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# Analysis of genetic heterogeneity of *Staphylococcus aureus* strains isolated from food and clinical samples from northern Jordan using VNTR, toxin profiles and antibiograms

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## ABSTRACT

**Aims:** *Staphylococcus aureus* is a Gram positive pathogen distributed worldwide and represents a rising problem for both hospitals and community. The aims of the study were to examine the antibiograms, toxin profiles as well as the genetic diversity of a set of *S. aureus* isolates from clinical and food samples.
**Methodology and results:** To get some insights on the genetic heterogeneity and test for the presence of certain virulence genes, all isolates were subjected to different PCR amplifications and antibiotic sensitivity analysis. The *mec*A gene was detected in both clinical and food isolates. Resistance to penicillin and amoxicillin was observed in both clinical and food isolates. About 88% of both food and clinical isolates harbored the toxin gene *sea,* while 70% and 29% of clinical and food isolates respectively, harbored *sec*. The *seb* gene was detected in 59% and 18% of clinical and food isolates, respectively. Dendrograms prepared from the VNTR, antibiograms and toxin profiles, revealed 89, 52 and 12 clusters, respectively. Thus, suggesting a very high heterogeneity among the isolates.
**Conclusion, significance and impact of study:** Strains used in this study showed high heterogeneity when examined by VNTR or antibiograms, while appeared less heterogeneous when dendrogram was generated based on toxin profiles. This study highlights the fact that methicillin resistance in *S. aureus* might be generated within the health institutions or the community. Obtained results also might help health authorities understand the origin of methicillin resistant clones within the study area.

*Keywords: Staphylococcus aureus*, toxins, antibiotic resistance, Variable Number Tandem Repeat (VNTR)

## INTRODUCTION

*Staphylococcus* is transient colonizer of skin and mucosal membranes of both humans and animals (Pantosti, 2012). Enterotoxin-producing *S. aureus* are recognized as the most frequent agents of the blood stream and skin infections (Vivoni and Moreira, 2005). Further, *S. aureus* is an important cause of pneumonia, postoperative wound infections as well as nosocomial infections (Lee, 2003). In addition, it is considered one of the most commonly food poisoning-associated pathogens. In fact, *S. aureus* is only second or third to *Salmonella* and *Clostridium perfringens* in causing food borne outbreaks worldwide (Atanassova *et al.*, 2001; Alarcon *et al.*, 2006). Symptoms like vomiting, diarrhea and abdominal pain usually appear within 2-6 h of ingesting the food that contain preformed toxin. *Staphylococcus aureus* is not an exclusive human pathogen (Alarcon *et al.*, 2006). Nasal cavity and skin of birds and warm-blooded animals, especially pigs, are considered reservoirs for *S. aureus* (Leonard and Markey, 2008). In addition, *S. aureus* is the major pathogen causing mastitis in dairy cows leading to contaminating milk from affected cows (Katsuda *et al.*, 2005). Indeed, *Staphylococcus* has been isolated from food animal carcasses such as pigs, sheep, goats, cows, camels and chickens (Leonard and Markey, 2008). In general, *Staphylococcus* reaches the

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food chain by unhygienic practices of food handlers and asymptomatic carriers. Therefore, the presence of this bacterium or its toxins in foods is usually indicative of lack of hygiene in the food production processes (Atanassova *et al.*, 2001: Alarcon *et al.*, 2006).

Bacterial pathogens frequently acquire resistance to commonly used antibiotics, making the control of their infections a problem. *Staphylococcus aureus*, particularly the Methicillin-resistant *S. aureus* (MRSA), is by far one of the most important bacterial pathogens responsible for nosocomial infections in health care settings with varying prevalence among different countries (Malachowa *et al.*, 2005; Chmelnitsky *et al.*, 2007). Studies conducted in Netherlands (Kadariya *et al.*, 2014) reported about 20-42% of human MRSA cases belong to a livestock strain that was termed livestock-associated MRSA. The livestock-associated MRSA appears to have crossed the species barrier, resulting in zoonotic transmission (Köck *et al.*, 2013). These findings highlight the fact that animals might be an important reservoir for MRSA infections. Further, besides being resistant to penicillin and methicillin, MRSA have been reported to develop multi-drug resistance profiles (Pandya *et al.*, 2014). The production of enterotoxins is another virulence factor that complicates the infections caused by these MRSA, as it is known that pathogenicity is related to a combination of toxin production, invasive capacity, and antibiotic resistance (Carfora *et al.*, 2015).

Molecular typing plays an important role in epidemiological studies, particularly in tracing the passage of pathogens from the food chain to humans and vice versa. Such information is vital to evaluate the efficacy of an outbreak detection and prevention; and to help in studying evolutionary relationship of MRSA and other pathogens (Tenover *et al.*, 2007). Further, understanding the relationship among pathogens from different sources is an important tool in understanding the mechanism of pathogen transfer between hospitals and community.

Multiple-Locus Variable Tandem Repeat (VNTR) analysis (MLVA) provide a good discriminatory power in molecular typing and classifying MRSA strain types and provide results comparable to those obtained by Pulse Field Gel Electrophoresis (PFGE) and other typing methods (Sabat *et al.*, 2003, 2006; Ishino *et al.*, 2007). The MLVA utilizes the fact that the numbers of repeat units at certain DNA loci varies among strains. This variation can be detected by PCR with specific primers that flank DNA loci containing the repeated sequences. This method can be used effectively to type methicillin resistant *S. aureus* and determine their diversity and genetic relatedness (Sabat *et al.*, 2003). Antibiograms profile-based dendrogram analysis is another method to study the heterogeneity among the isolates. The method was successfully implemented to study the relatedness of different serotypes of *Escherichia coli* based on antibiograms profiles (Sumathi *et al.*, 2008). In contrast, Kerouanton *et al.* (2007) used the method to study relatedness among a group of *S. aureus* using the antibiograms and reported that the method was not useful.

