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Research Article

Nosocomial Infection caused by Healthcare-Associated (HA) and Community-Associated (CA) Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Abstract

Between 01 June 2017 and 30 June 2018, at Security Forces Hospital (SFH), Saudi Arabia, 79 MRSA were isolated from Clinical specimens collected from Intensive Care Unit (ICU) patients with skin and soft tissue infection 69.6% (55/79), bloodstream infections 12.7% (10/79) and from tracheal 17.7% (14/79). During hospitalization we found the frequency of nares colonized by MRSA was 21.5% (17/79). Based on the Centers for Disease Control and Prevention (CDC) definition all the infections are nosocomial (NI) and all isolates could be healthcare associated (HA-MRSA) or community acquired (CA-MRSA) originated. Antimicrobial susceptibility testing were performed by microdilution (Dade Behring MicroScan, Sacramento, CA) using the Walk-Away-96 SI and interpreted according to the Clinical and Laboratory Standards Institute (CLSI). The % of antimicrobial susceptibility to the antibiotics tested; clindamycin (CD), erythromycin (E), (ciprofloxacin (CIP), gentamycin (GEN), fusidic acid (FUC), tetracycline (TET), and trimethoprim/Sulfamethoxazole (SXT), were; 72, 63% 66%, 81%, 80%, 79% and 96% respectively. According the antibiotic susceptibility results we founded 47% are susceptible to all antibiotic tested except oxacillin and one antibiotic from other family. 28% of the isolates were considered as multidrug resistant *S. aureus* (MDRSA), these strains were resistant to more than three category of antibiotic and 4% (3/79) were extensively drug-resistant *S. aureus* (XDRSA), these strains are resistant to at least one agent in all but remain susceptible to only one or two categories. According the antibiotic susceptibility, 68% of our strains are behave like healthcare associated- community acquired (HCA-CA-MRSA). The Macrolide-Lincosamide-streptogramin B (iMLS B) resistance by D test was put up for all the erythromycin-resistant and clindamycin-sensitive strains. The overall D test positive in MRSA isolates was 5% (4/79 strains). The mecA gene detected by GeneXpert real-time PCR platform (Cepheid). In this study all the isolates were susceptible to vancomycin, the: Minimal Inhibitory Concentration (MIC) of (56%) 44/79 strains were less than µg/ml and for (44%) 35/79 of the strains were µg/ml. In this study no vancomycin heteroresistance in *S. aureus* (hVISA) or Vancomycin intermediate S. aureus (VISA) was isolated from all the strains especially from those with vancomycin MIC 2 µg/ml by screening method. Also, no increasing of vancomycin MIC value after 15 days Re-incubation of the MicroScan panels at 35°C with the same strains. Extensive genotyping and molecular characterization of the strains are in processing.

Keywords: *Methicillin Resistance Staphylococcus aureus (MRSA); Community Acquired MRSA (CA-MRSA); Hospital Acquired MRSA (HA-MRSA); Multi-Drug Resistant S. aureus (MDRSA); Vancomycin Heteroresistance in S. aureus (hVISA); Health Care Associated Community Acquired Methicillin Resistance Staphylococcus aureus (HCA-CA-MRSA)*

Abbreviations

MRSA: Methicillin-Resistant *Staphylococcus aureus*; SCCmec: Staphylococcal Chromosomal Cassette mec Gene; HA-MRSA: Healthcare Associated MRSA; CA-MRSA: Community-Associated MRSA; HCA-CA-MRSA: Healthcare Associated-Community Acquired MRSA; NI: Nosocomial Infections; ICU: Intensive Care Unit; CDC: Centers for Disease Control and Prevention; EUCAST: European Committee on Antimicrobial Susceptibility Testing; FDA: Food and Drug Administration; CLSI: Clinical and Laboratory Standards Institute; MIC: Minimal Inhibitory Concentration; MDRSA: Multidrug Resistant *S. aureus*; XDRSA: Extensively Drug-Resistant *S. aureus*; VRSA: Vancomycin resistant *S. aureus*; VISA: Vancomycin Intermediate *S. aureus;* hVISA: Heterogeneous Vancomycin intermediate *S. aureus*; PVL: Panton-Valentine Leukocidin; CD: Clindamycin; E: Erythromycin; CIP: Ciprofloxacin; GEN: Gentamycin; FUC: Fusidic Acid; TET: Tetracycline; SXT: trimethoprim/Sulfamethoxazole; PCR: Polymerase Chain Reaction; iMLS B: Macrolide-Lincosamide-Streptogramin B

