Genetic variations of *Staphylococcus aureus* strains isolated from clinical specimens at Jaizan hospital in Saudi Arabia

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ABSTRACT: Among 65 *Staphylococcus* sp. strains isolated from clinical specimens, ten were confirmed to be *Staphylococcus aureus*. Antimicrobial susceptibility patterns revealed that isolates were resistant to methicillin and oxacillin from 75 to 90%. Genetic variation among the ten candidates displayed total of 32 RAPD fragments, 24 of which were polymorphic. The mean polymorphic percentage was 75%, whereas the percentage of primer 1 was higher with 83.33% followed by primer 2 (75%). However, primer 3 displayed lower percentage with 55.56%. Genetic similarity showed high similarity of isolate 8 with 10 (88%) followed by isolates 5 and 7 (86%). Isolates 2 and 4 showed the lowest with 35%. UPGMA dendrogram revealed two main clusters, the first included two isolates 3 and 4 with bootstrap (68.4). The second divided the residual 8 isolates into two sub-clusters.
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Key words: *Staphylococcus aureus* isolates, RAPD analysis, patient, clinical specimens.

1.INTRODUCTION:

Staphylococci are widespread in nature, they found in the air, dust, water, and humans. The main human reservoirs of these organisms are the skin and nasal cavity (Jay, 1986). About 40 to 44% of healthy humans carry staphylococci in the nose (Kluytmans and Belkum, 1997. In the healthy population ~20% of the individuals carry *S. aureus* persistently, ~60% intermittently and ~20% never carry this bacterium (Kluytmans and Belkum, 1997). It has long been recognized as one of the major human pathogens and one of the most common nosocomial organisms, being responsible for most post-surgical infections. *S. aureus* is an opportunistic bacterium, frequently part of the human microflora, causing disease when the immune system becomes compromised. Although *S. aureus* found in different parts of the body, anterior nares are the primary ecological niche in humans. Nasal carriage differs between individuals and is one of the major risk factors for *S. aureus* infection(Kluytmans and Belkum, 1997).

Staphylococcus aureus is a frequent cause of health care–associated infections in acute care hospitals. It is the most common cause of nosocomial pneumonia and surgical site infections and the second most common cause of bloodstream, cardiovascular, and eye, ear, nose, and throat infections (Haley et al., 1985 and Pittet et al., 1994).

S. aureus isolates have been distinguished by phenotypic methods such as biotyping, bacteriophage typing and antibiotic resistance. Typing systems based solely on phenotypic tests have limitations such as relatively low discriminatory power and modest reproducibility because phenotypic traits are inconsistently expressed. The shortcomings of phenotypically-based typing methods have led to the progressive development of genotypic strategies like random amplification of polymorphic DNA analysis (RAPD), which has been proposed as the method of choice for typing *S. aureus* isolates (Tenover et al., 1994) and considered one of the most reproducible and discriminatory typing techniques.

Accurate and rapid typing of *S. aureus* strains is crucial to the control of infectious strains. Numerous typing methods have been described(Yoshida et al., 1997 and van Leeuwen et al., 2003) among these is randomly amplified polymorphic DNA (RAPD) analysis. Several reports have been analyzed *S. aureus* strains using RAPD analysis for different approaches. For instant, test whether 100 clinical isolates harboring the toxin genes(Naffa et al., 3006)[8], assess genetic relationship of 80 isolates from different hosts (Reinoso et al., 2004 and Nikbakht et al., 2008), genotyping 20 strains isolated from mucous membrane of anterior part of nose of children (Baksheeva et al., 2011) and to detect the genetic identity of 6 isolates using five primers(Narmeen and Jubrael, 2009).

The aim of the present study is to investigate the genetic variations and similarity of the isolates using RAPD analysis as well as to assess the genetic relationship of isolates using USIN represented the genetic relationships using UPGMA dendrogram cluster analysis.

