JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Jaradat et al. 2013 : 2 (6) 2455-2461

STAPHYLOCOCCUS AUREUS ISOLATES FROM CAMELS DIFFER IN COAGULASE PRODUCTION, GENOTYPE AND METHICILLIN RESISTANCE GENE PROFILES

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ARTICLE INFO

Received 19. 2. 2013
Revised 2. 5. 2013
Accepted 10. 5. 2013
Published 1. 6. 2013

Short communication

OPEN ACCESS

ABSTRACT

Accurate and rapid typing of *S. aureus* is crucial to the control of its infections and minimizing its leakage to the food chain. The primary purpose of this research was to isolate *S. aureus* from camels' meat and nasal swabs and to characterize the isolates for coagulase production and the presence of methicillin gene using PCR-RFLP of coagulase gene. A total of 264 camel's meat and nasal swabs were collected from abattoirs or meat markets and were used in the study. Ninety two percent of samples showed typical colonies of *S. aureus* on Baird-Parker agar with a mean count $2.5 \times 10^4 \pm 1.8 \times 10^4$ CFU g^{-1}. Upon confirmation of the isolates using *S. aureus* specific thermonuclease gene (*nuc*) PCR primers, only 64 isolates contained the specific product and thus were confirmed as *S. aureus*. However, when tested for the presence of coagulase gene, only 48 of them were positive while the other 16 were coagulase negative. Coagulase gene-RFLP revealed 19 distinct patterns when the gene was digested with *Alu* I and *Cfo* I. The typing revealed that the 48 classified isolates were genetically diverse and comprised a heterogeneous population with 14 genotypes at a 44.4% similarity level. When the coagulase positive isolates were tested for the presence of methicillin resistance (*mec* A) gene, 37 of the isolates were positive while the other 11 isolates were negative. The high heterogeneity among *S. aureus* isolates might be due to cross contamination between camel carcasses in slaughter houses and from handlers and their utensils.

Keywords: Coagulase gene, *Staphylococcus aureus*, molecular typing, camel, MRSA

INTRODUCTION

Genus *Staphylococcus* contains 47 species and 24 subspecies, as reported in the List of Prokaryotic Names with Standing in Nomenclature as of the full update on September 24, 2012 (**www.bacterio.net**). Among the identified species, 19 species are of potential interest in food, and only six of the species are coagulase positive (**Kloos and Bannerman, 2005**). The most important species among the potential pathogens is *Staphylococcus aureus* subsp. *aureus* (*S. aureus*). *Staphylococcus aureus* by most are coagulase positive though coagulase negative strains are also common in animal hosts, humans, meat and milk and their products (**Matthews *et al.*, 1997; Woo *et al.*, 2001; Willey *et al.*, 2008;Tarazi *et al.*, 2009, Alaboudi *et al.*, 2012**).

Staphylococcus aureus is considered one of the main food-borne pathogens worldwide, as they produce coagulase, heat stable nuclease or enterotoxins (**Jay *et al.*, 2005**). It is also a major causative agent of clinical or subclinical mastitis of dairy ruminants (**Schlegelov *et al.*, 2003; Peles *et al.*, 2007**). Poultry, meat and egg products as well as milk and milk products have been reported as common foods that may cause staphylococcal food poisoning (**Le Loir *et al.*, 2003**).

Strains of *S. aureus* that exhibit resistance to methicillin (Methicillin Resistant *S. aureus* [MRSA]) are among the most life-threatening antibiotic resistant pathogens. Meat products are usually not considered a significant source of MRSA (**Lozano *et al.*, 2009**). However, new studies conducted in Canada and Europe have shown that the MRSA is widespread among livestock, farmers, and meat (**Shuaihua *et al.*, 2009**). In the same token, the Dutch Food Safety Agency sampled various kinds of meat collected from retail trade and reported the presence of MRSA isolates in 11.9% of the analyzed samples (**de Boer *et al.*, 2009**).

Camel (*Camelus dromedaries*) meat is a good source of meat in areas where climate adversely affects rearing other animals (**Knoess, 1977**). In Jordan, there are approximately 14,000 one-humped camels being raised mainly for meat production.