Introduction

In the past decade Methicillin-resistant *Staphylococcus aureus* (MRSA) became a major pathogen in hospitals and in the community. *Staphylococcus aureus* is one of the most important isolated human bacterial pathogens. Methicillin-resistant *S. aureus* (MRSA), are usually resistant to other B-lactam antimicrobial drugs. By definition and according the CDC, MRSA considered as health care associated, if the strain was isolated only after hospitalization for ≥ 72h and if the year before the present hospitalization, the patient had any one of the following: hospitalization, surgery, residency in a long-term care facility, and hemodialysis or peritoneal dialysis, or at the present admission had indwelling percutaneous devices or catheters [1]. MRSA, first identified in the 1960s and traditionally was associated with healthcare facilities and called HA-MRSA, now it's prevalence has reportedly increased in the community and called Community-Associated or community-acquired MRSA (CA-MRSA). They are clinically, microbiologically, and genetically distinct from Healthcare-Associated MRSA (HA-MRSA) [2]. The first case of CA-MRSA infection in the United States was reported in 1980 [3]. Several studies demonstrated that methicillin resistance *Staphylococcus aureus* was acquired through different genes in CA-MRSA and HA-MRSA isolates. Specifically, *Staphylococcal* chromosomal cassette mec (SCCmec) types I, II, and III confer methicillin resistance in HA-MRSA whereas SCCmec types IV and V confer methicillin resistance in CA-MRSA [4]. The SCCmec types carried by HA-MRSA are larger than those carried by CA-MRSA and confer resistance to additional non–B-lactam antibiotics. CA-MRSA is therefore susceptible to a broader range of antibiotics than HA-MRSA [4]. A study of pathogens isolated at Canadian hospitals between 2007 and 2009 found the susceptibility of CA-MRSA to trimethoprim-Sulfamethoxazole 100.0%, gentamicin 98.7%, and clindamycin 86.1% to be greater than that of HA-MRSA (86.5%, 85.5%, and 27.8%), respectively. Antibiotic sensitivity profiles can consequently be used as an inexpensive means of classifying MRSA as health care associated or community associated [5,6]. For example, clindamycin susceptibility is predictive of CA-MRSA with 95% sensitivity, 80% specificity, and a likelihood ratio of Methicillin-resistant *S. aureus* isolates that are resistant to 3 or more non–B-lactam antibiotics can safely be categorized as HA-MRSA [5,6]. In Canada, more than 20% of nosocomial MRSA infections are caused by CA-MRSA. In our hospital this percent reach 74.5%. A recent study from Alberta found 27.6% of such hospital-onset MRSA infections were caused by CA-MRSA and 27.5% of community-associated infections were caused by HA-MRSA [7,8]. There is rational evidence that CA-MRSA is more likely than HA-MRSA to be associated with Skin and Soft Tissue Infections (SSTIs). [9,10]. CA-MRSA is more likely than HA-MRSA to carry Panton Valentine leukocidin, a known virulence factor [7,10,11] often associated with tissue necrosis SSTIs [5,10]. CA-MRSA and HA-MRSA can be differentiated in several ways. These include presumed location of acquisition (i.e. community or hospital), [12]. Antibiotic susceptibility pattern [4] and genotyping [13,14] the latter being the most definitive. Since two decades some strains of MRSA with reduce susceptibility to vancomycin was reported from different country in the different contents, they include vancomycin intermediate *S. aureus* (VISA), heterogeneous hVISA and vancomycin-resistant *S. aureus* [15,16]. The first strain of hVISA was isolated from Saudi male patient presenting with severe sepsis immediately after admission in our ICU at SFH, Riyadh Saudi Arabia [17].

Materials and Methods

Nosocomial infection case and Specimen nature

79 strains of MRSA were isolated from wound and soft tissue, bloodstream infections, tracheal According to the infection control criteria, all our strains considered as HA-MRSA infection, the infection by MRSA appear 72h after the present admission.

Prevalence of nasal carriers of MRSSA among our patients

All the patients were screened at the time of admission for MRSA from nose swab at the present hospitalisations and one week after admission. The presence of MRSA was done by the present of *mec*A gene which encodes PBP2a detected by rapid real-time Polymerase chain reaction (RT-PCR) the Cepheid GeneXpert as recommended by the CLSI. All positive case treated by Mupirocin for 5 days.

Identification and antimicrobial susceptibility

Identification and antimicrobial susceptibility was done by microdilution (Dade Behring MicroScan, Sacramento, CA) using the Walk-Away-96 SI. The susceptibility results were interpreted according to CLSI criteria [18,19]. The MIC against vancomycin was also determined by MicroScan and by Etest (AB-Biodisk, Solna, Sweden) and by microdilution using a 0.5 McFarland standard suspension. Macro-Etest was also performed using a 2.0 McFarland suspension, as previously described [20]. These determinations were performed according to the manufacturer's instructions for Etest and interpreted according to CLSI recommendations [20]. All the panel of MicroScan showed MIC for vancomycin 2 µg/ml were re-incubated at 35°C for 15 days to cheek if any growth on the well with high concentration of vancomycin.