The aims of this study were to investigate and compare the frequency *S. aureus* toxin genes (*sea, seb, sec, sed, see*) as well as the antibiotic resistance profiles of strains isolated from food and clinical samples collected from the northern Jordan. The heterogeneity among the isolates was also investigated using the toxin profiles, antibiograms and was compared to the VNTR profiles.

## MATERIALS AND METHODS

### Isolation of *S. aureus* from food samples

A total of 360 raw meat samples (camels, beef, sheep, goats, chicken and fish), cow's and goat's milk were randomly collected and examined during a two years period, between 2007 and 2009. Meat samples were obtained from abattoirs from 4 different governorates in Northern Jordan. Samples (250 g) were collected from different locations of each carcass. Chicken and fish samples were purchased from local supermarkets and were processed similar to meat samples. Milk samples were collected under aseptic conditions in sterile containers. Samples were inoculated onto Baird-Parker agar base (BPA) supplemented with egg yolk-tellurite emulsion (Oxoid UK) and incubated for 24 h at 37 °C. Typical *S. aureus* colonies were picked from each sample and tested for coagulase production using latex coagulase test kit (Plasmatec, Canada) as per manufacturer's instructions. Table 1 shows the samples used in the study and their sources.

### *Staphylococcus aureus* from clinical samples

To study the heterogeneity and to understand the pattern of methicillin resistance among *S. aureus* in the northern city of Irbid, 50 clinical isolates of *S. aureus* were randomly selected from a pool of clinical samples. The clinical isolates were collected from different human samples (blood, urine, pus/wound, tissue, body fluids, ear and eye cultures, prosthetic infections, nasal swabs, sputum, and breast discharges) collected from patients referred to King Abdullah University Hospital or University of Jordan hospital laboratories at the same period the food samples were being collected. The identity of the patients from which the clinical isolates were collected were kept anonymous, and only the isolate's source of infection were presented; and thus an IRB approval was waved. Those isolates were identified biochemically using the Validity Index of Traditional Environmental Knowledge (VITEC) (Table 1).

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**Table 1:** Summary of the samples used in the study with their sources.

| Sample Group | Sample Source | Number of Samples | Details |
|---|---|---|---|
| Food | Chicken | 6 | F5, F7, F8, F9, F10, F11 |
| | Sheep's milk | 1 | A29 |
| | Lamb | 1 | F22 |
| | Fish | 3 | F61, F62, F63 |
| | Cow's milk | 21 | F64-F68, F70-F78, F80, F85, A23-A28 |
| | Goat's meat | 12 | A1-A12 |
| | Camel's meat | 10 | A13-A22 |
| Clinical | Pus | 17 | 1C, 3C, 4C, 12C, 27C, 34C, 35C, C53, C63, C79, C81, C89, C163, C165, C169, C178, C179 |
| | Blood Culture and CVS | 11 | 2C, 11C, 22C, 25C, 28C, 30C, C52, C71, C76, C77, C171 |
| | Wound Culture | 3 | 5C, 16C, 33C |
| | Eye Culture | 3 | 6C, C32, C157 |
| | Ear Culture | 1 | 31C |
| | Sputum | 2 | 8C, C159 |
| | Urinary Catheter and Urinary Tract infections | 7 | 10C, 17C, 21C, 24C, 29C, 32C, C127 |
| Total | | 105 | |

**Molecular confirmation of the presumptive *S. aureus* and *mec*A testing**

DNA from confirmed *S. aureus* colonies was extracted using Genomic Wizard DNA extraction kit (Promega, Wisconsin, USA) following the manufacturer's instructions. Coagulase positive isolates were then tested by PCR using primers specific for the thermonuclease gene, *nuc*, which is specific for *S. aureus* as described by Brakstad *et al.* (1992) (see Table 2). The isolates were then tested for the presence of methicillin resistance gene (*mec*A) following the method of Smyth *et al.* (2005) (Table 2).

**Multiple-Locus Variable-Number Tandem Repeat (VNTR) Analysis**

*Staphylococcus aureus* isolates were analyzed for MLVA using a set of 5 pairs of PCR primers described by Sabat *et al*. (2003) that simultaneously amplify the hyper variable VNTR regions of *spa, sspA, clfA, clfB* and *sdr* genes (Table 2).

**Antibiotic resistance of the isolates**

The resistance profiles of the 105 *S. aureus* isolates were determined using the disk diffusion method following the instructions of the Clinical and Laboratory Standards Institute (CLSI, 2007). All isolates were tested for resistance against the following 15 antibiotics; Cefoxitin (FOX), Vancomycin (VA), Chloramphenicol (C), Trimethoprim (SXT), Gentamycin (GN), Amikacin (AK), Teicoplanin (TEC), Tobramycin (TOB), Amoxicillin (AMC), Penicillin (P), Tetracyclin (TE), Rifampicin (RA), Clindamycin (DA), Azithromycin (AZM), Erythromycin (E).

**Testing the isolates for the presence of enterotoxin genes**

All the isolates were tested for the presence of some enterotoxin genes *sea*, *seb*, *sec*, *sed* and *see* by PCR using the primers and amplification conditions described by Johnson *et al.* (1991) (Table 2). These genes were selected based on their importance. For instance, *seb* is a potential warfare agent while *sec* is implicated in endocarditis (Otto, 2014).

**Statistical analysis and phylogenetic tree development**

Results of the VNTR, the antibiograms and toxin profiles analysis were subjected to cluster analysis. Similarity analysis was performed from a combined binary matrix based on the absence (0) or presence (1) of an amplified DNA band or the resistance (1) or sensitivity (0) of the isolates to antibiotics using the Jaccard coefficient (Naffa *et al.*, 2006). Strain clustering was performed by the unweighted pair group method with arithmetic mean (UPGMA) analysis. The statistical program SPSS, version 23 (IBM, USA) was used for the analysis. A 70% similarity was arbitrarily used as a discriminating threshold to define homologous clusters.


