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Examination methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence in cockroaches from hospital in Chaharmahal-va-Bakhtiari province, Iran by polymerase chain reaction (PCR)

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This study gives the reported prevalence of cockroaches and the medical importance of the insects due to the transmission of nosocomial infections. Besides, one of the major reasons of hospital and community infections all over the world is methicillin-resistant *Staphylococcus aureus* (MRSA). The aim of this study was to determine inset the hospital cockroaches as the main factor for MRSA infections transmission and also determine antibiogram pattern MRSA. In this study, going to hospitals, over 100 cockroaches were collected using hand and Telesticky methods and enriching the intended strain on a specific medium. Then was a designed primer for mecA gene and amplification in polymerase chain reaction (PCR). The disk diffusion method was used for specifying resistance patterns in line with guidelines of Clinical and Laboratory Standards Institute (CLSI). Results showed that a total of 44 (62.86%) of 100 samples were contaminated with *S. aureus* isolated from cockroaches hospitals, also with molecular sieve of PCR, an addition of 8 (19.56%) of the strain contained the mecA gene. The overall resistance of isolated MRSA strains to antimicrobial agents was 8 (100%) for methicillin, 7 (87.5%) for cefixime and 6 (75%) for vancomycin, which had more resistance, respectively. This study implies that cockroaches, as a potential factor in transmission of MRSA function and medical resistance pattern of MRSA are different in different areas.

Key words: Antibiogram, resistance, methicillin-resistant *Staphylococcus aureus* (MRSA), Chaharmahal-va-Bakhtiari hospitals, polymerase chain reaction (PCR).

INTRODUCTION

In nineteenth century, the role in the transmission of disease to humans from insects was demonstrated (Service, 1979). Among these insects are cockroaches, which can influence human health; the reason of which medical importance of insects is of top priority among the food habits and stooling at various intervals, poor bites especially between the toes, that is potentially dangerous to human health (Zaeim et al., 2008). Cockroaches are normally carriers of about 40 different species of pathogenic bacteria that infect vertebrates (Vatandoost and Mousavi, 2009). They have ability to transfer at least 7 species of intestinal worms, among which include bilharziver, teniasis, askaryazys, necatoriasis (Doroodgar et al., 2005) and the transmission of poliomyelitis and

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Aspergillus fungi. More than what is thought, naturally, cockroaches are infected with pathogenic bacteria, thus causing leprosy, yeki, bloody diarrhea, pimples, Hungarian unitary tract abscesses, and food poisoning. Almost all cockroaches have co-existence with 150 species of bacteria and 60 species of yeast species, and 90 species protozoa and 45 species of the pathogenic ring worm (Salehzadeh et al., 2007). So far, numerous pathogenic bacteria, including *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* have been isolated from cockroaches (Fotedar et al., 1991; Foster 2004).

Staphylococcus aureus is a Gram-positive bacterium, and about 20% of the human population carries this commensal bacterium without any clinical symptoms. However, *S. aureus* has the potential to cause a broad range of infections, including wound infections, skin abscesses, pneumonia, bacteremia, meningitis, and toxic flick syndrome (Iandolo 1989). It is well documented that strains of *S. aureus* produce a variety of extracellular protein toxins, including enterotoxins, toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ET), hemolysins, and coagulase. The two ETs, exfoliative toxins A and B (ETA and ETB), in continued or independently, are implicated in the cause of staphylococcal scalded-skin syndrome (Iandolo 1989). In the early 1960s, only 2 years after the introduction of methicillin as a drug versus *S. aureus* infections, hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) strains were first isolated.

