

Detection of inducible clindamycin resistance (MLS_{Bi}) among methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from health care providers

Masoumeh Navidinia*

Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Corresponding Author: email address: dr.navidinia@sbmu.ac.ir (M. Navidinia)

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important agents producing nosocomial diseases in hospitalized children. Then, screening of health care providers who are in direct contact with patients in hospital is necessary. The objective of this study was to investigate MRSA collected isolates for MLS_B phenotypes, in particular inducible clindamycin resistance (MLS_{Bi}). Two hundred and twenty nine health care providers were examined and nasal samples for *S. aureus* culture and sociodemographic data were obtained from them during one year August 2012 - July 2013. After MRSA identification, all isolates were examined for antibiotic resistant pattern. *Staphylococci* were isolated from 27 samples. Twenty one of them were MRSA. All isolates sensitive to linezolid and vancomycin. D-tests identified 6 isolates (28.6%) with inducible resistance to clindamycin (MLS_{Bi} phenotype). Carrier samples screening are considered less than clinical samples. Treatment of variety of infectious diseases due to resistant bacteria is difficult. So, annual screening of these individuals, detecting the carriers and decolonizing them to reduce transmission of *S. aureus* in the hospital is necessary.

Keywords: Methicillin-resistant; *Staphylococcus aureus* (MRSA); Inducible clindamycin resistance (MLS_{Bi}); Health care providers.

INTRODUCTION

Staphylococcus aureus find nearly 25-30% on skin or nose of healthy people. MRSA is a type of *Staphylococcus* that is resistant to certain antibiotics such as methicillin, cloxacillin, dicloxacillin, oxacillin, nafcillin, and closely related class of drugs such as cephalosporins (e.g., cephalexin). One of the most important reasons of MRSA expansion is unnecessary and broad-spectrum antibiotic overuse for less serious infections. Unfortunately, these MRSA isolates which susceptible only to glycopeptides antibiotics such as vancomycin, are becoming multidrug resistant [1].

At present, low level resistance to vancomycin is appearing and increasing [2]. The possible predisposing factors of MRSA emergence are, long time hospitalization, consumption of antibiotics without doctor prescription, lack of awareness, receipt of antibiotics before coming to the hospital

and etc [3]. MRSA serious infections have been increased in the world. Infected patients and health care providers carriers play important role in spreading and transferring this superbug in hospital [4]. Today, emergence of multiple drug resistance and monitoring of disease transmission by MRSA isolates not only in hospitals but also in communities is the major challenge [5]. A considerable increase in the prevalence of MRSA has been reported from every region of world. Treatment of the infections due to MRSA is difficult because of the restricted spectrum of antimicrobials of proven efficacy. A macrolide-lincosamide-streptogramin B (MLS_B) antibiotic, clindamycin, is a good substitute to treat these infections. But, there are reports of resistance to this drug too. A variety of *erm* genes, which may be expressed either constitutively (MLS_{Bc} phenotype) or inducibly (MLS_{Bi} phenotype) may cause resistance to macrolide (MLS_B).

Active efflux pump encoded by *msr A* gene (MS phenotype) is other mechanism of resistance. Because in treatment with clindamycin *in vivo* may result failure, thus detect this resistance by D test (double disc diffusion test) is necessary. Conventionally, laboratory susceptibility test for clindamycin usually cannot detect inducible clindamycin resistance and erythromycin resistant - clindamycin sensitive [6]. Variation of the prevalence of inducible clindamycin resistance in different geographical regions and different hospitals, we decide to study MRSA in health care providers for MLSB phenotypes, in particular inducible clindamycin resistance (MLSB_i).