2.MATERIALS AND METHODS:

2.1.Materials:

2.1.1. Subjects:

The study samples were swabs of respiratory secretions, nose, skin, wounds and blood samples were

collected from each patient at public hospital in Saudi Arabia during the period from February to June 2012.

2.1.2. Samples:

The study was conducted on sterile culture swabs (Becton-Dickinson, Sparks, MD) were used in order to obtain samples. Each swab was moistened with 0.9% NaCl (w/v) and then rubbed across a pre-determined surface area, then introduced into tubes. After sampling, each swab was stored in a holder containing a moistened sponge (provided by Becton-Dickinson) and analyzed within 3 h.

In the present study, ten *S. aureus* strains were isolated as follow: 1, 2 and 3 from wounds; 4, 5 and 6 from skin and respiratory system; 7, 8 and 9 & 10 isolates from nasal swabs and blood, respectively.

2.2. Methods:

2.2.1. Isolation of bacteria from Clinical samples

Each sample was streaked on the surface of a tryptic soy agar^{+} 5% sheep blood plate. After 48 h incubation at 35°C, the plates were examined for hemolytic colonies. Each hemolytic colony type was re-striked onto a tryptic soy agar^{+} 5% sheep blood plate, incubated for 48 hours at 35°C. Then, cell morphology and Gram reaction were recorded. Bacterial slants of nutrient agar (as stock culture) were prepared for further biochemical testing. By conventional biochemical tests such as gram staining, catalase production, bound and free coagulase and DNAase activity (Bannerman,2003), ten of 65 *Staphylococcus* sp. strains isolated from different patients were confirmed to be *Staphylococcus aureus*. The isolates were kept at -20^{o}C until use.

2.2.2.Methicillin and oxacillin sensitivity

Characteristic of colonies that were identified as *S. aureus* were tested for resistance to methicillin (5 µg) and oxacillin (1 µg) by using Mueller–Hinton agar and Disc-diffusion test according to NCCLS (2003).

2.2.3.DNA extraction and RAPD analysis of *S. aureus* isolates

Genomic DNA was extracted from overnight cultures of ten *S. aureus* isolates using the Wizard Genomic DNA Purification kit (Promega) as recommended by the manufacturer. RAPD analysis was performed using three 10-mer random primers (Metabion, Martinsried, Germany) as shown in Table (1). PCR amplification was carried out in a DNA thermocycler (Biometra, Germany) according to Nariman *et al.* (2008) for 30 cycles each. The PCR reaction was carried out in a final volume of 25 µl with 1X PCR buffer containing 10 mM Tris-HCl, 25 mM $MgCl_2$, 1 µl of template DNA, 0.2 mM dNTPs, 1 to 2 µM (each) primer and 0.5 U of *Taq* DNA polymerase (Promega).

PCR conditions consisted of an initial denaturation at 95°C for 2 min followed by 95°C for 1 min, annealing to primers at 35°C for 1 min and extension at 72°C for 1 min with a final extension step at 72°C for 5 min. PCR-amplified products were separated using agarose gel electrophoresis in 1% TBE buffer and stained with 0.2 µg/ml ethidium bromide according to (Sambrook *et al.*, 1989). Amplified fragments were detected and photographed under UV light.

Table 1. Names and sequences of the three primers used for RAPD-PCR analysis.

RAPD primers		Primer sequences (5′-3′)
1	MN-45	AAGACGCCGT
2	KAY-1	AGCAGCCTGC
3	EPO-15	ACAACTGCTC

2.2.4.Genetic analysis

RAPD fragments were scored as present (+) or absent. The data was used for similarity-based analysis using the program MVSP (version 3.1b) from www.kovcomp.com. RAPD analysis was analyzed using the Nei genetic similarity index(Nei and Li, 1979) based on the equation:

$$\text{Similarity} = 2N_{ab}/(N_a + N_b),$$

Where:

$\mathbf{N_{ab}}$ = number of scored amplified fragments with the same molecular size shared between a and b,

$\mathbf{N_a}$ **and** $\mathbf{N_b}$ = number of scored amplified fragments in a and b, respectively.