Since the 1990s, virulent association-acquired MRSA (CA-MRSA) strains, which are characterized by the presentment of the toxin Panton-valentine leukocidin (PVL), and have been encountered in the community and health care (Deurenberg and Stobberingh 2008). MRSA is an eminent cause of nosocomial infections worldwide and has also emerged as a community-associated pathogen (Chambers and Deleo, 2009). The risk agents for infection with these pathogens that are unique to the hospital population are well established (Lowry, 1998). Over the last few decades, there has been an enormous gain and emergence of MRSA as a cause of infection in the community in patients who have never been hospitalized and have no other known risk factors, for MRSA infection causes significant worry. Infections caused by these organisms have been described in earlier studies as having specified strain, virulence, and epidemiologic properties (Diep et al., 2006a; Diep et al., 2006b; Tenover et al., 2006). According to serological classification, to date, six staphylococcal enterotoxin (SE) groups have been recognized: staphylococcal enterotoxin A, B, C, D and E (SEA SEB, SEC, SED and SEE) (Martins et al., 2007) and the recently described SEH (Mehrotra et al., 2000). Community-associated MRSA (CA-MRSA) strains differ from health care-associated *S. aureus* strains in that they are more often improved from skin and soft tissue sources, with at least two clones, designated USA300 and USA400.

For both groups of patients with CA-MRSA (*S. aureus* resistant to methicillin patients) and patients with CA-MSSA (sensitive methicillin patients with *S. aureus*) skin infections were the most common location (80% of patients infected with MRSA and 93% of patients infected with MSSA), other infection sites included the respiratory tract (13% for MRSA-infected patients and 6% for MSSA infected patients), blood (4% for MRSA infected patients and 1% for MSSA infected patients), and urine (3% for MRSA-infected patients) (Davis et al., 2007). The *S. aureus* strains resistance to these antibiotics is attributed to the presence of mecA, whose product is a 78 kDa protein called penicillin binding protein 2a (Mehrotra et al., 2000), whose transcriptional control may be mediated by a repressor (mecI) and a sensor/inducer (mecR1). The mecI-mecR1-mediated induction of mecA takes various hours, rendering the strains phenotypically susceptible in spite of the presence of the resistance gene. Therefore, it has been proposed that the full resistance to b-lactams observed in many contemporary clinical *MRSA* strains requires a non-functional mecI-mecR1 regulatory system. The mecA gene is embedded in a large chromosomal cassette (the SCCmec element) for which several structural types have been characterize.

The MRSA characteristic phenotype is due to the presence of mecA which encodes a penicillin-binding protein (PBP, PBP2a), with degraded affinity for b-lactams (Oliveira and De Lencastre, 2011). Methicillin resistance is associated with the presence of a chromosomal mobile genetic element entitled the staphylococcal cassette chromosome mec (SCCmec) (IWG-SCC 2009). So far has been identified five different types of Sccm (V, IV, III, II and I). 20 KP to 68 KP have the variable type III which is the most dangerous (Martins et al., 2007; Zhang et al., 2005). Some epidemic MRSA clones, typically expressing full b-lactam resistance, carry SCCmec elements that contain an intact mecI-mecR1 locus (for example, SCCmec types II and III) (Oliveira and De Lencastre 2011). Of cause, one of the efficient ways of controlling the spread of MRSA is through determination of the genotypic characteristics as well as species genetic relatedness in geographic other regions (Gomes et al., 2006; Enright et al., 2002). Since the hospital environments provide them with suitable temperature, humidity and a ready source of food, presence of cockroaches here is uncommon.

Many researches in recent years have shown that drug resistant bacteria are of great importance in hospitals that are potential carriers of microorganisms, and their presence makes the problem more significant. The aim of this research was to use PCR to determine the prevalence MRSA in cockroaches from a hospital in Chaharmahal VA Bakhtiar, Iran. The study also tried to determine the pattern of antibiotic resistance in summer of 2011.