MATERIAL AND METHODS

In this descriptive study, two hundred and twenty nine health care providers (such as nurses and health care workers) were examined from different wards in Mofid children hospital, Tehran, Iran. Office personnel were excluded. Nasal samples for examination of *S. aureus* isolated from persons during one year august 2012 - july 2013. All subjects in this study were without underlying diseases and were not taking antibiotics two weeks before sampling a sterile moistened swab was inserted into each nostril to approximately 1 cm depth, and rotated five times. The samples were transferred quickly to the laboratory and were inoculated onto mannitol salt agar medium and incubated at 35 °C for overnight. The isolates were identified as *S. aureus* based on morphologic and biochemical tests [7]. All the strains were screened for methicillin resistance by oxacillin (1µg) and cefoxitin (30 µg) disk diffusion test based on standard guidelines [8].

Antibiotic resistant pattern

The resistant patterns of MSSA and MRSA strains were determined by disk diffusion method (Kirby-Bauer). The antibiotics panel was: penicillin (10units), cefpodoxime (10µg), oxacillin (1µg), vancomycin (30µg), linezolid (30µg), clindamycin (2µg), ciprofloxacin, rifampicin (5 µg), teicoplanine (30 µg), cefepime, erythromycin (15µg), cefotaxim (30µg), azithromycin (15µg), and ceftazidim (30 µg), minocycline (30µg), doxycycline (30µg), trimethoprim-sulfamethoxazole (25µg), ceftriaxone (30µg). Zone diameters were

measured after 24 h incubation at 35°C. Zone inhibition diameters as recommended by clinical and laboratory standards institute (CLSI 2012) American type culture collection (ATCC) 29213 *S. aureus* was used as the control strain [8]. All the isolates which showed clindamycin-erythromycin discordant sensitivity results were further subjected to D test as per CLSI guidelines. Briefly, erythromycin (15 µgm) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µgm) disc on Muller- Hinton agar, previously inoculated 0.5 Mcfarland bacterial suspensions. Following overnight incubation at 37°C, flattening of zone (D shaped) around clindamycin in the area between the two discs indicated inducible clindamycin resistance [6].