A dendrogram was constructed based on the similarity matrix data by unweighted pair group method with average (UPGMA) cluster analysis.

3.Results

Among 65 *Staphylococcus* sp. strains isolated from patient at Jazan public hospital in Saudi Arabia, ten were confirmed to be *Staphylococcus aureus*, isolated as follow:

1, 2 and 3 …….from wounds,

4 & 5 ……from skin,

6,7,8 and 9 & 10 isolates…..from nasal cavity and blood, respectively.

Antimicrobial susceptibility patterns revealed that isolates were resistant to methicillin and oxacillin from 75 to 90%.

3.1. Genetic characterization of clinical *S. aureus* isolates by RAPD analysis

A total of 32 amplified DNA fragments ranging in size from 100 to 2000 bp were detected by using three random primers; 1, 2 and 3, whereas 26 fragments were polymorphic and the other 6 amplified fragments were regularly identified among the ten *S. aureus* isolates (Table3**)**.

Table 2. RAPD analysis of the polymorphic fragments of ten *S. aureus* isolates using three primers

Primer name	*P%	Fragment №	Fragment Bs	*Staphylococcus aureus* isolates 1	2	3	4	5	6	7	8	9	10
		1	1050		+			+	+	+	+	+	+
		2	1000		+			+	+	+	+	+	+
		3	900		+	+	+				+	+	+
1		4	800	+	+								
	83.33	5	700	+	+			+	+	+	+	+	+
		6	600	+		+	+						
		8	350				+	+	+	+	+	+	+
		10	200					+		+			
		11	150	+	+	+			+		+	+	+
		12	100	+	+	+		+	+	+	+	+	+
Variable bands = 10, **Total № = 12**				**7**	**9**	**6**	**5**	**8**	**8**	**8**	**9**	**9**	**9**
		1	1100	+	+				+		+	+	+
		2	1000					+		+		+	
		3	870	+	+			+	+		+		+
2		4	790	+			+		+		+		+
		5	710	+	+				+		+		
	75	6	550			+	+	+		+		+	+
		7	460	+	+				+	+		+	+
		10	220	+	+	+	+	+	+	+	+		+
		11	150	+	+	+	+	+	+		+	+	+
Variable bands = 9, **Total №= 11**				**9**	**8**	**5**	**6**	**7**	**9**	**6**	**8**	**7**	**9**
		1	2000				+	+	+	+	+	+	+
		3	1400	+	+	+							
3	55.56	4	910		+								
		5	740	+	+			+	+	+	+	+	
		6	600	+									
Variable bands = 5, **Total №= 9**				**7**	**7**	**5**	**5**	**6**	**6**	**6**	**6**	**6**	**5**
Total variable bands = 24 **Overall total bands = 32**				Mean polymorphic percentage of the three primers= **75%**									

Primer name	*P%	Fragment №	Fragment Bs	*Staphylococcus aureus* isolates 1	2	3	4	5	6	7	8	9	10
		1	1050		+			+	+	+	+	+	+
		2	1000		+			+	+	+	+	+	+
		3	900		+	+	+				+	+	+
1		4	800	+	+								
	83.33	5	700	+	+			+	+	+	+	+	+
		6	600	+		+	+						
		8	350				+	+	+	+	+	+	+
		10	200					+		+			
		11	150	+	+	+			+		+	+	+
		12	100	+	+	+		+	+	+	+	+	+
Variable bands = 10, **Total № = 12**				**7**	**9**	**6**	**5**	**8**	**8**	**8**	**9**	**9**	**9**
		1	1100	+	+				+		+	+	+
		2	1000					+		+		+	
		3	870	+	+			+	+		+		+
2		4	790	+			+		+		+		+
		5	710	+	+				+		+		
	75	6	550			+	+	+		+		+	+
		7	460	+	+				+	+		+	+
		10	220	+	+	+	+	+	+	+	+		+
		11	150	+	+	+	+	+	+		+	+	+
Variable bands = 9, **Total №= 11**				**9**	**8**	**5**	**6**	**7**	**9**	**6**	**8**	**7**	**9**
		1	2000				+	+	+	+	+	+	+
		3	1400	+	+	+							
3	55.56	4	910		+								
		5	740	+	+			+	+	+	+	+	
		6	600	+									
Variable bands = 5, **Total №= 9**				**7**	**7**	**5**	**5**	**6**	**6**	**6**	**6**	**6**	**5**
Total variable bands = 24 **Overall total bands = 32**				Mean polymorphic percentage of the three primers= **75%**									