Detection of inducible clindamycin resistance

Testing for inducible clindamycin macrolide-Lincosamide-streptogramin B ($iMLS_B$) resistance was accomplished by the agar disk diffusion (D test) method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [20].

Surveillance and detection of hVISA and VISA from MRSA with 2 µg/ml vancomycin MIC

BHIA6V screening: In house BHI Agar plates with 6 mg/ml of vancomycin were prepared. 10 µl of 0.5 McFarland suspensions of each of the isolate was inoculated as spot of 15 mm in diameter. These plates were incubated at 35°C for 24 and 48h and were observed carefully in transmitted light for growth. Isolates were considered VISA/VRSA; hVISA or VSSA, if there was confluent growth, countable growth or no growth respectively after 48h of incubation [21]. In this study we re-incubation the MicroScan panels with MRSA having MIC-vancomycin 2 µg/ml for 15 days at 35°C, to see if there is any increase of MIC which could reflect the presence of vancomycin reduce susceptibility MRSA (VISA or hVISA).

Results

79 strains of MRSA were isolated from wound and soft tissue 69.6% (55/79), bloodstream infections 12.7% (10/79) and from tracheal 17.7% (14/79) (Table 1). Out of the 79 patient, the prevalence of the MRSA nasal colonization in this study was 21%. The patterns of susceptibility of these strain were comparable to those isolated from the clinical specimens of the patients. As recommended Mupirocin was applied to the anterior nares twice daily for 5 days. The % of antimicrobial susceptibility to the antibiotics tested; CD, E, CIP, GEN, FUC, TET, and SXT, were; 72, 63%, 66%, 81%, 80%, 79% and 96% respectively (Table 2). 28% of the isolates were considered as multidrug resistant *S. aureus* MDRSA and 4% considered as XDRSA, according the antibiotic susceptibility all are considered as hospital originated (Table 3). According to the susceptibility results 68% of our strains are behave like HCA-CA-MRSA and 32% as HA-MRSA (Table 4). The $iMLS_B$ resistance by D test was put up for all the erythromycin-resistant and clindamycin-sensitive strains. The overall D test positive in MRSA isolates was 5% (4/79 strains). After 15 days re-incubation of the MicroScan panels at 35°C, we did not detecting any increasing of vancomycin MIC value. By vancomycin screening agar with 4 and 6 µg/ml method, we did not detect any decreased vancomycin susceptibility in MRSA isolates include in this study especially from those with vancomycin MIC 2 µg/ml. these results reflect the absence of decreased vancomycin susceptibility in MRSA isolates in our study. After reviewing the patients' medical record no any failure treatment by vancomycin was occurred. Molecular typing for studying the a unique toxin is commonly found produced by CA-MRSA; Panton-Valentine leukocidin (PVL) which is rarely found in healthcare-associated infections; and *the SCC mec* elements to differentiate between HA-MRSA types (I, II, and III) and CA-MRSA type (IV) [22] *are in* processing.

Type of Infections	Sample	Number & % of Simple
Bloodstream	Blood	10 (12.7%)
Respiratory tract	Tracheal	14 (17.7%)
Wound and soft tissue	Swab and Biopsy	55 (69.6%)

Table 1: *79 Nosocomial Infections Caused By CA-MRSA and HA-MRSA.*

VAN	SXT	GEN	FUC	TET	E	CD	CIP	MET
100	94	81	80	79	63	72	66	0

Table 2: *% of antibiotic susceptibility for (79 CA-MRSA and HA-MRSA)*

SXT: Trimethoprim/Sulfamethoxazole; GEN: Gentamycin; FUC: Fusidic Acid; TET: Tetracycline; E: Erythromycin; CD: Clindamycin; CIP: Ciprofloxacin; MET: Methicillin.

Number	CD	E	CIP	GEN	TET	SXT	V	FUS
12	S	S	R	S	S	S	S	R
2	S	S	R	R	R	S	S	R
9	R/4s	R	S	S	S	S	S	S
10	R	R	R	S	S	S	S	R
3	R	R	R	R	R	R	S	R
26	S	S	S	S	S	S	S	S
5	S	S	S	R	R	S	S	R
5	S	S	S	R	S	S	S	S
7	S	S	S	S	R	S	S	S

Table 3: *Antibiotic susceptibility pattern of NI-MRSA (N; 79 strains).*

SXT: Trimethoprim/Sulfamethoxazole; GEN: Gentamycin; FUC: Fusidic Acid; TET: Tetracycline; AZT: Azithromycin; E: Erythromycin; CD: Clindamycin; CIP: Ciprofloxacin; LEV: Levofloxacin; MOX: Moxifloxacin.

R/4s: Four Strain were D-test positive.