Typing pathogenic bacteria is an important part of the control of food poisoning. With proper typing, the source of infection can be traced and consequently better control measures can be implemented (**Symms *et al.*, 1998**). Earlier, health authorities used to use biotyping to classify incriminated *S. aureus* strains into human, poultry, bovine, ovine and nonspecific biovars (**Devriese, 1984**). However such classification fails short of tracing the origin of many isolates due to the untypability of many strains (**Tarazi *et al.*, 2009**). Other phenotypic methods are also inadequate to trace any particular *S. aureus* strain. Molecular typing provides alternative methods to these traditional typing methods and provides more assurance of the origin of an outbreak strain (**Hookey *et al.*, 1998**).

Among the methods used for molecular typing of *S. aureus* isolates, pulsed-field gel electrophoresis (PFGE) is considered to be the gold standard and compares favorably with other typing techniques. However, PFGE is technically demanding, time-consuming, expensive, and lacks an agreed criterion for the interpretation of banding patterns, especially in multi lab projects (**Symms *et al.*, 1998**). Multilocus sequence typing (MLST) is a reliable method but it is expensive and needs an extensive work where seven housekeeping genes need to be sequenced (**Enright *et al.*, 2000**). Multiple loci VNTR fingerprinting is a convenient method of typing *S. aureus,* with results compared to PFGE or spa typing, yet it needs a large set of PCR primers (**Luczak-Kadlubowska *et al.*, 2008**). Coagulase production is believed to aid in the virulence of coagulase positive *S. aureus* (**Baddair *et al.*, 1994**). Restriction Fragment Length Polymorphism (RFLP) of coagulase gene provides a simple method of typing *S. aureus* isolates (**Walker *et al.*, 1998**). This method is based on polymerase chain reaction amplification of the variable region of the numerous variants of the coagulase gene (**Goh *et al.*, 1992**) followed by restriction enzyme digestion and the subsequent analysis of the generated RFLP patterns. This method is fast, easy, relatively inexpensive, and differentiates coagulase positive from coagulase negative strains. In addition, it can be used as an epidemiological marker of studied isolates (**Hookey *et al.*, 1998; Walker *et al.*, 1998**). However, the

inability to type coagulase negative strains disadvantages the method and decreases its usage.

Staphylococcus aureus has been isolated, tested for antibiotics resistance and classified virtually from all sources including camels (**Monecke *et al.*, 2011; Shuiep *et al*., 2009**). However in Jordan, *S. aureus* isolated from camel carcasses were not tested thoroughly or classified using molecular typing methods to study their genetic relatedness.

The objectives of the study were to; i) isolate *S. aureus* from nasal swabs and camel meat and testing the isolates for presence of coagulase (*coa*) and methicillin resistance (*mec* A) genes and ii) type the isolates using PCR-RFLP of coagulase gene to understand their genetic relatedness.

MATERIAL AND METHODS

Sample collection

A total of 264 camel meat and nasal swab samples were collected randomly from 5 sporadic camel herds around the city of Irbid in Jordan and examined between June 2007 and the end of July 2008. The meat samples (147) were purchased either from the local retail stores (30) or obtained directly from Al Ramtha-Jordan Abattoir (117). Each meat sample represented one camel in which 250 grams of thin meat pieces were collected aseptically from five different locations of each carcass. The nose swabs (117) were collected also randomly by inserting sterile swabs moistened in 0.1% Buffer Peptone (Hi-Media, India) in the external openings of the nose. The nasal swab samples were not necessarily taken from the same camels that the meat was taken from. All samples were transported as soon as possible to the laboratory under aseptic cooled conditions and examined within 6 hours of collection time.

Isolation of *Staphylococcus*

Staphylococcus aureus isolation was performed by mixing 25g of each meat sample with 225 ml of 0.1% sterile buffered peptone water, and homogenized in stomacher (Seward, UK) at 230 cycles for 1.5 min. Serial dilutions were prepared in buffered peptone and 0.1 ml of selected dilutions of each sample was spread onto Baird-Parker agar base (**Bird, 1996**) supplemented with egg yolk-tellurite emulsion (Oxoid, UK). Plates were incubated at 37° C for 24-48 hours. Nasal swabs were enriched by incubation in Brain Heart Infusion broth (BHI) (Hi-Media, India) overnight at 37° C followed by subsequent streaking over Baird-Parker Agar, and incubation at 37° C for 24-48 hours. Black to dark grey colonies with opaque zones, surrounded by clear halo zone was considered presumptive *S. aureus*. Prior to subjecting the isolates to molecular typing, presumptive colonies were subjected to coagulase production using the Latex Coagulase Kit (Plasmatec, Canada) as per manufacturer's instructions.