+ = Present of amplified bands, Bs = Molecular size by base pair, P% = Polymorphic percentage, Total = Total number of amplified bands

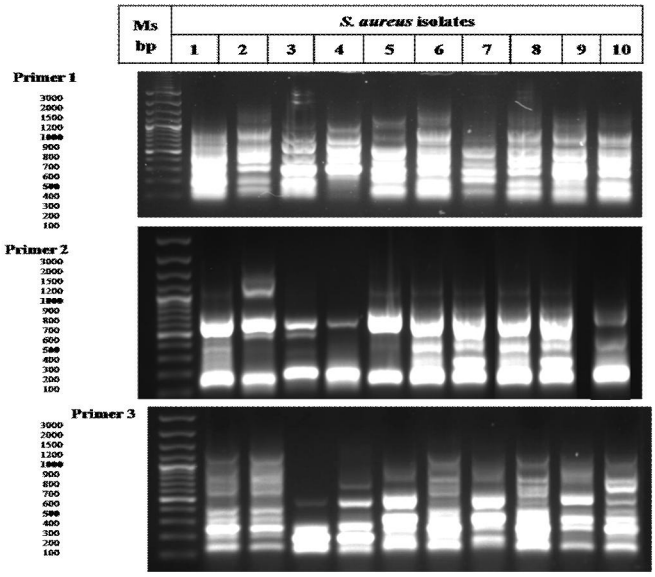

Fig. 1. RAPD amplification profiles of ten *S. aureus* isolates using three primers, Ms= 100 bp DNA ladder.

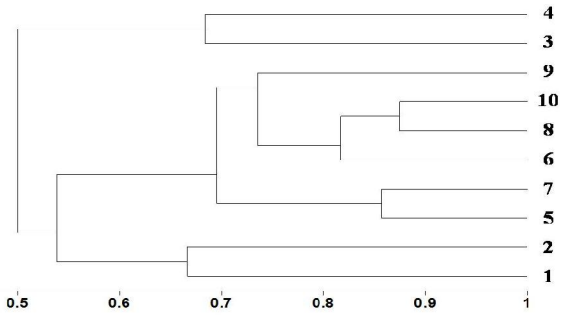

Fig. 2. Dendrogram represented the genetic relationships among ten *S. aureus* isolates using UPGMA cluster analysis of Nei genetic similarity coefficients generated from three RAPD primers.

The three primers showed a mean polymorphism of 75%, whereas the polymorphic percentage of primer 1 was higher (83.33) followed by primer 2 (75); however primer 3 displayed unexpected lower percentage with 55.56.

Primer 1 revealed 12 fragments, 10 of which were polymorphic with sizes ranging from 100 to 1050 bp; two fragments with 250 and 500 bp were frequently observed among the ten isolates (Fig. 1; Table 2). The total number of amplified fragments of the isolates varied considerably. For instance, in isolates (2, 8, 9 and 10) 9 fragments with different molecular sizes were amplified and the three later isolates were similar in fragment sizes. Eight fragments were amplified in isolates (5, 6 and 7), whereas 5 and 7 showed similar fragment profiles. The remaining 3 isolates showed the lower numbers with 5, 6 and 7 fragments. On the other hand, one fragment was detected distinctively in two isolates: 800 bp in isolates 1 and 2 & 200bp in 5 and 7.