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**Table 2:** Primers used in the study with their product size, sequence and their running conditions.

| Primer target | Primer sequence | Running conditions | Product size (bp) | References |
|---|---|---|---|---|
| *mecA* | F: 5'GCAATCGCTAAAGAACTAAG<br>R; 5'GGGACCAACATAACCTAATA | Pre-denaturation 94 °C for 3 min for the first cycle; 94 °C for 10 sec (denaturation) and 53 °C for 20 sec (annealing) for the next 30 cycles, and a final extension step at 72 °C for 5 min | 222 | Smyth *et al.* (2001) |
| *nuc* | F5' GCGATTGATGGTGATACGGTT<br>R5'AGCCAAGCCTTGACGAACTAAAGC | Pre-denaturation at 94 °C for 1 min, annealing at 55 °C for 30 sec and extension at 72 °C for 1.5 min. amplification was for 37 cycles | 270 | Brakstad *et al.* (1992) |
| *clfA* | F 5'-GATTCTGACCCAGGTTCAGA<br>R5'-CTGTATCTGGTAATGGTTCTTT | Pre-denaturation 94 °C for 5 min, followed by 20 cycles of 30 sec at 94 °C, 30 sec at 55 °C, 30 s at 72 °C with final extension at 72 °C for 5 min | Multiple | Sabat *et al.* (2003) |
| *clfB* | F5'-ATGGTGATTCAGCAGTAAATCC<br>R5'-CATTATTTGGTGGTGTAACTCTT | | | |
| *sdr* | F5'-GTAACAATTACGGATCATGATG<br>R5'-TACCTGTTTCTGGTAATGCTTT | | | |
| *spa* | F5'-AGCACCAAAAGAGGAAGACAA<br>R5'-GTTTAACGACATGTACTCCGT | | | |
| *sspA* | F5' -ATCMATTTYGCMAAYGATGACCA<br>R5'-TTGTCTGAATTATTGTTATCGCC | | | |
| *sea* | F; 5'- TTGGAAACGGTTAAAACGAA<br>R; 5'-GAACCTTCCCATCAAAAACA | Pre-denaturation at 94 $^{o}$C for 2 min followed by annealing for 2 min at 55 $^{o}$C and extension for 1 min at 72 $^{o}$C and a final extension step at 72 $^{o}$C for 7 min. total 40 cycles | 120 | Johnson *et al.* (1991) |
| *seb* | F; 5'-TCGCATCAAACTGACAAACG<br>R;5'-GCAGGTACTCTATAAGTGCC | | 478 | |
| *sec* | F;5'-GACATAAAAGCTAGGAATTT<br>R;5'-AAATCGGATTAACATTATCC | | 257 | |
| *sed* | F;5'-CTAGTTTGGTAATATCTCCT<br>R;5'-TAATGCTATATCTTATAGGG | | 317 | |
| *see* | F;5'-TAGATAAAGTTAAAACAAGC<br>R;5'-TAACTTACCGTGGACCCTTC | | 170 | |

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## RESULTS AND DISCUSSION

### Isolation and confirmation of the *S. aureus* isolates

All isolates used in the study were characterized phenotypically by Gram stain, colony morphology on Bird Parker Agar (BPA) and for the presence of free plasma coagulase. All isolates used in this study were typical *S. aureus* strains as they appeared as dark grey to jet black colonies with opaque zones, surrounded by clear halo on BPA. Also, all isolates appeared as Gram-positive cocci under the microscope and tested positive for the coagulase test. To confirm the identity of the isolates, they were tested for the presence of thermonuclease gene (*nuc*), which is specific for *S. aureus* (Brakstad *et al.*, 1992). As a result, 55 food and 50 clinical isolates were confirmed as *S. aureus*.

### Testing isolates for antibiotic resistance

The antibiograms of all the isolates were depicted in Table 3. All the 50 tested clinical isolates were resistant to penicillin while 44 isolates were resistant to the amoxicillin, but none of the isolates were resistant to either vancomycin or teicoplanin. These results are comparable to the results reported by Wu *et al.* (2010), who also reported that 100% of the community acquired MRSA isolates were resistant to penicillin but not to vancomycin. Out of the 55 food isolates, 34 were resistant to penicillin and 32 to amoxicillin. Interestingly, there were 12 food isolates resistant to vancomycin and 23 isolates were resistant to rifampicin, which might indicate that these antibiotics are included in animal feed or used for animal disease treatment.

A multi-drug resistant isolate is defined as being resistant to three or more antibiotics. When the isolates were scrutinized for percentages of isolates exhibiting multi-drug resistance, 80% and 70% of the food and clinical isolates were multi-drug resistant, respectively. Among the food isolates, F63 was resistant to all the 15 tested antibiotics while one isolate (F70) was resistant to 10 antibiotics, and the rest showed resistance against 2 to 8 antibiotics. One food isolate was sensitive to all tested antibiotics. Two clinical isolates, C76 was resistant to 11 antibiotics, and C127 was resistant to 10 antibiotics while the other isolates showed resistance against 1 to 8 antibiotics. Nevertheless, the similar proportion of multi-drug resistance among both clinical and food isolates observed in this study (80% vs 70%) contradicts the results obtained by Reinoso *et al.* (2008) who reported that resistance was pronounced among clinical *S. aureus* than those from food samples.

### Testing isolates for presence of *mec*A gene by PCR

MRSA infections were reported in pet animals, in hospitalized patients and in community (Strommenger *et al.*, 2006). However, it is not well known if the pet animals serve as reservoir for MRSA and transmit it to humans or *vice versa* (Pantosti, 2012).

