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Determining of antibiotic resistance profile in *Staphylococcus aureus* isolates

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ABSTRACT

Objective: To determine the pattern of antibiotic resistance among *Staphylococcus aureus (S. aureus)* isolates from clinical specimens and to identify community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in specimens that have been collected from patients referring to one of the hospitals of Ahvaz. **Methods:** *S. aureus* isolates from a hospital in Ahvaz were screened for resistance to various antibiotics including methicillin. The susceptibility of the isolates was determined by Kirby-Bauer disc diffusion method. The MRSA was also treated with ethidium bromide to find the origin of resistance. **Results:** Among the bacterial isolates, all of 11 *S. aureus* were resistant to methicillin and cefixime, 2 were resistant to ciprofloxacine, 6 were resistant to tetracycline and the reminder were sensitive or intermediate to other antibiotics. The treated isolates were reminded resistant to methicillin and this suggested that the plasmid was not the origin of resistance in these isolates. **Conclusions:** These results showed that infection due to MRSA is widespread in Ahvaz and with respect to the spread of vancomycin resistance among MRSA and appearance of overwhelming infections. It is necessary to identify continuously the profile of antibiotic resistance among *S. aureus* isolates in other regions and finding appropriate antibiotic for infection control and eradication.

1. Introduction

Bacterial resistance to antimicrobial agents is an out growing problem in the treatment of bacterial infections. This is due to the alteration of resistance mechanisms, acquisition of resistance genetic element from other bacteria and genetic changes in bacteria[1,2]. Hence, many researchers have been focusing on recognition of resistance mechanisms among bacterial isolates.

Historically, *Staphylococcus aureus(S. aureus)* has been recognized as an important cause of disease around the world and it has become a major pathogen associated with both hospital- and community-acquired infections. In the past, the availability of antibiotics, invasive infections caused by *S. aureus* were often fatal. The introduction of penicillin greatly improved the prognosis for patients with severe staphylococcal infections, but after a few years of clinical use, resistance appeared owing to production of β-lactamases that hydrolyze the penicillins. Therefore, penicillins that were resistant to the action of β-lactamases, such as methicillin, were developed to treat staphylococcal infection caused by β-lactamase-producing strains.

Methicillin was designed to resist β-lactamase degradation, but methicillin-resistant *Staphylococcus* aureus (MRSA) strains that were resistant to all β-lactam antibiotics were identified soon after methicillin was introduced into clinical practice[3]. Although there are three known mechanisms for which *S. aureus* becomes resistant to methicillin-hyperproduction of β-lactamases[4]; modification of normal penicillin-binding proteins (PBPs)[5] and the presence of an acquired penicillin-binding protein, PBP2a[6] of most clinical isolates present the latter mechanism.

Until recently, MRSA was predominantly a nosocomial pathogen causing hospital-acquired infections, but MRSA strains are now being increasingly isolated from community-acquired infections as well[7]. Although

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community-acquired-MRSA (CA-MRSA) strains cause mostly skin abscesses and furunculosis, severe necrotizing pneumonia and shock resulting in death has also been associated with CA-MRSA[8,9]. These new CA-MRSA strains are usually resistant to β-lactams but susceptible to other antimicrobial classes and carry mostly staphylococcal cassette chromosome *mec* (SCC*mec*) type IV. CA-MRSA strains are also more likely to possess unique combinations of virulence factors and seem to be genetically different from hospital-acquired-MRSA (HA-MRSA)[8-11]. Investigators have suggested that CA-MRSA strains have arisen from different genetic backgrounds rather than from the worldwide spread of a single clone[12].

Vancomycin has been the antibiotic of choice to treat MRSA infections, and the emergence of vancomycin-nonsusceptible *S. aureus* reported in recent years is a cause of great public health concern and has made therapy of MRSA infections even more challenging for clinicians[7].

Obtaining data about MRSA isolate and their pattern of resistance can be useful guideline for tropical infection control, antibiotic selection and more importantly to prognosis the resistant isolates in future.