As a result, RAPD analysis revealed that the amplified PCR products of most of the 10 isolates vary in molecular size patterns even with the equal total fragments.

Primer 2 revealed 11 fragments, 9 of which were polymorphic with sizes ranging from 150 to 1100 bp (Fig. 1; Table 2). The total fragment numbers of the ten isolates varied significantly in their fragments in a descending order from 9 to 5. Three isolates 1, 6 and 10 revealed the highest with 9 fragments, followed by isolates 2 and 8 with 8, while isolate 3 revealed fives. Primer 3 revealed 9 fragments, 5 of which were polymorphic with sizes ranging from 240 to 2000 bp and four fragments were commonly detected among all isolates (Fig. 1; Table 2). The total number of fragments of the ten isolates varied from 5 in isolates 3, 4 and 10 to seven in two isolates (1 and 2), while six fragments were detected in the remaining five isolates. Some distinctive amplified fragment were *S. aureus* isolate-specific, for instance 600 and 910 bp were uniquely detected in isolates 1 and 2, respectively.

3.2. Genetic similarity of the ten *S. aureus* isolates using RAPD analysis

Genetic similarity between each two pairs of the ten *S. aureus* was performed using the Nei similarity index on the basis of RAPD amplified fragments using the three random primers (Table 3). Genetic similarity percentages were calculated from the amplified fragment data using un-weighted pair group method with averages (UPGMA).

The constructed UPGMA dendrogram of the three primers showed two main clusters, the first included

two isolates 3 and 4 with bootstrap (68.4) and the second includes 8 isolates. The second cluster divided the 8 isolates into two sub-clusters, whereas the first contained two isolates 1 and 2 with similarity (66.7%). The second contained 6 isolates and subsequently divided to two branches, the first branch containing isolates 5 and 7 (85.7) and the second divided more to two sub-branches. One contained isolate 9 and the second showed three isolate 6 and the two isolates 8 and 10 with 87.5% (Fig. 2).

Table 3. Genetic similarity percentages of *S. aureus* isolates based on RAPD amplified products.

Isolates	1	2	3	4	5	6	7	8	9
2	67								
3	46	39							
4	46	35	68						
5	45	54	50	57					
6	63	67	41	52	75				
7	41	50	46	52	**86**	71			
8	57	61	52	58	68	**83**	58		
9	43	52	54	54	71	65	74	72	
10	55	59	56	63	72	**80**	68	**88**	**83**

Using the three primers, isolate 8 revealed most high similarity with isolate 10 (88%) followed by isolates 5 and 7 (86%). Additionally, isolates 6 and 8 showed similar percentage (83%) like isolates 9 and 10 as shown in Table (3). In contrast, isolates 2 and 4 showed the lowest similarity percentage with 35%, followed by isolates 2 and 3 (39%). All the other remaining pairs showed intermediate similarity percentages.

4. DICUSSION

S. aureus is one of the infectious causes most commonly spread in hospitals(Hugo and Russell, 1995, Mohanasoundaram & Lalitha, 2008 and Marchese et al., 2009) and in the intensive care units (Paterson, 2006). During the past decade, an increasing number of MRSA cases have been encountered globally among healthy community (Kluytmans-Vandenbergh and Kluytmans, 2006). Methicillin resistance in *Staphylococcus* is mainly mediated by the over-production of PBP2a, an additional modified penicillin binding protein (PBP) with low affinity for ß-lactam antibiotics. The *mecA* gene, the structural determinant that encodes PBP2a, is therefore considered as a useful molecular marker of putative methicillin resistance in *S. aureus* (Swenson and Tenover, 2005).