**Table 3:** Antibiotic resistance profiles to clinical and food *Staphylococcus* isolates.

| Antibiotic | Clinical Isolates (50) | | Food Isolates (55) | |
|---|---|---|---|---|
| | R | S | R | S |
| Vancomycin (VA) | 0 | 50 | 12 | 43 |
| Chloramphenicol (C) | 3 | 47 | 1 | 54 |
| Trimethoprim (SXT) | 9 | 41 | 19 | 36 |
| Gentamycin (GN) | 4 | 46 | 9 | 46 |
| Amikacin (AK) | 6 | 44 | 4 | 51 |
| Teicoplanin (TEC) | 8 | 42 | 14 | 41 |
| Tobramycin (TOB) | 7 | 43 | 9 | 46 |
| Amoxicillin (AMC) | 44 | 6 | 32 | 23 |
| Penicillin (P) | 50 | 0 | 34 | 21 |
| Tetracyclin (TE) | 20 | 30 | 22 | 33 |
| Rifampicin (RA) | 4 | 46 | 23 | 32 |
| Clindamycin (DA) | 11 | 39 | 9 | 46 |
| Azithromycin (AZM) | 18 | 32 | 16 | 39 |
| Erythromycin (E) | 21 | 29 | 10 | 45 |
| Cefoxitin (FOX) | 24 | 26 | 17 | 38 |

When the isolates were tested for the presence of the *mec*A gene, 36 out of 50 clinical isolates (72%) were positive, while 42 out of 55 food isolates (76%) were positive. These numbers appeared to be very high in comparison to results of other studies (Tsen *et al.*, 1998; de Boer *et al.*, 2009). In fact, Tsen *et al.* (1998) reported a complete absence of MRSA in food isolates in Taiwan. The high number of isolates harboring the *mec*A gene and exhibiting multidrug resistance to most antibiotics used in this study suggests a major role for horizontal gene transfer. There is evidence that the *mec*A gene resides on a mobile genetic element that also harbor genes, which encode resistance to non-beta-lactam antibiotics, causing the observed multi-resistance (Wielders *et al.*, 2002).

The presence of high percentage of MRSA in food isolates in Jordan might signify the widespread of this pathogen in the environment and consequently in the food animals and their products. In addition, it is well known that the presence of the *mec*A gene doesn't necessarily indicate a phenotypic methicillin resistance (Martineau *et al.*, 2000). The difficulty in the detection of the MRSA phenotype stems from the heterogeneous expression of the *mec*A gene in strains of *Staphylococcus,* where each cell in a population may carry the genetic information but only 1 in $10^5$-$10^7$ cells may express the resistance phenotypically (Yamazumi *et al.*, 2001).

Although resistance to cefoxitin was used as an indicator for methicillin resistance (Anand *et al.*, 2009), in our study we did not observe any correlation between resistance to cefoxitin and presence of *mec*A gene. Only 24 out of 50 clinical isolates showed resistance to cefoxitin while 35 isolates harbored the *mec*A gene.

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Among the food isolates, only 17 out of 55 food isolates exhibited resistance to cefoxitin while 42 isolates harbored the *mec*A gene. In a study reported by Chauhan *et al.* (2013), all MRSA tested positive for cefoxitin. However, the authors did not test for the presence of the *mec*A gene by PCR and just depended on the phenotyping of the isolates by the traditional antimicrobial testing.

**Distribution of enterotoxin genes**

When the isolates were tested for the presence of the major enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*), the distribution of these genes varied between the food and clinical isolates (Figure 1). The *sea* gene, which was the most abundant, was found in (88%) of the clinical and food isolates, followed by *sec* (70 and 29%) for clinical and food isolates, respectively. This result appeared in accordance with results reported by Kerouanton *et al.* (2007) who reported that *sea* was the most frequent in human isolates. In contrast, our results appeared contradicts results reported by Akineden *et al.* (2008) who stated that *sec* is mainly found in *S. aureus* isolated from food samples.

Nevertheless, these results were similar to reports by Mehrotra *et al.* (2000) and Becker *et al.* (2003). The high prevalence of toxin genes in strains isolated from food and clinical samples suggest that humans could have acquired the toxin harboring strains from contamination food products. As for the distribution of *sea*, similar results were reported by Omoe *et al.* (2002) who showed that 76% of isolates from food poisoning harbored the *sea* gene. The presence of *seb* genes was third with 56% and 18% of clinical and food isolates harbored the gene, respectively. The *sed* gene was only found in one clinical isolate (2%), while *see* was found in 22 % of the food isolates and only 4% of the clinical isolates. Although only one isolate harbored the *sed* toxin gene, Morandi *et al.* (2009) reported that *sed* gene was the most frequent gene at 30% of *S. aureus* from dairy products harboring that gene. This indicates that high variation among the isolates is based on location and origin of samples.

When evaluating the distribution of toxin genes among the isolates, almost all isolates (103/105, 98%) harbored at least one type of toxin genes, except two isolates originated from food samples (A27 and F22) were devoid of any toxin gene. Our result appeared in agreement with results reported by two studies (Ferry *et al.*, 2005; Morandi *et al.*, 2009). Ferry *et al.* (2005) reported that 84% of the *S. aureus* clinical isolates harbored at least one toxin gene and Morandi *et al.* (2009) reported that 79% of *S. aureus* from dairy products harbored at least one toxin gene. In contrast, Peles *et al.* (2007) reported that only 15% of *S. aureus* isolated from milk samples in Hungary harbored at least one toxin gene. This could be due to the homogeneity of the *S. aureus* isolates used in their study. The *sea* gene was detected in the majority of isolates from clinical (44 out of 50) and food (49 out of 55) samples. Similar results were reported by Akineden *et al.* (2008) who found that most of tested clinical samples harbored the *sea* gene.

When the isolates were analyzed for the number of toxin genes they harbored, 40 isolates contained one toxin gene while, 34 isolates contained two toxin genes, 26 isolates contained 3 toxin genes and only 3 clinical isolates contained 4 toxin genes. Collectively, our results and results reported by others suggest that toxin gene profiles vary from place to place and there is no rule to whether food or clinical isolates harbor a certain type of toxin genes.