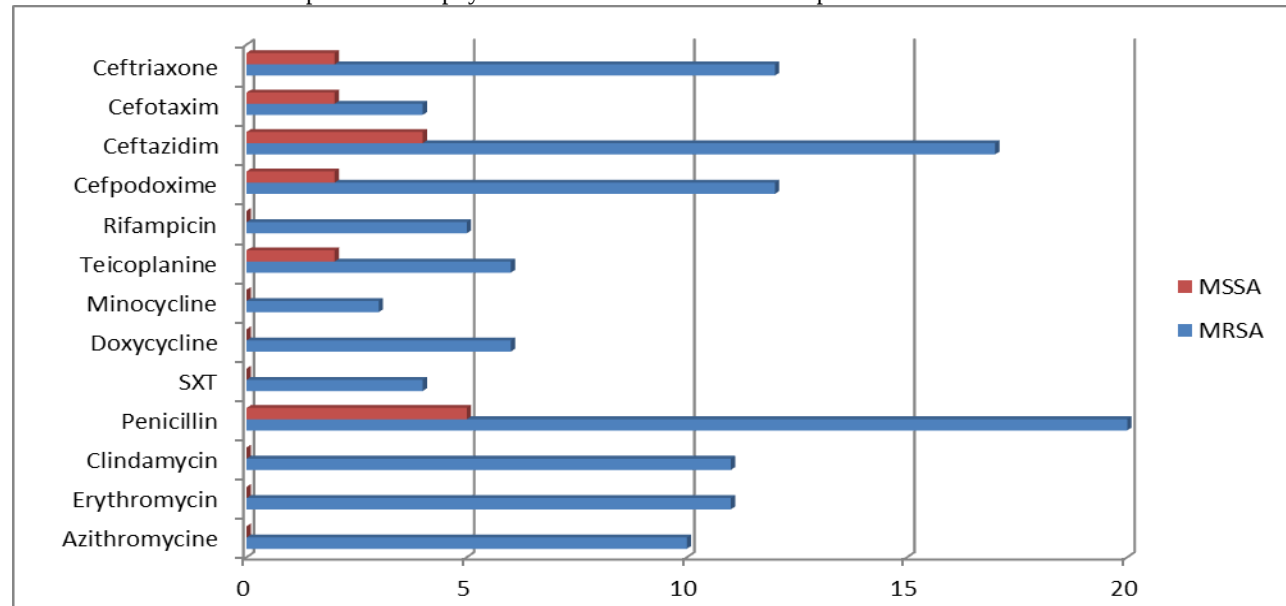
Statistical analysis

Statistical analysis was conducted using the SPSS version 16. Fisher's exact test was used to evaluation relation between MRSA and MSSA. P-value of less than 0.05 was considered as significant.

RESULTS

In this study, 229 health care providers (23-49 years old) from different hospital wards (infectious, gastrointestinal, pediatric intensive care unit, neonatal intensive care unit, endoscopy ,neonatal, hematology, neurology, surgery, nephrology , respiratory, dialysis, emergency, laboratory, radiology and pediatric infectious research center)were studied.

Two hundred (87.33%) were female and 29 (12.66%) were male. No significant differences were observed in MRSA colonization between health care providers in various wards. Staphylococci were isolated in 27 cases (12%). MRSA were 21 cases (77.7%) and 6 cases (22.3%) were MSSA. No significant difference was between the age ($p = 0.920$), sex ($p = 0.315$) and different wards in of MRSA and MSSA carriers. Antibiotic resistant pattern in MRSA was more than MSSA but no significant difference was between them .MSSA was sensitive to most antibiotics. All strains in this study were sensitive to linezolid and vancomycin and the rate of penicillin resistance was high in both groups (Table1).

Table 1. Antibiotic resistant pattern of Staphylococci isolated from health care providers

DISCUSSION

The worldwide emergence of MRSA is a remarkable challenge for public health [9-11]. Based on centers for disease control (CDC) reports, 1 % of all Staphylococcal infections and 50 % of healthcare-associated Staphylococcal infections are caused by MRSA [3]. In examination of 229 samples, 21(12%) MRSA was detected. Similar with our study conducted in Germany 2007[12] and west of Iran 2013[13], the prevalence of MRSA isolates among health care carriers was reported 11.3% and 17.57%, respectively.

Compared to Germany (6.5%), Dutch(1.4%), a study in Shiraz, Iran(5.3%), the other study in PIRC,Iran(3.2%),Switzerland(3.3%), the USA (3.4%), France (6.6%) and the UK(6.7%), the prevalence of MRSA was less than our study[14-21]. Rezaei and, etal considered colonization with methicillin resistant and methicillin sensitive *Staphylococcus aureus* subtypes in patients with atopic dermatitis .They found a higher rate (33%) of MRSA colonization in the nasal cavity. Because, MRSA is one of the most organisms that find on their skin. The high percentage of MRSA in health care providers, especially who do not exhibit any symptoms or signs of severe disease is very dangerous. Because they can cause epidemic,

raise the occurrence of severe diseases among patients, and enhance mortality rates by transfer the strains to patients [22]. Linezolid is one of the most effective oral medications used for outpatient treatment of MRSA infections that is resistant to other antibiotics. In this study, there was no resistance against linezolid in both groups [23]. MRSA nasal colonization isolates showed variable resistance to clindamycin, ceftriaxone, cefpodoxime, azithromycine, and erythromycin [23]. Resistant to penicillin and clindamycin [23, 24] was similar with the other studies .Moderate resistance to other conventional antibiotics (such as azithromycin, erythromycin, clindamycin, cefpodoxim, ceftriaxon were detected in MRSA [22]. By definition, all MRSA isolates carry the *mecA* gene, which confers resistance to all beta-lactam antibiotics, including cephalosporins and carbapenems .In our study and similar studies some MRSA are susceptible to some beta lactams such as cephalosporins[25].