In the present study, the ten *Staphylococcus aureus* clinical strains isolated from patient in Jaizan Hospital, Saudi Arabia revealed DNA polymorphism using the three RAPD primers; either in the occurrence of amplified fragments or in the variable genetic similarities of each isolate with the others. Despite the fact that they should display narrow and low variation due to the genomic structure of the *Staphylococcus aureus* species and the structure of the 10 mer-RAPD primers. Eventually, the fluctuation of genetic similarity values each of the ten isolates with others using the three primers evidently revealed the divergent genetic backgrounds of such isolates with their DNA polymorphism patterns. Moreover, the results revealed that the 10 isolates were genetically different and some primers used such as primers 1 and 2, showed high polymorphism. These results were in agreement with several reports, which stated polymorphism among isolates using RAPD fingerprinting. For instance, ten out of 100 primers showed polymorphism among 18 different isolates of *S. aureus* from Nigeria generating 88 bands, 51 of which were polymorphic(Onasanya et al., 2003). In another study, Singh et al., 2006) detected polymorphisms between isolates and they suggested that biotyping often lacks discriminatory power because of variations in gene expression and random mutations that may alter biologic properties of microorganisms. RAPD-PCR grouped 35 *S. aureus* isolated from food raw materials and workers' hands into five clusters (A-E) showing 19 RAPD types with discrimination indexes (D) of 0.949(Ye et al., 2012).

Marked genetic variability among 130 *S. aureus* strains isolated from different raw-milk dairy products (122 isolates) and human samples (eight isolates)(Morandi et al., 2010). (Nikbakht *et al.*,2008) detected 43 RAPD profiles of MRSA isolates collected from clinical specimens and noses of 460 staff and inpatients. The isolates clustered into 18 taxa with 50% similarity, indicating the heterogeneity of their test isolates.

5. REFERENCES

1. Baksheeva SS, Avetisian LP, MIu C and Shaginian IA (2011). Genetic diversity of *Staphylococcus aureus* strains that colonize nose mucous membrane of children living in a large industrial city. Zh Mikrobiol. Epidemiol. Immunobiol. 6: 52-54.
2. Bannerman TL (2003). Staphylococcus, Micrococcus and other catalase-positive cocci that grow aerobically. In: Manual of Clinical Microbiology. Murray P.R., Baron E.J., Jorgensen J.H., Pfaller M.A. and Yolken R.H. [Eds.]. 8th ed. ASM Press, Washington D.C. pp: 384-402.
3. Haley RW, Culver DH, White JW, Morgan WM, Emori TG (1985). The nationwide nosocomial infection rate: a new need for vital statistics. Am J Epidemiol., pp. 121:159.
4. Hugo WB, Russell AD (1995). Pharmaceutical Microbiology, 5th ed. Blackwell scince, pp. 391-402.
5. Jay JM (1986). Staphylococcal gastroenteritis. In, Jay JM (Ed): Modern Food Microbiology. 3rd ed.,