Molecular Confirmation of the *Staphylococcus*

Presumptive *S. aureus* isolates were tested by amplifying the *nuc* gene using PCR analysis as described by **Brakstad *et al.*, (1992)**. The primer sequence for the *nuc* gene used are presented in Table 1. Briefly, PCR amplifications were conducted in tubes containing 25µl Ready Mix™ Taq PCR reaction mix (Sigma®, USA), 1µl of 1 mM $MgCl_2$ (Promega, USA), 1µl from each primer (10 pm) (Table 1) and 5µl Bacterial DNA template with the addition of nuclease free water (Promega, USA) to a final volume of 50 µl (**Pinto *et al.*, 2005**). Negative controls were performed with 5 µl of water instead of DNA template while VITEC confirmed *S. aureus* isolates were used as the positive control. Reactions were carried out in Mygenie 96 Thermal Block thermal cycler (Bioneer, Korea). PCR products were separated by electrophoresis on 2% agarose (Bio basic, Markham, Ontario, Canada) gel and visualized under U.V light.

Molecular typing by PCR and RFLP

Molecular typing of the isolates was carried out based on polymerase chain reaction amplification of the variable region of the coagulase gene (*coa* gene) followed by *Alu* 1 and *Cfo* I restriction enzyme digestion and analysis of restriction fragment length polymorphism as described below. Primers and conditions of running are listed in Table 1.

Amplification of the coagulase gene and the restriction digestion of the isolates

Amplifications of coagulase gene were conducted in PCR tubes containing 25µl Ready Mix™ Taq PCR reaction mix (Sigma®, USA), 1 µl from each primer (10 pm) and 5 µl DNA template. Nuclease free water was added to a final volume of 50µl. PCR products (10 µl) were electrophoresed on 2% agarose gel in Tris- borate-EDTA (TBE) buffer in the presence of ethidium bromide (Promega, USA). A 100-bp DNA ladder (Bio basic, Markham, Ontario, Canada) was included in each run. The DNA bands were visualized using UV transilluminator and photographed using the Vilber Lourmat detection system (Marne-la-Vallée Cedex, France). Positive and negative control strains were used for further confirmation.

Restriction fragment length polymorphism (RFLP) of coagulase gene

PCR amplified coagulase gene products (7 to 10 µl) were digested in tubes containing 2 µl of restriction endonuclease enzyme *Alu* I or *Cfo* I (10 U μl^{-1}) (Fermentas, EU). The mixture was adjusted to 20 µl by adding nuclease free water, and incubated at 37° C for 90 min in a water bath. Twenty microliters of digested PCR products were electrophoresed on 3% agarose gels in TBE buffer in the presence of ethidium bromide, and the band patterns and sizes were scored (**Hookey *et al.*, 1998**).

Table 1 Oligonucleotide primer pairs and PCR running conditions

Gene	Primers 5'…….3'	Amplification conditions			Product size (bp)	Reference
		Temp	Time	No. of Cycles		
Methicillin resistance (mecA)	***mec*A F** 5-'GTAGAAATGACTGAACGTCCGATGA 3' ***mec*A R** 5'CCAATTCCACATTGTTTCGGTCTAA 3'	94° C	4 min	1	310	**Geha *et al.*, (1994)**
		94° C	45 s			
		50° C	45 s	35		
		72° C	60 s			
		72° C	2 min	1		
Coagulase (coa)	***Coa* F** 5'-ATA GAG ATG CTG GTA CAG G-3' ***Coa* R** 5'-GCT TCC GAT TGT TCG ATG C-3'	94° C	45 s	1	Variable [a]	**Hookey *et al.*, (1998)**
		94° C	20 s			
		57° C	15 s	35		
		72 °	15 s			
		72° C	2 min	1		

[a] 875, 660, 603, or 547 bp

Computer analysis of RFLP data

RFLP banding patterns of the 48 isolates of *S. aureus* were examined and bands were scored, with the data coded as a factor of 1 or 0, representing the presence or absence of bands, respectively. A similarity matrix among *S. aureus* isolates was produced using the Jaccard coefficient as:

$$S_j = \frac{n_{xy}}{n_x + n_y}$$

Where n_{xy} is the number of shared restriction fragments between two samples, n_x and n_y are the total number of fragments observed in samples x and y without repeating, respectively. A dendrogram showing the genetic relatedness among the isolates was constructed from the resulting data using SPSS version 16 (SPSS, IBM Inc., NY, USA). The cutoff for the dendrogram was selected based on the average of the mean similarity matrix.