The aim of this study is to determine the pattern of antibiotic resistance among *S. aureus* isolates from clinical specimens and to identify CA-MRSA in specimens that have been collected from patients referring to one of the hospitals of Ahvaz.

2. Materials and methods

2.1. Sample collection

Bacterial strains were collected from October 2006 till May 2007 from patients that have been refereed to one of the hospitals of Ahvaz. The samples were collected from urine, blood and wound infection. After culturing and bacterial isolation, the isolates were identified by standard biochemical tests such as morphology of colony, gram staining, catalase reaction, oxidase reaction, growth on MRSA, mannitol fermentation, coagulase production, resistance to bacitracin and sensitivity to furazolidone. These isolates were suspended in skim milk (10%) suspension and stored at -20 ℃, till antimicrobial resistance testing.

2.2. Antibiotic susceptibility test

To determine the susceptibility of these isolates, a culture was prepared in trypticase soy broth (TSB, Merck) after thawing the suspensions and incubated at 37 ℃ until reaching to turbidity equal to 0.5 Mc Farland turbidometer. A lawn culture was prepared from each isolate on Muller-Hinton Agar (Merck) and standard antibiotic discs with 6 mm diameter including methicillin, cefixime, tylosine, tetracycline, trimethoprim/sulfamethoxazole, ciprofloxacine and norfloxacine were placed on the culture. The plates incubated at 37 ℃ for 24h. After incubation, the average diameters of inhibition zone around each disc were recorded based on mm. The sensitivity of these isolates was determined by comparison of the above results with available data.

2.3. Determining the genetic origin of resistance

In order to determine if there was a plasmid responsible for resistance to methicillin or this trait was a chromosomal trait, ethidium bromide treatment was used for plasmid inactivation. This treatment would lead to plasmid elimination[13,14]. For this purpose 100 μg/mL concentration of ethidium bromide was used[15]. The isolates that were resistant to methicillin were cultured in TSB and the above mentioned concentration of ethidium bromide was added to each tube. The cultures were incubated at 37 ℃. After 24 h a culture was done from each tube on nutrient agar and after colony formation, one colony was transferred to Muller-Hinton broth. After reaching to 0.5 Mc Farland turbidity a lawn culture was prepared and methicillin discs were placed on the agar. Those isolates that were not able to grow in the presence of the methicillin were considered to be antibiotic resistance with plasmid origin.

3. Results

Table 1 showed the results of antibiotic susceptibility testing. Among 83 samples cultured during this survey 11 isolates of *S. aureus* were collected and identified by standard biochemical tests. Then, the antibiotic susceptibility of these isolates was assessed. All of the *S. aureus* isolates were determined based on the inhibition zone diameter (mm) as methicillin resistant strains (resistance: ≤ 9 mm, Intermediate: 9≤ inhibition≤14 mm, susceptible: 14 mm). Then all of these isolates can be considered as MRSA and with regarding to that they were isolated from patients without any previous hospitalization were named as CA-MRSA. Another finding was that all of these isolates were resistant to cefixime, a β-lactam antibiotic. This suggested that all of these isolates were β-lactamase producers and they may be resistant to other β-lactam antibiotics. Table 1 showed that the majority of isolates were multi-resistant to other tested antibiotics.

All of 11 isolate resistant to methicillin were cured with ethidium bromide in order to find the genetical origin of resistance. After curing all of them were remained resistant to methicillin and did not inhibit by methicillin disc in second antibiotic susceptibility test. So it can be concluded that the origin of resistance in all of the *S. aureus* isolates in this survey was chromosomal.

Table 1
The susceptibility pattern of *S. aureus* isolates to common antibiotics (mm).