Van Nostrand Reinhold Company Inc., New York, pp. 437-458.
6. Kluytmans J, van Belkum A, Verbrugh H (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms and associated risks. Clin. Microbiol. Rev. 10, pp. 505–520.
7. Kluytmans-Vandenbergh MF, Kluytmans JA (2006). Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. Clin Microbiol Infect. 12(7), pp. 697-8.
8. Marchese A, Gualco L, Maioli E, Debbia E (2009). Molecular analysis and susceptibility patterns of meticillin-resistant *Staphylococcus aureus* (MRSA) strains circulating in the community in the Ligurian area, a northern region of Italy: emergence of USA300 and EMRSA-15 clones. Int. J. Antimicrob. Agents 34(5), pp.424-428.
9. Mohanasoundaram MK, Lalitha MK (2008). Comparison of phenotypic versus genotyic methods in detection of methicillin –resistant *S. aureus*. Indian. J. Med. Res. 127(1), pp. 78-84.
10. Morandi S, Brasca M, Lodi R, Brusetti L, Andrighetto C. and Lombardi A. (2010). Biochemical profiles, RFLP, RAPD and multilocus variable number tandem repeat analysis (MLVA) for typing *Staphylococcus aureus* isolated from dairy products. Research in Veterinary Science 88: 427-435.
11. Naffa RG, Bdour SM, Migdadi HM and Shehabi AA (2006). Enterotoxicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan. Journal of Medical Microbiology 55: 183-187.
12. Nariman AH Aly, Effat AM Soliman and Tahany El-Kawokgy (2008). RAPD identification of local *Bacillus thuringiensis* isolates toxic to *Spodoptera littoralis* and *Culex pipiens* using universal primers for *cry* genes. Middle Eastern and Russian Journal of Plant Science and Biotechnology (Japan) 2: 60-66.
13. Narmeen SM and Jubrael MS (2009). Isolation and identification of *Staphylococcus aureus* using classical and molecular methods. J. Duhok Univ. 12: 10-16.
14. National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria that grow *aerobically*. Approved standard, 6th edition, NCCLS document M7-A6. Wayne, PA: National Committee for Clinical Laboratory Standards, (2003).
15. Nei M and Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269–5273.
16. Nikbakht M, Nahaei MR, Akhi MT, Asgharzadeh M and Nikvash S (2008). Molecular fingerprinting of meticillin-resistant *Staphylococcus aureus* strains isolated from patients and staff of two Iranian hospitals. Journal of Hospital Infection 69: 46-55.
17. Onasanya A, Mignouna HD and Thottappilly G (2003). Genetic fingerprinting and phylogenetic diversity of *Staphylococcus aureus* isolates from Nigeria. African Journal of Biotechnology 2: 246-250.
18. Paterson DL (2006). Resistance in gram-negative bacteria: Enterobacteriaceae. Am. J. Med. 119, S20e8. discussion S62e70.
19. Pittet D, Tarara D, Wenzel RP (1994). Nosocomial bloodstream infection in critically ill patients, excess length of stay, extra costs, and attributable mortality. JAMA, 271:1, pp. 598-601.
20. Reinoso E, Bettera S, Frigerio C, Direnzo M, Calzolari A (2004). Bogni, RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human hosts. Microbiol Res. 159, pp. 245-255.
21. Singh A, Goering RV, Simjee S, Foley SL and Zervos MJ (2006). Application of molecular techniques to the study of hospital infection. Clinical Microbiology Reviews 19: 512-530.
22. Swenson JM, Tenover FC (2005). Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. J. Clin. Microbiol. 43, pp. 3818-3823.
23. Tenover F, Arbeit R, Archer G, Biddle J, Byrne S, Goering R. (1994). Comparison of traditional and molecular methods of typing isolated of *Staphylococcus aureus*. J. Clin. Microbiol. 32: 407-415.
24. van Leeuwen WB, Jay C, S. Snijders, Durin N, Lacroix B, Verbrugh HA, Enright MC, Troesch A, van Belkum A (2003). Multilocus sequence typing of *Staphylococcus aureus* with DNA array technology. J. Clin. Microbiol. 41, pp. 3323-3326.
25. Ye Y, Jiang Q, Wu Q, Zhang J, Lu J and Lin L (2012). The characterization and comparison of *Staphylococcus aureus* by antibiotic susceptibility testing, enterobacterial repetitive intergenic consensus-polymerase chain reaction, and RAPD-PCR. Foodborne Pathog. Dis. 9: 168-171.
26. Yoshida T, Kondo N, Abu-Hanifah Y, Hiramatsu K (1997). Combined use of ribotyping, PFGE typing and IS431 typing in the discrimination of nosocomial strains of methicillin-resistant *Staphylococcus aureus*. Microbiol Immunol 41, pp. 687–695.