Detection of the methicillin resistance gene (*mec* A) by PCR

The presence of *mec* A gene was tested following the procedure described by **Geha *et al.*, (1994)**. Briefly, 5 µl of template DNA was added to the PCR mixture containing 1X master mix (Promega, USA) and 50 pmol of the forward and

reverse *mec* A primers (Table 1). Nuclease-free water (Promega, USA) was added to reach a final reaction volume of 50 µl. DNA amplification was carried out using conditions outlined in Table 1. Positive and negative control reference strains were used for confirmation.

PCR products were electrophoresed on 2% agarose gels in the presence of ethidium bromide and TBE buffer.

RESULTS

Isolation and characterization of the isolates

One hundred and forty three out of 147 (97.27%) camel meat samples examined showed typical *S. aureus* colonies on BPA while 100 of 117 (85.4%) nasal swabs showed typical *S. aureus* colonies on BPA. Ninety two percent of samples showed typical colonies of *S. aureus* on Baird-Parker agar with a mean count $2.5 \times 10^4 \pm 1.8 \times 10^4$ CFU g^{-1}. When the 243 presumptive *S. aureus* isolates were tested for the presence of *S. aureus* specific-thermonuclease gene (*nuc*), only 64 isolates (26%) were positive while the rest did not show any product, and thus were considered non-*S. aureus*. Among these 64 confirmed *S. aureus* isolates, there was only 11 isolates obtained from nasal swabs while the other 53 were obtained from meat samples (Table 2). Out of the nasal swabs, 7 isolates (4, 12, 15, 25, 120, 134, 143) were coagulase positive while the other 4 isolates (22, 33, 96, 100) were coagulase negative. Similarly, out of the meat samples, 41 isolates were coagulase positive while the other 12 isolates were coagulase negative. The entire coagulase positive but one isolate, exhibited one single fragment for the coagulase gene ranging in size from 500 to 900 bp. The most frequent product size was 600 bp which was found in 46% of the isolates. This was followed by 700 bp product which was found in 35% of the isolates while the rest of the PCR products (500, 550, 895, 900) were found in only 19% of the isolates. PCR-amplified coagulase gene products of all tested coagulase positive isolates were digested with *Alu* I and *Cfo* I restriction enzymes, and the resulted fragments were separated on 3% agarose gel electrophoresis (Figure 1).

Table 2 Number of isolates used in the study, their source, presumptive positive isolates, negative isolates, confirmed positive by *nuc* and coagulase positives and negatives

Isolate Source and Type	Camels Meat	Camels Nasal Swabs	Total
Number of tested samples	147	117	264
Number of typical colonies	143	100	243 (92%)
Confirmed positives by Microbat system	58	16	74 (28%)
Confirmed by PCR (*nuc* gene)	53	11	64 (24.2%)
Coagulase positive (PCR)	41	7	48 (18.2%)
Coagulase negative (PCR)	12	4	16 (6.1%)