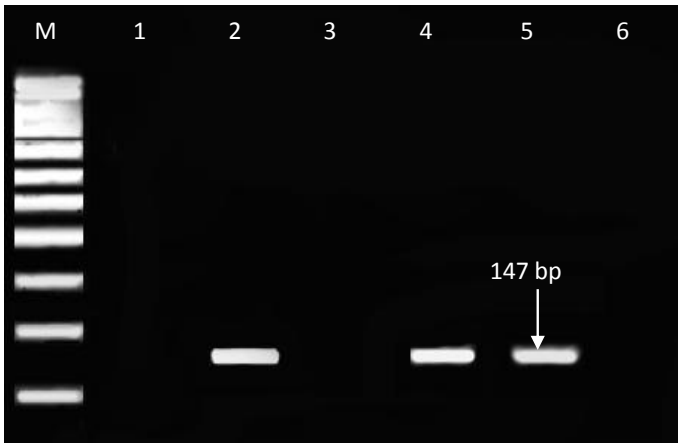

Figure 1. Agarose gel stained with ethidium bromide, with PCR products of MRSA isolates. Line M is 100 bp DNA ladder, line 1 is negative control, line 2 is positive control, line 3 and 6 are MRSA negative samples, line 4 and 5 are MRSA positive samples.

Table 1. Antimicrobial resistance of isolated *S. aureus* strains from different samples to studied antibiotics.

Antimicrobial agent	Resistance (%)
Nitrofurantoin	25 (56.8)
Methicillin	32 (72.72)
Vancomycin	26 (59.09)
Cefixime	39 (88.63)
Trimethoprim sulfamethoxazole	16 (36.37)
Amikacin	3 (6.81)
Ciprofloxacin	1 (2.27)
Gentamcin	5 (11.36)

MATERIALS AND METHODS

Sample collection

A total of 100 cockroaches were collected over a period of two month, from different wards of six hospitals located in Chaharmahal VA Bakhyiari province (Hajar Shahrekord, Ayatollah Kashani Shahrekord, Shohada Farsan, Imam Javad Naghan, Imam Reza Lordagan and Valiasr Boroujen). In these cockroaches' samples, 21, 24, 14, 12, 14, 15 specimens were obtained from hospitals, respectively. The collection of sample was done using manual and sticky trap methods from kitchens, wardrobe departments, and pediatric patients and they were transmitted to the Biotechnology Research Center of Shahrekord Islamic Azad University using separate sterile tube to prevent any contamination mixing of the samples.

Picking up the cultivation of susceptibility

The collected samples were incubated in sterile conditions on beard parker environment for 24 h at 37°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles were determined by the dilution method on Mueller-Hinton agar, according to the guidelines of Clinical and Laboratory Standards Institute (CLSI 2012). The antimicrobial agents tested included nitrofurantoin, methicillin, vancomycin, cefixime, trimethoprim sulfamethoxazole, amikacin, ciprofloxacin and gentamcin. Clinical and Laboratory Standards Institute breakpoints were used for minimum inhibitory concentrations (MIC) interpretation (CLSI 2012). The results were interpreted after 24 h of incubation at 37°C, as sensitive, intermediately sensitive, and resistant according to the zone diameter around each antibiotic disk.

PCR

To confirm the presence of MRSA mecA gene, PCR test was performed. Therefore, the final volume of 25 microliter PCR reaction containing 2 microliter of deoxyribonucleic acid (DNA) template, $MgCl_2$ concentration of 1.5 mM, 2 micromoles dNTPS, 2 mMol of each primers mecA-F: 5´-AACAGGTGAATTATTAGCACTTGTAAG-3 and mecA-R: 5´-ATTGCTGTTAATATTTTTTGAGTTGAA-3, and a single DNA polymerase was performed; thermal PCR conditions consisted of 5 min at 95°C and then 31 cycles Vasrsht initial temperature of 94°C, temperature of 61 and 72°C connector at each end for 1 min, and final extension was for 5 min at 72°C. For analysis of PCR products, the amplification products were analyzed in 1% agarose gel electrophoresis. Electrode buffer was TBE (Tris-base 10.8 g, 89 mM, boric acid 5.5 g, 2 mM EDTA (pH 8.0) 4 ml of 0.5 MEDTA (pH 8.0), with all components combined in sufficient H_2O and stired to dissolve). Gels were stained with ethidium bromide, aliquots of 10 µl of PCR products were applied to the gel. Constant voltage of 80 V for 30 min was used for products separation. After electrophoresis, images were obtained in ultra violet imager (UVI) doc documentation systems (UK).