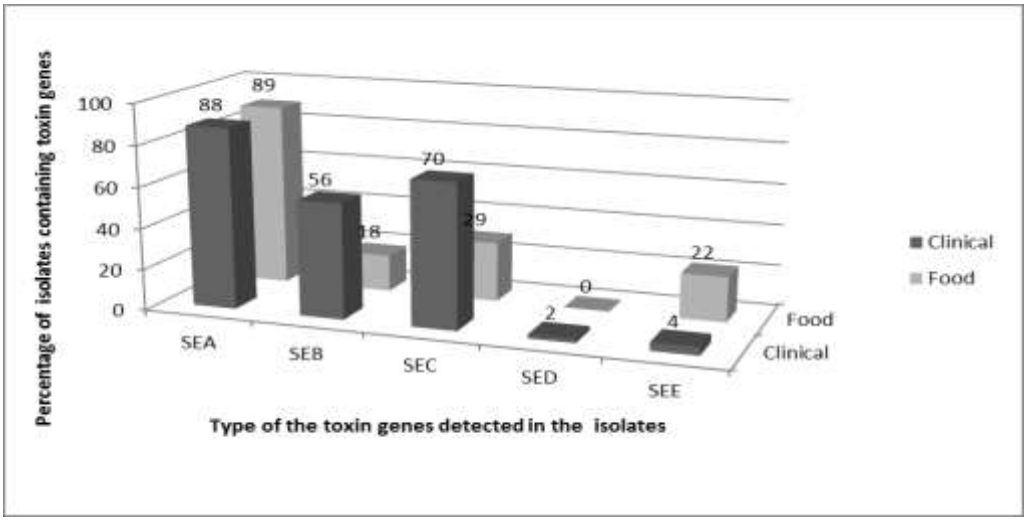


**Figure 1:** Distribution of enterotoxin genes as detected by PCR among food and clinical *S. aureus* isolates.

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**Phylogenetic analysis of the isolates using VNTR, antibiograms and toxin profiles**

In this study, we used VNTR analysis to study the genetic heterogeneity among our collection of food and clinical isolates. PCR amplification of extracted DNA gave multiple bands (4-8 bands) with a size ranging from 90 bp to 2156 bp for all the isolates. Genomic heterogeneity among the isolate is considerable and is reflected in the high number of banding patterns, as the 105 isolates generated 92 patterns or subtypes when a dendrogram was constructed from the VNTR analysis data. When a 70% similarity cut-off was arbitrarily set for the dendrogram analysis, the isolates were clustered in 89 clusters indicating a very high heterogeneity, which deem the method non-discriminatory among the isolates. These results contradict the results published by Morandi *et al.* (2010) who reported that this method was capable of differentiating between food and clinical isolates. Tenover *et al.* (2007) also used the same method to type 103 clinical and community associated MRSA and was able to identify 13 clusters with PFGE typing from 42 banding patterns using 75% similarity cut off. In the same study, when the same isolates were typed by VNTR, 9 clusters were identified with 36 unique VNTR patterns (Tenover *et al.*, 2007). These differences between our results and the results reported by Tenover *et al.* (2007) and Morandi *et al.* (2010) highlight the high heterogeneity of the isolates examined in this work.

As an alternative to the VNTR analysis, the antibiograms for the isolates were used to generate a dendrogram. A 70% similarity cut-off was also used to identify the clusters. The antibiograms generated 52 clusters with 33 single-isolate clusters while the other 19 clusters contain multiple isolates (Table 4). Some of these isolates contained only clinical isolates while others contain only food isolates and the rest contained mixed isolates. Observing the patterns of the antibiograms for each cluster shows major differences between clusters (e.g clusters 12, 25 and 30) while there is similarity in the antibiograms within some clusters (e.g cluster 5, 42 and 47) indicating the limited usefulness of this method to study the antibiograms patterns of a collection of isolates. This finding is in agreement with Kerouanton *et al.* (2007) who arrived at the same conclusion. Nevertheless, the toxin profiles were used to generate a dendrogram using a 70% similarity cut-off (Figure 2). The toxin profiles generated only 12 clusters with 3 clusters (3, 5 and 8) contains 32, 17 and 23 isolates, respectively (Table 5). The rest of the isolates were distrusted among the other 9 clusters with two clusters each contain one isolate. Noticeably, the toxin profiles in each cluster were identical for all the isolates in that cluster irrespective of the isolate origin. This method exhibits a good discriminatory power as opposed to the VNTR and the antibiograms profiles. These results are consistent with the concept of host specialization where some animal isolates were grouped in clusters while clinical isolates were grouped in other clusters (Reinoso *et al.*, 2004). In addition some clusters contained isolates from food and clinical origin with the same toxin gene profiles indicating some genetic relatedness.

**Table 4:** The clusters of the tested bacteria based on antibiograms profiles.

| Cluster Number | Isolates | Antibiotic resistance profile at 70% similarity | Remarks |
|---|---|---|---|
| 1 | A21, A29, 22C | A21, A29; P, TOB, TEC<br>22C; E, P, TOB, TEC | Mixed isolates |
| 2 | C63, F11 | C63: TE, P, AMC, TEC, OX<br>F11: TE, P, AMC, TEC | Mixed isolates |
| 3 | A13, A25, A1 | A13; TE, P, AMC, TEC, VA<br>A25: E, TE, P, AMC, VA<br>A1: P, AMC, TEC, VA | All are food/animal isolates |
| 4 | 10C, 32C | 10 C; P, AMC, TOB<br>32C; TE, P, AMC, TOB | Both are clinical isolates |
| 5 | A11, A23, A2, 2C, 34C, 27C, 29C, 21C, 25C, 11C, 17C, 8C, 12C, 5C | P* , AMC | Majority are clinical with only 3 food isolates with identical antibiograms |
| 9 | A10, A12 | A10; FOX, TE, P, AMC, SXT, MET<br>A12; FOX, TE, P, AMC, SXT | Both are food isolates |
| 11 | A9, A19, A5 | P, AMC, SXT | All are food isolates with identical antibiograms |
| 12 | A22, A27, A8, A15 | A22, A27, A8; TE, P, AMC, SXT<br>A15; TE, P, AMC, TEC, SXT | All are food isolates |
| 14 | C79, F9 | C79; E, TE, P, AMC<br>F9; E, TE, P, AMC | Mixed |
| 16 | A20, A26 | A20; E, DA, TE, P, AMC, SXT<br>A26; E, DA, TE, P, AMC, TEC, SXT, VA | Both food isolates |