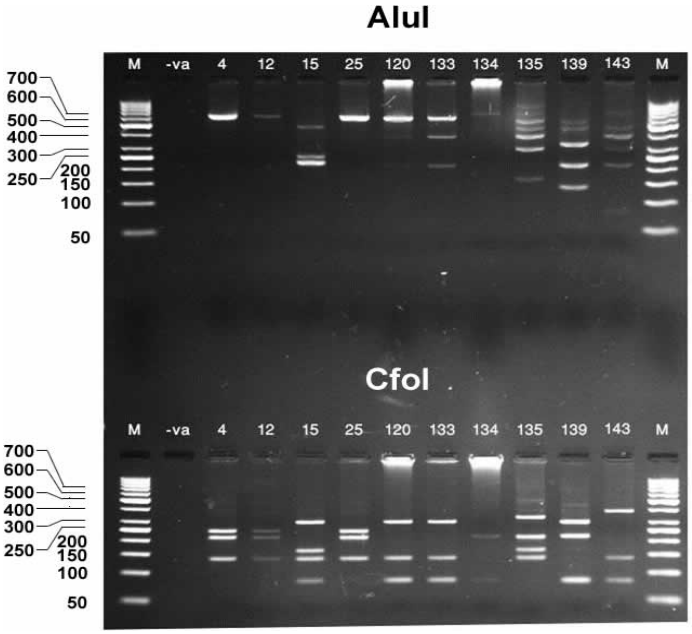

Figure 1 Restriction digestion of *Coa* gene in representative *S. aureus* isolates by *Alu* I and *Cfo* I enzymes. M, Molecular markers, -ve, negative control, numbers 4-143 representing 10 *S. aureus* isolates selected randomly from the total number of isolates in the study.

PCR-RFLP Analysis

Eleven distinct RFLP patterns were observed upon digestion with *Alu* I enzyme with the number of fragments varying from one (undigested product; 4, 12, 25,134, 145, 146, 147, 151, 152, 153, 158, 161, 169, 174, 175, 176, 177, 178, 180) to five, with sizes of the fragments varied from 85 to 650 bp. Ten distinct patterns where observed using the *Cfo* I enzyme. The number of fragments varied from two to four, and fragment sizes varied from 80 to 475 bp. However, the agarose gel analysis of the combination of both *Alu* I and *Cfo* I fragments of the RFLP patterns revealed 19 different types (Figure 2). Types 1, 2, 3, 4, 5, 6, 8, 10, 12, 15 and 19 were the most common and accounted for 83.3% of the isolates while type 12 alone accounted for 23% of the isolates. The remaining 8 isolates each fall in a single cluster by itself (2.1% of the total).

Phylogenetic and cluster analysis

These fragments were used for cluster analysis and phylogenetic study. The genetic similarity indices were calculated based on Jaccard coefficient and were in the range of 0.0-1.0. The average mean similarity index was 0.444 indicating that the isolates shared 44.4% of their RFLP fragments. The Upweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was constructed on the basis of similarity indices among *S. aureus* isolates using the 19 RFLP patterns to show genetic relatedness among the isolates (Figure 3). The dendrogram revealed three major clusters that were completely separated from each other (0% similarity).

Cluster I contained sub clusters A, B, and C joined at 9% similarity, while cluster II contained two major groups, IIa and IIb which also joined at 9% similarity. Group IIa contained sub clusters D and E joined at 23% similarity, while group IIb contained sub clusters F, G, H, I and J that were joined together at a similarity level of 20%. Cluster III contained sub clusters K, L, M and N joined together at about 9% similarity. When a cut-off line was drawn at a 44.4 % similarity value, as calculated from the Jaccard similarity index, 14 genotypes were recognized. A large number of *S. aureus* isolates belonged to genotype E with 14 isolates sharing a similarity of 0.5-1.0.

The other 34 isolates were distributed as following; genotype I with 6 isolates sharing 55% similarity while genotypes D, F, and G containing 4, 5, and 4 isolates, respectively, and an identical similarity of 1.0 shared among them. Furthermore, genotype K contained 3 isolates sharing 63% similarity and genotype M contained 2 isolates sharing 48% similarity. Other genotypes contained only a small number of isolates such as in genotypes A, B and C. They all contained two isolates that share an identical similarity of 1.0, while genotypes H, J, L, and N each contained only one isolate. It is worth mentioning that all but one of the isolates from swabs (isolate number 143) falls into cluster II.

When coagulase positive isolates were tested for the presence of *mec* A gene, about 37 isolates (76%) were positive while only 11 isolates were devoid of the gene. The negative isolates were scattered among the clusters with 3 isolates (120, 157, 172) in genotype I with another 3 isolates (4, 145,176) appearing in genotype E. Most of these *mec* A sensitive isolates were clustered along with *mec* A resistant isolates sharing identical RFLP patterns (similarity 1.0). For instance, isolate 161 that is *mec* A negative shared identical similarity with isolates 134, 174 and 175 which were positive for *mec* A resistance.