Isolate	Antibiotics						
	SXT	ME	CP	CFM	TY	T	NFX
S83	23	0	28	0	20	0	32
S5	19	0	29	0	24	0	32
S2	13	0	31	0	22	25	31
S21	21	0	29	0	22	26	33
S11	26	0	33	0	25	24	34
S26	0	0	0	0	0	0	15
S76	25	0	18	0	15	0	20
S77	0	0	10	0	17	0	15
S73	22	0	34	0	28	16	33
S57	20	0	20	0	17	18	16
S29	26	0	17	0	15	0	17

Note: STX: Trimethoprim/Sulfamethoxazole, ME: Methicillin, CP: Ciprofloxacine, CFM: Cefixime, TY: Tylosine, T: Tetracycline, NFX: Norfloxacine.

4. Discussion

Since methicillin resistance emerged, MRSA has become widespread in hospitals worldwide, causing bacteremia, pneumonia, surgical site infections, and other nosocomial infections[16-20]. MRSA strains produce PBP2a that is not part of the intrinsic set of PBPs of *S. aureus* but is a unique, inducible, acquired protein that has a molecular weight of approximately 76 kDa, and it is produced only by methicillin resistant staphylococci[21]. PBP2a has low affinity for β-lactam antibiotics and, therefore, is capable of substituting the biosynthetic functions of the normal PBPs even in the presence of the β-lactams, thereby preventing cell lysis. Isolates containing the PBP2a-mediated resistance mechanism are clinically resistant to all available β-lactams, including penicillins, cephalosporins, β-lactam/β-lactamase inhibitor combinations, monobactams, and carbapenems[21,22]. PBP2a is encoded by the *mec*A gene, which is not present in methicillin susceptible strains, and it is believed to have been acquired from a distantly related species, although the exact origin has not been found yet[21, 23]. Bal *et al*[24] first sequenced *mec*A in 1987, and now it is known that this gene is carried on a mobile genetic element, the SCC*mec*[25]. In addition to carrying the *mec*A gene, the SCC contains regulatory genes, the IS431mec insertion sequence, and the recombinase genes *ccr*, which are responsible for the integration and excision of SCC*mec*[26]. The *mec* gene complex has been classified into four classes, and the *ccr* gene complex into three allotypes[23]. Different combinations of *mec* gene complex classes and *ccr* gene complex types have so far defined four types of SCC*mec* elements, labeled SCC*mec* types I-IV. With respect to the nature of these genetic elements responsible for resistance to methicillin, MRSA is increasingly grown and CA-MRSA will become more common. Most CA-MRSA isolates harboring SCC*mec* type IV are usually resistant to β-lactam and macrolide antibiotics, but susceptible to other classes[10]. Recently a novel type V SCC*mec* has been described in a CA-MRSA strain isolated in Australia[27].

It is clear that the emergence of CA-MRSA isolates is changing the management of clinical infections potentially caused by *S. aureus*. So rapid methods for accurate detection of MRSA are necessary to promptly identify patients and implement contact precautions as well as appropriate treatment.

Hence, survey is necessary for determining the rate of staphylococcus infections among different populations and also determining the source of resistance, plasmid or chromosome, is very important because plasmid resistance genes can more rapidly spread among bacterial isolate and cause rapid distribution of MRSA specially at hospital environments.

The results of this study revealed that *S. aureus* infection is widespread in patients referring to hospitals. All of the isolates were resistant to methicilin and with regard to the lack of any previous hospitalization in these patients, these strains can be named as CA- MRSA. The treatment of isolates with ethidium bromide showed that the genetic element responsible for resistance to methicilin was located on chromosome and plasmid did not have any role in this resistance. The plasmid mediated resistance can be rapidly spread specially in bacterial biofilms and health care centers. The occurrence of resistance to multiple antibiotics in the isolates in this study can be an alarm for infection treatment and can cause a major challenge for clinicians. Furthermore, these results necessitate continuous and regular monitoring and screening of *S. aureus* isolates from out-patients and as well as hospitalized patients for determining the prevalence of infection due to this agent, its antibiotic resistance pattern and finding appropriate antibiotic for treatment of these infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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