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| | | | |
|---|---|---|---|
| 17 | F7, A17, 31C, 33C | DA, TE, P, AMC | Mixed source but identical antibiograms |
| 19 | 3C, 28C | P, AMC | Both are clinical with identical antibiograms |
| 21 | C169, C171, C163, C165, C167 | FOX, P, AMC, TEC, SXT | All are clinical with identical antibiograms |
| 25 | C76, C127, C53, F63 | C76; E, AZM, DA, FOX, TE, P, AMC, TOB, AK, GEN, SXT<br>C127; E, AZM, FOX, TE, P, AMC, TOB, AK, GEN, SXT<br>C53; E, AZM, RA, FOX, TE, P, AMC, TOB, AK, GEN, SXT<br>F63**; E, AZM, DA, RA, FOX, TE, P, AMC, TOB, TEC, AK, GEN, SXT, C, VA | Mixed source, and different antibiograms |
| 30 | C159, C178, C74, C89, C177, C157, C158, C174 | C159, C178, C74, C89; E, AZM, FOX, TE, P, AMC<br>C177; E, AZM, FOX, TE, P, AMC, TEC<br>C157; E, AZM, FOX, TE, P<br>C158; E, AZM, FOX, TE, P, AMC<br>C174; E, AZM, DA, FOX, TE, P, AMC, SXT | All are clinical but with different antibiograms |
| 32 | 11C, F5, F8, 24C | 11C, F5; E, AMZ, DA, RA, FOX, P, AMC<br>F8; E, AMZ, DA, RA, FOX, TE, P, AMC<br>24C; E, AMZ, DA, RA, FOX, P, AMC, AK | Mixed source and different antibiograms |
| 42 | F72, F74, F67 | AMZ, RA, FOX, TOB, TEC, GEN | All are food isolates with identical antibiograms |
| 43 | F61, F77 | F61; AMZ, RA, TOB, GEN<br>F77; AMZ, RA, TOB | Both are food isolates |
| 47 | F76, F80, F66 | AMZ, RA | All are food isolates with similar antibiograms |
| 6, 7, 8, 10, 13, 15, 18, 20, 22, 23, 24, 26, 27, 28, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 44, 45, 46, 48, 49, 50, 51, 52 | A28, 1C, A24, A4, A6, C71, F22, 30C, C52, C159, A7, 35C, F70, C77, C81, C68, F65, 6C, 4C, F10, C32, A3, F62, F85, F75, F78, F64, F73, F68, F71, A16, A18, A14 | Variable | All of these are clusters with single isolate in each with different antibiograms |
| Total 52 | Total 105 | | |

*All are similar
**Resistant to all tested antibiotics


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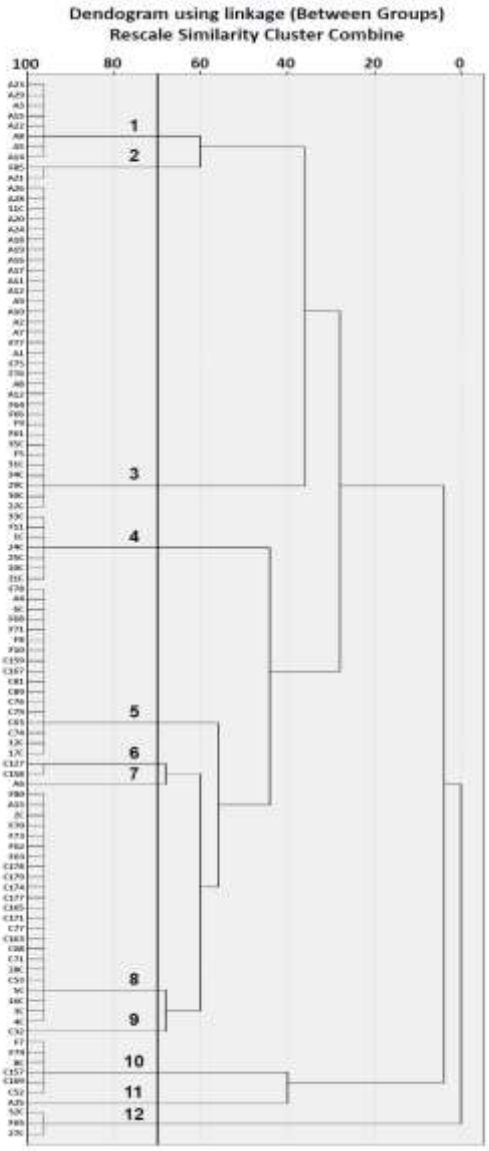


**Figure 2:** Dendrogram generated using unweighted pair group method with arithmetic average analysis using toxin gene patterns. Similarity analysis was performed from combined binary matrix based on the presence of a toxin (1) or the absence of the toxin (0) using the Jaccard coefficient performed by unweighted pair group method with arithmetic mean (UPGMA) analysis. The cut-off was arbitrarily set at 70% similarity level and classified the isolates into 12 different clusters 1-12. The scale at the top show the similarity index as a discriminating threshold to define homogenous clusters.