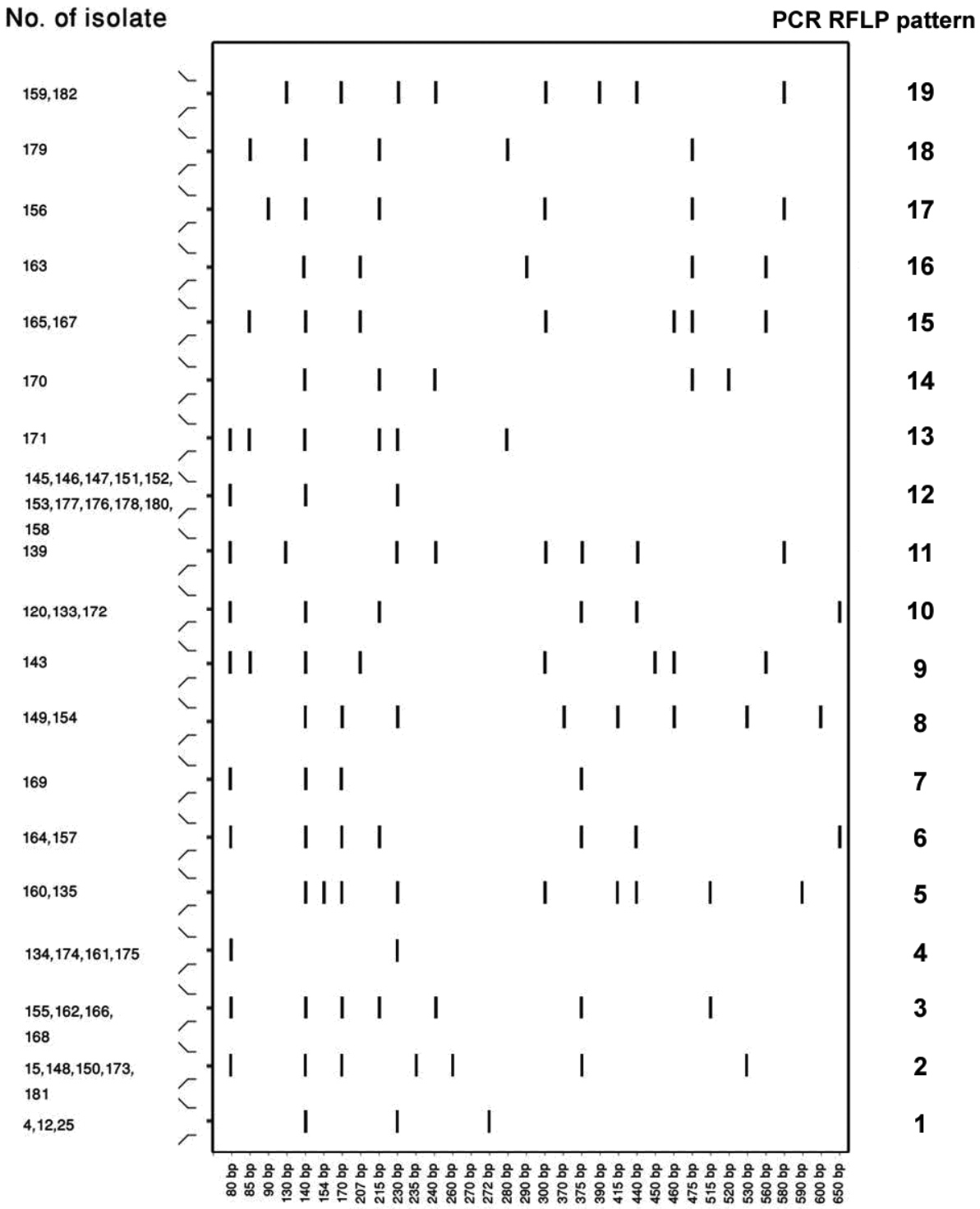

Figure 2 Schematic representations showing different restriction patterns of coagulase gene among *S. aureus* isolates isolated in this study digested with *Alu* I + *Cfo* I enzymes