Statistical analysis

The numbers of cockroaches presenting airsacculitis and the prevalence of re-isolation of *S. aureus* from the swap were analyzed by the chi-square test using the statistical package for social sciences (SPSS) 17 (SPSS Inc. Chicago, IL, USA) software. The probability level for significance was $p \leq 0.05$.

RESULTS

The quality of extracted DNA from samples was examined by electrophoretic analysis through a 2% agarose gel. Of 100 cockroaches collected from hospital in this study, 44 samples (62.86%) were infected with *S. aureus*. The mecA gene of MRSA was successfully amplified with the MecA-F and MecA-R primers. Agarose gel electrophoresis of the PCR amplified products is show in Figure 1. From 44 *S. aureus* samples that assayed by PCR in this research, only 8 samples (19.56%) were positive to MRSA (147 bp fragment).

There were antimicrobial susceptibility pattern of isolated *S. aureus* strains to studied antibiotics as shown in Table 1. The overall susceptibility of isolated *S. aureus* strains to antimicrobial agents was 97.73% for ciprofloxacin, 93.19% for amikacin, 88.64% for gentamicin, 63.63% for trimethoprim sulfamethoxazole, 43.2% for nitrofurantoin, 40.91% for vancomycin, 27.28%

Table 2. Antimicrobial resistance of isolated MRSA strains to studied antibiotics.

Antimicrobial agent	Resistance (%)
Nitrofurantoin	5 (62.5)
Methicillin	8 (100)
Vancomycin	6 (75)
Cefixime	7 (87.5)
Trimethoprim sulfamethoxazole	3 (37.5)
Amikacin	0 (0.0)
Ciprofloxacin	0 (0.0)
Gentamcin	0 (0.0)

Table 3. Comparison of resistance pattern of *S. aureus* strains to antimicrobial agents in different studies.

Antimicrobial agent	Resistance (%) present study (Iran, 2011)	Resistance (%) Ekrami & Samarbafzadeh (Iran, 2007; Ekrami et al., 2010)	Resistance (%) R Baral & B Khanal (Nepal, 2008; Baral et al., 2011)	Resistance (%) Khalili (Iran,2008; Soltani et al., 2010)
Nitrofurantoin	56.8	-	-	-
Methicillin	72.72	-	26	-
Cefixime	88.63	-	-	-
Trimethoprim sulfamethoxazole	36.37	-	-	-
Ciprofloxacin	2.27	68.2	11.36	41.9
Gentamicin	11.36	30.6	22	44.9
Amikacin	6.81	25	-	39.3
Vancomycin	59.09	0	0	0

(Ekrami et al., 2010), (Baral et al., 2011); (Soltani et al., 2010).

for methicillin, and 11.37% for cefixime. According to these results, ciprofloxacin, amikacin, and gentamicin were the most effective agents against isolated *S. aureus*. It also showed that isolated *S. aureus* of cockroaches from hospital has more resistance to vancomycin rate of 59.09%, rather than other studies. Antimicrobial susceptibility of MRSA isolates from cockroaches is shown in Table 2. Gentamcin (100%), amikacin (100%), ciprofloxacin, and trimethoprim sulfamethoxazole (62.5%) were the most effective agents against these isolates. Comparison of resistance pattern of *S. aureus* strains to antimicrobial agents in different studies is shown in Table 3.

DISCUSSION

S. aureus is a gram-positive bacterium that can be part of the normal human microbiota as a colonizer of the mucosal membranes and skin. However, *S. aureus* has the potential to reason a wide range of infections, including wound infections, skin abscesses, pneumonia, bacteremia, meningitis, and toxic shock syndrome (Foster, 2004). MRSA is the origin cause of nosocomial infection worldwide (Lowry, 1998). MRSA is a main nosocomial isolate in hospitals that is responsible for higher morbidity. Sources of MRSA are infected patients, asymptomatic colonized hospital party and hands of health care workers serving in intensive care units (ICUs) on MRSA positive cases (Nickersin et al., 2009). Variegation types of the staphylococcal cassette chromosome mec (SCCmec) are known to confer methicillin resistance on the human pathogen *S.*

Aureus (Chlebowicz et al., 2010). *S. aureus* is responsible for skin infection in mice (Nippe et al., 2011) and finding genes in *S. aureus* strain isolated from raw and pasteurized milk (Rall et al., 2008).