Some additional auxiliary factors, increase MRSA susceptibility to beta-lactams or other clinically used antibiotics. These auxiliary genes including *femX* (*fmhB*), *murE*, *pbp2*, *SAV1220*, *SAV175* and *femD* (*glmM*) were loyalty identified to give back beta-lactam susceptibility of MRSA strain context. [26] In every region, rating of resistance or

sensitivity of MRSA against conventionally antibiotics is different. When antimicrobials are considered for therapy, susceptibility testing for antibiotics for every isolate of MRSA should be done. This study showed that all MRSA isolates were significantly less sensitive to antibiotics compared with MSSA isolates [24]. A remarkable result in this study was high percentage of MRSA in health care providers. Unfortunately, it is thought that rate of MRSA in health care providers carriers are less than clinical samples. So, MRSA screening in these persons often don't study or seldom examine in Iran. The best program for monitoring of MRSA spread and infection remains to debate formally. However, studies have consistently indicated that screening is advantageous in high-risk units to discover the reservoir and to begin contact cautions. Management programs may be useful in decreasing to occur the MRSA infection in health care providers' carriers [27]. Current studies show highly change carrier rate ranging from 0% to 29% [23, 28-36].

Neerja Jindal and et al. in 2013, studied the prevalence of inducible clindamycin resistance among clinical isolates of Mrsa in Malwa region of Punjab (north India). Of the total of 288 Staphylococcal isolates studied, 116(40.27%) were found to be MRSA. 54 isolates were resistant to erythromycin but sensitive to clindamycin. D test showed that it was positive in 21(18.1%) indicating inducible clindamycin resistance. In MSSA, inducible clindamycin resistance was observed in 10(5.81%). It was concluded that clindamycin could be used for the treatment of both MRSA and MSSA infections but after doing a simple, inexpensive D test ,and ruling out inducible resistance to clindamycin [6]. In study Deepa and et al. in 2013, among the 373 clinical isolates of Staphylococci which were studied, 134 isolates showed a discordant resistance pattern. 45 (33.6%) isolates were D-test positive, which had inducible

clindamycin resistance and belonged to the inducible macrolide lincosamide streptogramin-B phenotype (MLS_{Bi}). 89 (66.4%) isolates were D-test negative and they belonged to the macrolide streptogramin phenotype (MS). Among the MLS_{Bi} phenotypes, 6 (13.3%) isolates were methicillin-resistant *Staphylococcus aureus* (MRSA). So, the D-test becomes an imperative part of the antimicrobial susceptibility tests for all the Staphylococcal isolates on a routine basis. Thus, clindamycin can be removed in patients with infections due to MLS_{Bi} phenotype, to avoid possible therapeutic failures. The increasing of the inducible resistance (MLS_{Bi}) compared with the constitutive (MLS_{Bc}) resistance among Staphylococci and the indiscriminate use of antimicrobials has deteriorated the sensitivity pattern [37,38]. Mahima Lall and et al. In 2014, 16.6% of MRSA showed constitutive resistance and 37.5% inducible MLS_{Bi} resistance. Community associated MRSA (CA-MRSA) represented 10% of all isolates and had lower prevalence of MLS_{Bi} than hospital associated MRSA (HA-MRSA). They found a high prevalence of 20.3% of MLS_{Bi} amongst all staphylococcal isolates [39].

Conventional methods for MRSA screening need to be reconsidered and only use of phenotypic approaches for detection should be abandoned.

Given the high rates of MRSA in health care providers in this study, detecting the carriers and decolonizing them to reduce transmission of *S. aureus* in the hospital is important. Annual screening of these persons along with patients is recommended.

ACKNOWLEDGMENT

This work was supported by pediatric infection research center (PIRC) of Mofid children hospital in Tehran. We thank Professor A. Karimi and Dr S. Maham and staff of PIRC for their support.

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