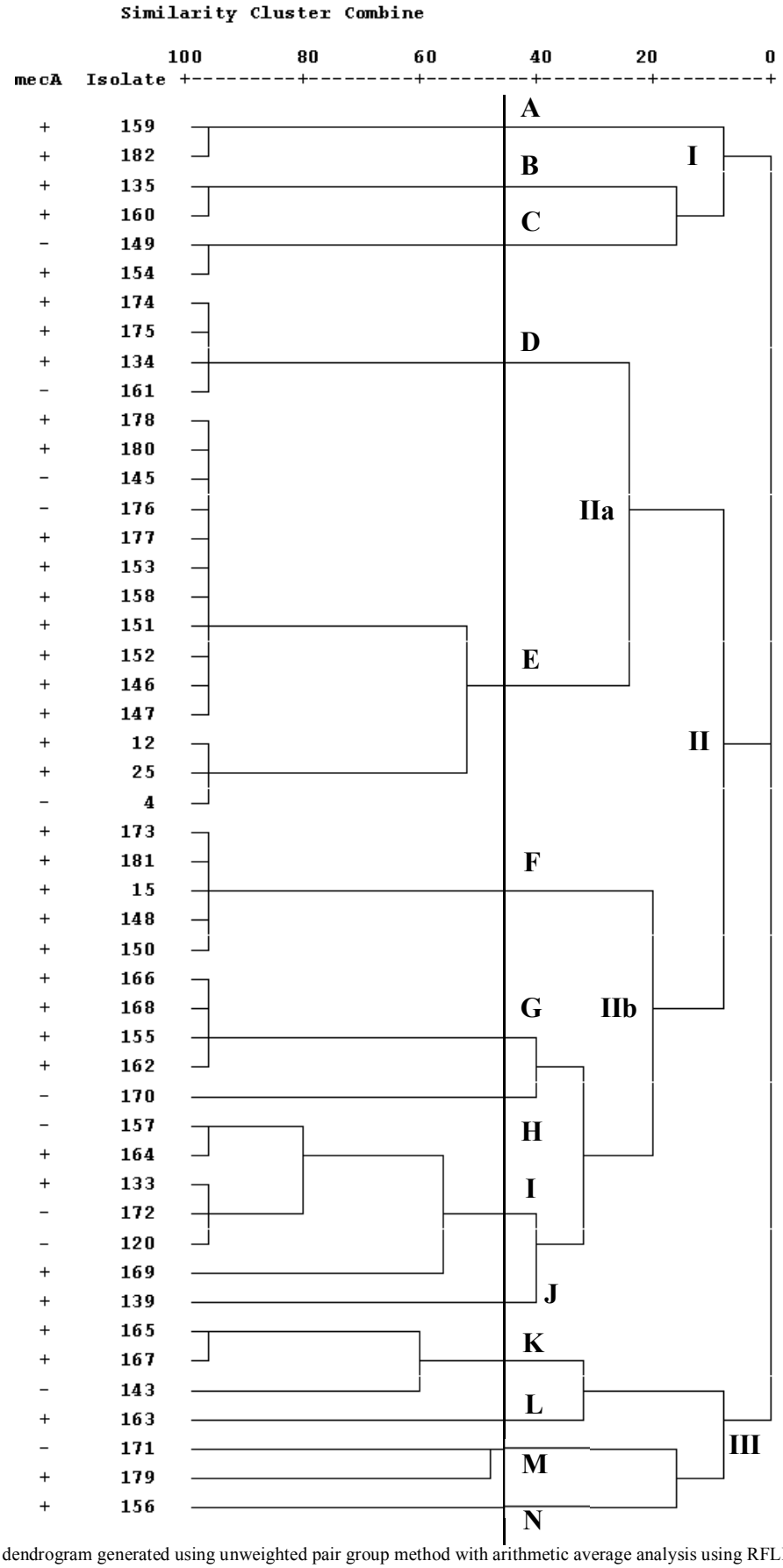

Figure 3 UPGMA dendrogram generated using unweighted pair group method with arithmetic average analysis using RFLP patterns of the coagulase gene and combining both *Alu*I+*Cfo*I enzymes. The cut-off was set at 44.4% similarity level and classified the isolates in 14 different groups (A - N). The scale at the top shows the similarity index

DISCUSSION

This study was undertaken to investigate the presence of *S. aureus* in camel meat and nasal swabs and to understand the genetic relationship among these isolates based on PCR-RFLP of the coagulase gene polymorphisms as well as to investigate the prevalence of *S. aureus* positive for *mec* A gene among the confirmed isolates. Coagulase production is considered a virulence factor that enables the pathogen to evade the host's immune system and is used frequently to identify *S. aureus* (**Baddair *et al.*, 1994**). Therefore, the variability of the 3' end of the coagulase gene is used for the genotyping of *S. aureus* isolates and studying their genetic relationship (**da Silva and da Silva, 2005**).