The results of the present conciliate revealed a contamination of almost all cockroaches collected from hospitals with different microorganism that is significantly higher in collation to control group (Salehzadeh et al., 2007). Although in the present study, only one isolate showed infections to cockroaches, *S. aureus* and MRSA, but reports from other studies indicate that infections can be seen in more bacteria. For instance, (Fotedar et al., 1991) showed that bacterial pathogens like *Klebsiella* spp, *P. Aeruginosa* and *S. aureus* were infections to cockroaches. This is similar with the study of Salehzadeh et al. (2007) that reported *S. aureus* of (16.5%) isolated from cockroaches of hospital of Hamadan Iran (Salehzadeh et al., 2007).

In a study performed by Fotedar (1991) in India, fewer than 5% of all *S. aureus* isolates were reported as MRSA (Fotedar et al., 1991). In the present study, also, a high percentage of test cockroaches (62.86%) were showed to carry *S. aureus*, some of them are of medical importance. However, only few numbers of cockroaches collected from hospitals (19.56%) showed to have MRSA contamination. It appears that cockroaches infected with MRSA are important endemic pathogen in our hospitals. Virtually all *S. aureus* strains were susceptible to penicillin G until 1994 when the first reports of penicillin-resistant *S. aureus* were reported, and today virtually all strains of *S. aureus* are resistant to natural penicillins.

Methicillin and other resistant penicillins were developed to treat infections reasoned by penicillin-resistant *S. aureus* and met with initial success; however, over time, strains of MRSA began to spread (Rice, 2006). Table 3 shows reported resistance pattern of *S. aureus* strains to antibiotics in some other studies compared to the present document. In a related study in Taleghani Burn Hospital of Ahvaz, Iran Medical University, MRSA was resistant to gentamycin (64.3%), ciprofloxacin (81.2%) (Ekrami and Kalantar, 2007). But in our study, MRSA was resistance to ciprofloxacin (0.0%) and gentamycin (0.0%) were high levels. It appears that MRSA has emerged as an important endemic pathogen in our hospitals. According to the reports, burst of MRSA is increasing in Europe. In Austria, 21.6%; Belgium, 25.1%; Spain, 30.3%; and France, 33.6% of isolated *S. aureus* strains are methicillin resistant (Ekrami et al., 2010). In a survey performed in Pakistan, 61.29% of isolated *S. aureus* strains were resistant to oxacillin (Farzana and Hameed 2006). In a survey performed by H. Khalili in Iran, 100% of strains sensitive to vancomycin was recorded among isolated *S. aureus* strains (Soltani et al., 2010). In the study performed in Pakistan, 100% of isolated *S. aureus* strains were sensitive to vancomycin (Farzana and Hameed, 2006). The difference in the current study was that about 75% of MRSA and 59.09% *S. aureus* isolates were sensitive to vancomycin. In Iran, vancomycin resistance rates of 11, 21, and 42.5% have been reported in different studies that have evaluated pediatric population (Mamishi et al., 2005; Haghi-Ashteiani et al., 2007; Kalantari et al., 2007).

CONCLUSION AND RECOMMENDATION

Finally, as stated earlier, our study showed isolated *S. aureus* of cockroaches from hospital have more resistance to vancomycin rate of 59.09%, than other studies. Amikacin, ciprofloxacin, and gentamcin seems to be the only antimicrobial agent that showed 100% sensitivity and may be used as the drug of choice for treating multidrug resistant MRSA infections. However, regular monitoring of amikacin, ciprofloxacin, and gentamcin sensitivity, and routine testing of other newer glycopeptides like teicoplanin should be carried out. Furthermore, the regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern of MRSA and formulation of definite antibiotic policy may be helpful for reducing the incidence of MRSA infection.

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