of this SCCmec type among ST239/CC8 strains that reported positive for IEC genes in Iran (49, 50) and other countries (51, 52) warns of the possibility of the presence of wild animal-related S. aureus clones in regional hospitals and transferring other virulence genes from them to human-adapted strains.

Conclusion

The results of the current study highlight the value of close monitoring of MRSA strains in the hospital setting for the presence of antiseptic resistance genes and their associated phenotypic resistance. Typing and screening the clones circulating in a hospital is strongly recommended for better understanding the epidemiology of antiseptic resistant MRSA strains, especially S. aureus clones that are also common among wild animals.

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Not applicable.

Authors’ Contributions

HGM Performed conceptualization, project administration, and wrote the original draft; AA Helped with investigation and resources; VD, SN, and SS Provided investigation; AS Helped review and edit; AVB Helped write, review, and edit, and gave scientific advice.

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Ethical Approval

Sample collection was performed according to the rules and regulations set by the Ethical Committee of North Khorasan University of Medical Sciences (project number: IR.NKUMS.REC.1394.027).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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