HCA-CA-MRSA	HCA-HA-MRSA
68	32

Table 4: *% of hospital and community acquired MRSA (NI) 79 MRSA 06/2017-06/2018.*

HCA-CA MRSA: Health Care Associated Community Acquired MRSA; HCA-HA MRSA: Health Care Associated Hospital Acquired MRSA; NI: Nosocomial Infection.

Discussion

This study demonstrate that a high % of NI-MRSA patients identified in our hospital ICU had HCA-CA-MRSA infections (68%), these results reflect the spread of MRSA in the community and the most of NI were caused by these strains. Most of CA-MRSA infections were from the skin and soft tissue (69.6%) which respond to wound care (cleaning, incision and drainage) and oral antibiotherapy. CA-MRSA

showed susceptibility to the different antibiotics ranged from 66% for Ciprofloxacin to 96% for SXT. In The present study we demonstrate that the most of MRSA caused NI are not originated in the healthcare facility but coming from the community. Because of long hospitalized; 21% of our patients were found colonized with health care-associated pathogens CA or HA-MRSA. Decolonization prevents both vertical and horizontal transmission, depending on the method. There are several decolonization methods, such as nasal, topical, and oral decontamination, with many different products, Mupirocin still remains the gold standard agent for nasal decolonization of *S. aureus*, but there is concern about Mupirocin resistance, and alternative agents are needed. According the Lists of antimicrobial categories proposed for antimicrobial susceptibility testing were created using documents and breakpoints from the (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the United States Food and Drug Administration (FDA) [23], 28% of our isolates were are considered as MDR and 4% as XDR. Antibiotic susceptibility of CA-MRSA revealed that majority of the isolates were sensitive to the routinely used antibiotics except the resistance to lactams or with one antibiotic from another group. In the present study, there was significant difference in the susceptibility pattern of CA-MRSA and HA-MRSA to most of the antibiotics; macrolide, tetracycline, gentamicin, and ciprofloxacin. Different studies from different countries including ours demonstrated the increasing of the MRSA in the community and caused HCA-MRSA infections. Several other reports have quoted similar antibiotic susceptibility pattern, suggesting CA-MRSA to have wider antimicrobial susceptibility pattern compared to HA-MRSA [15,19-21]. In this study, we showed a significant difference in the antibiotic susceptibility pattern of CA-MRSA and HA-MRSA and the most of NI in our facility caused by CA-MRSA (68%). D-test was positive in 5% (4/79 strains) in both HCA-CA-MRSA which is similar to the other study reported in the other area [24]. As erythromycin and clindamycin are considered treatments of choice, however, resistance to erythromycin with false susceptibility to clindamycin *in vitro* may lead to therapeutic failure. In this study it's very interesting for cotrimoxazole which found non active only against extensive drug resistant *S. aureus* (XDRSA). The reasons for the increasing incidence of MRSA in the hospital and community could be multifactorial. Selection pressure due to overuse of antibiotics could have contributed to the emergence of these pathogens. In this study and according the criteria of CLSI and EUCAST about MIC of vancomycin, the MRSA with MIC 2 µg/ml still susceptible and we still advise to treat a patient infected by these strains but under controlling the patient condition and the clinical response. Since the first reports of hVISA/VISA [25], their prevalence differed among geographic regions the incidence of hVISA was 6.81% in Asia and 5.60% in Europe/America, and that of VISA was 3.42% and 2.75%, respectively. In 2010, a methicillin-resistant *Staphylococcus aureus* strain with reduced susceptibility to vancomycin (hVISA), Was isolated from a 69-year-old Saudi male patient presenting with severe sepsis immediately after admission in our ICU [17]. In this study we demonstrated that MRSA with vancomycin MIC 2 µg/ml still susceptible but we advise to control the treatment of patients infected by these strains [26]. Finally, the use of glycopeptides antibiotic should be limited and prolonged courses should be avoided if possible as this is strongly associated with the selection of glycopeptides resistance.

Conclusion

The prevalence of CA-MRSA appears to be on the rise globally and become a worldwide major health problem both in community and hospital predominantly associated with purulent SSTIs. In this study and according the antibiotic susceptibility, probably 68% of Nosocomial Infection-MRSA are HCA-CA-MRSA. CA-MRSA strain has already disseminated into the hospital and has probably adopted multiresistant genes from the hospital strains which explain the replacement of HA-MRSA by CA-MRSA infections in the hospital. This study demonstrated that the cotrimoxazole could be as a good marker for the XDR-MRSA. Vancomycin MIC values is recommended to control the probable failure treatment. The absence of hVISA and VISA among our isolates of MRSA with MIC 2 µg/ml never exclude there existence. This study suggests that efficient infection control protocols and Antibiotic Stewardship program should be considered and adopted in hospitals to prevent the spread of these strains between patients, in the facility, in the country and globally.

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