## CONCLUSIONS

In conclusion, it appears that when using 70% similarity, the VNTR as well as the antibiograms profiles did not provide enough discriminatory power to cluster the isolates based on the origin, and thus very high cluster numbers were obtained, which indicates a very high heterogeneity among the isolates. In contrast, profiling the isolates based on the presence of toxin genes provided a far better discriminatory power to cluster the isolates based on their origin. The high heterogeneity of the isolates and the apparent clustering of either food or clinical in separate clusters revealed that the origin of the *S. aureus* isolates in clinical and food are different. Nevertheless, the toxin patterns of some food and clinical isolates were identical indicating a very close relationship among those. Further, it is a possibility that some human infections were caused by consuming contaminated food products made from these animals.

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## REFERENCES

**Anand, K. B., Agrawal, P., Kumar, S. and Kapila, K. (2009).** Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mec*A gene for detection of MRSA. *Indian Journal of Medical Microbiology* **27, 27-29.**

**Akineden, O., Hassan, A. H., Schneider, E. and Usleber, E. (2008).** Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats milk cheese. *International Journal of Food Microbiology* **124, 211-216.**

**Alarcon, B., Vicedo, B. and Aznar, R. (2006).** PCR-based procedures for detection and quantification of *Staphylococcus aureus* and their application in food. *Journal of Applied Microbiology* **100, 352-364.**

**Atanassova, V., Meindl, A. and Ring, C. (2001).** Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham- a comparison of classical culturing detection and RFLP-PCR. *International Journal of Food Microbiology* **68, 105-113.**

**Becker, K., Friedrich, A., Lubritz, G., Weilert, M., Peters G. and von Eiff, C. (2003).** Prevalence of genes encoding pyrogenic toxin superantigens and exofoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *Journal of Clinical Microbiology* **41, 1434-1439.**

**Brakstad, O. G., Aback, K. and Mealand, J. A. (1992).** Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *Journal of Clinical Microbiology* **30, 1654-1660.**

**Carfora, V., Caprioli, A., Marri, M., Sagrafoli, D., Boselli, C., Giacinti, G., Giangolini, G., Sorbara, L., Dottarelli, S., Battisti, A. and Amatiste, S. (2015).** Enterotoxin genes, enterotoxin production, and

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

methicillin resistance in *Staphylococcus aureus* isolated from milk and dairy products in central Italy. *International Dairy Journal* **42, 12-15.**

**Chauhan, P., Bais P. S. and Gupta N. (2013).** Prevalence of methicillin resistant *Staphylococcus aureus* (mecA gene) among patients admitted in intensive care unit. *International Journal of Bioassays* **2, 1256-1259.**

**Chmelnitsky, I., Navon-Venezia, S., Leavit, A., Somekh, E., Regev-Yochai, G., Chowers, M., Shitrit, P. and Carmeli Y. (2007).** SCCmec and spa types of methicillin-resistant *Staphylococcus aureus* strains in Israel. Detection of SCCmec type V. *European Journal of Clinical Microbiology Infectious Diseases* **27, 385-390.**

**Clinical and Laboratory Standards Institute (CLSI) (2007).** Performance standards for antimicrobial susceptibility testing; 175th informational supplement. *CLSI document* M100-S17, Vol 27.

**de Boer, E., Zwartkruis-Nahuis, J. T. M., Wit, B., Huijsdens, X. W., de Neeling, A. J., Bosch, T., van Oosterom, R.,A.,A., Vila, A. and Heuvelink A. E. (2009).** Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of Food Microbiology* **134, 52-56.**

**Ferry, T., Thomas, D., Genestier, A-L., Bes, M., Lina, G., Vandenesch, F. and Etinne J. (2005).** Comparative prevalence of superantigen genes in *Staphylococcus aureus* isolates causing sepsis with and without septic shock. *Clinical Infectious Diseases* **41, 771-777.**

**Ishino, K., Tsuchizaki, N., Ishikawa, J. and Kusefulness, H. (2007).** Usefulness of PCR-restriction fragment length polymorphism typing of the coagulase gene to discriminate arbekacin-resistant methicillin-resistant *Staphylococcus aureus* strains. *Journal of Clinical Microbiology* **45, 607-609.**

**Johnson, W. M., Taylor, S. D., Ewan, E. P., Ashton, F. E., Pollard, D. R. and Rozee, K. R. (1991).** Detection of genes for enterotoxins, exfoliative toxins and toxic shock syndrome toxin 1 in S*taphylococcus aureus* by the polymerase chain reactions. *Journal of Clinical Microbiology* **29, 426-430.**

**Kadariya, J., Smith, T. C. and Thapaliya D. (2014).** *Staphylococcus aureus* and Staphylococcal disease: An ongoing challenge in public health. *Biomed Research International* **2014 ID827965.**

**Katsuda, K., Hata, E., Kobayashi, H., Kohmoto, M., Kawashima, K., Tsunemitsu, H. and Eguchi, M. (2005).** Molecular typing of *Staphylococcus aureus* from bovine mastitic milk on the basis of toxin genes and coagulase gene polymorphisms. *Veterinary Microbiology* **105, 301-305.**

**Kerouanton, A., Hennekinne, J. A., Letertre, C., Petit, L., Chesneau, O., Brisabois, A. and De Buyser, M. L. (2007).** Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *International Journal of Food Microbiology* **115, 369-375.**

**Köck, R., Schaumburg, F., Mellmann, A., Köksal, M., Jurke, A., Becker, K. and Friedrich, A. W. (2013).** Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. *PLOS One* **8, e55040.**

**Lee, J. H. (2003).** Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology* **69, 6489-6494.**

**Leonard, F.C. and Markey B. K. (2008).** Methcillin-resistant *Staphylococcus aureus* in animals: A review. *The Veterinary Journal* **175, 27-36.**

**Malachowa, N., Saabat, A., Gniadkowski, M., Krzyszton-Russjan, J., Empel, J., Miedzobrodzki, J., Kosowska-shick, K., Appelbaum, P. C. and Hryniewicz, W. (2005).** Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence typing for clonal characterization of *S. aureus* isolates. *Journal of Clinical Microbiology* **43, 3095-3100.**

**Martineau, F., Picard, F. J., Lansac, N., Menard, C., Roy, P. H., Ouellette, M. and Bergeron, M. G. (2000).** Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *S. epidermidis. Antimicrobial Agents and Chemotherapy* **44, 231-238.**

**Mehrotra, M., Wang, G. and Johnson W. M. (2000).** Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *Journal of Clinical Microbiology* **38, 1032-1035.**

**Morandi, S., Brasca, M., Andrighetto, C., Lombardi, A. and Lodi, R. (2009).** Phenotypic and genotypic characterization of *Staphylococcus aureus* strains from Italian dairy products. *International Journal of Microbiology* **Article ID 501362.** DOI:10.1155/2009/501362.

**Morandi, S., Brasca, M., Lodi, R., Brusetti, L., Andrighetto, C. and Lombardi, A. (2010).** Biochemical profiles, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and multilocus variable number tandem repeat analysis (MLVA) for typing *Staphylococcus aureus* isolated from dairy products. *Research in Veterinary Science* **88, 427-435.**

**Naffa, R. G., Bdour, S. M., Migdadi, H. M. and Shehabi, A. A. (2006).** Enterotoxicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan. *Journal of Clinical Microbiology* **55, 183-187.**

**Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D-L., Ueda, S. and Shinagawa, K. (2002).** Detection of *seg*, *she*, and *sei* genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S.*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

*aureus* isolates harboring *seg*, *she* or *sei* genes. *Journal of Clinical Microbiology* **40, 857-862.**

**Pandya, N., Chaudhary, A., Mehta, S. and Parmar, R. (2014).** Characterization of Methicillin Resistant *Staphylococcus aureus* from various clinical samples at tertiary care hospital of rural Gujarat. *Journal of Research in Medical and Dental Science* **2, 49-53.**

**Pantosti, A. (2012).** Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health. *Frontiers in Microbiology* **3, 127.**

**Peles, F., Wagner, M., Varga, L., Hein, I., Rieck, P., Gutser, K., Kereszture, P., Kardos, G., Turcsanyi, I., Beri, B. and Szabio, A. (2007).** Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. *International Journal of Food Microbiology* **118, 186-193.**

**Reinoso, E. B., El –Sayed, A., Lämmler, C., Bogni, C. and Zschöck, M. (2008).** Genotyping of *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. *Microbiological Research* **163, 314-322.**

**Reinoso, E., Bettera, S., Frigerio, C., Direnzo, M., Calzolari, A. and Bogni, C. (2004).** RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human hosts. *Microbiological Research* **159, 245-255.**

**Sabat, A., Krzyszton-Russjan, J., Strzalka, W., Filipek, R., Kosowska, K., Hryniewicz, W., Travis, J. and Potempa, J. (2003).** New method for typing *Staphylococcus aureus* strains; Multiple-Locus Variable-Number Tandem Repeat Analysis of polymorphism and genetic relationships of clinical isolates. *Journal of Clinical Microbiology* **41, 1801-1804**.

**Sabat, A., Malachowa, N., Miedzobrodzki, J. and Hryniewicz, W. (2006).** Comparison of PCR-based methods for typing *S. aureus* isolates. *Journal of Clinical Microbiology* **44, 3804-3807.**

**Smyth, R. W., Kahlmeter, G., Olsson Liljequist, B. and Hoffman, B. (2001).** Methods for identifying methicillin resistance *in Staphylococcus aureus. Journal of Hospital Infections* **48, 103-107.**

**Strommenger, B., Kehrenberg, C., Kettlitz, C., Cunny, C., Verspohl, J., Witte, W. and Schwarz, S. (2006).** Molecular characterization of methecillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. *Journal of Antimicrobial Chemotherapy* **57, 461-465.**

**Sumathi, B. R., Amitha, R. G. and Krishnappa, G. (2008).** Antibiogram profile based dendrogram analysis of *Escherichia coli* serotypes isolated from bovine mastitis. *Veterinary World* **1, 37-39.**

**Tenover, F. C., Vaughn, R. R., McDougal, L. K., Fosheim, G. E. and McGowan, J. E. (2007).** Multiple-Locus Variable-Number Tandem-Repeat Assay Analysis of Methicillin-Resistant *Staphylococcus aureus* Strains. *Journal of Clinical Microbiology.* **45, 2215-2219.**

**Tsen, H. Y., Yu, G. K., Wang, K. C., Wang, S. J., Chang, M. Y. and Lin, L. Y. (1998).** Comparison of the enterotoxigenic types, toxic shock syndrome toxin (TSST-1) strains and antibiotic susceptibility for enterotoxigenic *Staphylococcus aureus* strains isolated from food and clinical samples. *Food Microbiology* **15, 33-41.**

**Vivoni, A. M. and Moreira, B. M. (2005).** Application of molecular techniques in the study of *Staphylococcus aureus* clonal evolution- a review. *Memórias do Instituto Oswaldo Cruz* **100, 693-698.**

**Wielders, C. L. C., Fluit, A. C., Brisse, S., Verhoef, J. and Schmitz, F. J. (2002).** *mecA* gene is widely disseminated in *Staphylococcus aureus* population. *Journal of Clinical Microbiology* **40, 3970-3975.**

**Wu, D., Wang, Q., Yang, Y., Geng, W., Wang, Q., Yu, S., Yao, K., Yaun, L. and Shen X. (2010).** Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* from skin/soft tissue infections in a children's hospital in Beijing, China. *Diagnostic Microbiology and Infectious Diseases* **67, 1-8**.

**Yamazumi, T., Furuta, I., Diekema, D. J., Pfaller, M. A. and Jones, R. N. (2001).** Comparison of the Vitek Gram-positive susceptibility 106 card, the MRSA-screen latex agglutination test, and *mec*A analysis for detecting oxacillin resistance in a geographically diverse collection of clinical isolates of coagulase-negative staphylocci. *Journal of Clinical Microbiology* **39, 3633-3636.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538