The observed high number of presumptive *S. aureus* in this study, and the lower numbers of confirmed isolates by *nuc* gene, signified the weakness of this method for the detection and identification of *S. aureus*. At the same time, it implied that most of the isolates may belong to other *Staphylococcus* species, which is not uncommon as they inhabit the skin of humans and higher animals (**Willey *et al.*, 2008**). In addition, based on the literature, there is a similarity in the *nuc* gene sequence in CoNs and CoPs Staphyloccoci that reaches up to 96% , with the most similar pair was *S. pseudintermedius* and *S. delphini* group B (95.9%). This striking similarity might be responsible for the differences in the presumptive and confirmed isolates (**Sasaki *et al.*, 2010**).

Earlier, *S. aureus* used to be biotyped using Divers Scheme (**Divers, 1984**) or phage typing, however, these systems were incapable of typing some coagulase positive strains. Molecular techniques depending on RFLP of coagulase gene classified these as untypable isolates. Nevertheless, not all the confirmed *S. aureus* isolates contained the *coa* gene, as about 25% of the isolates appeared be devoid of it, indicating lower pathgenicity of these isolates. Previously, coagulase-negative staphylococci were regarded as non-pathogenic however, their implication in many hospitals acquired bacteremia and their role in prolonging the hospital stay for many patients highlighted their important role as pathogens (**Martin *et al.*, 1989: Huebner and Goldmann, 1999**). Although coagulase-positive *Staphylococcus aureus* is the main agent responsible for food intoxication, some researches emphasize that coagulase-negative staphylococci (CNS) are also able to produce staphylococcal enterotoxins and may be a potential cause of food poisoning. In one study, CNS were isolated from food and the toxigenic capacity of the strains determined, where a total of 88 different food samples including meat and milk were analyzed, and 22.7% were found positive for CNS strains (**de Lourdes Ribeiro de Souza da Cunha *et al.*, 2006**). However, the incidence of Coagulase positive *Staphylococcus* vary considerably depending on the geographical location of the isolates and food types (**Karahan and Cetinkaya, 2007**).

When the isolates were tested for the presence of coagulase gene by PCR, five different gene sizes in a range of 500-900 kb were observed with the majority of the isolates exhibiting *coa* gene size of either 600 or 700 Kb.

The predominance of a particular strain of *S. aureus* might be the result of its increased resistance to the host immune response compared to those with the rare genotypes which could have lower resistance (**Moon *et al.*, 2007; Mullarky *et al.*, 2001**). Studies carried out by other researchers (**Hookey *et al.*, 1998; Kalorey *et al.*, 2007; Reinoso *et al.*, 2008; Salasia *et al.*, 2004**) using the same primer pairs, also showed that different coagulase gene types exist. Our results appeared very similar to the results reported by **Morandi *et al.* (2010)** who reported product sizes of the *coa* gene of their isolates to be between 420-800 bp with the 560 bp accounting for 50% of the isolates. However, though different restriction enzymes have been used, this study and our results appeared somewhat different from the results obtained by **Karahan and Cetinkeya, (2007)** who reported a single PCR product for their isolates with a larger size range of the fragments (500 to 1400 bp), which indicates the high heterogeneity of the *coa* gene among isolates from different regions. The reason for this polymorphism in the *coa* gene among *S. aureus* isolates could be due to certain deletion or insertion mutations by which a portion of the 3' end region of the *coa* gene is deleted or several nucleotides are inserted and consequently changed the *coa* gene size and probably antigenic properties of the coagulase enzyme (**Dastmalchi Saei *et al.*, 2009**).

Amplified *coa* gene products were subjected to restriction analysis by *Alu* I and *Cfo* I enzymes. RFLP of coagulase gene revealed 11 distinct patterns when the gene product was digested with *Alu* I while 10 distinct patterns were obtained when amplified *coa* gene was digested with *Cfo* I enzyme. It is worth mentioning that 19 isolates out of the 48 isolates were not digested by the *Alu* I enzyme. This could be due to the lack of restriction sites for the enzyme in the variable region of the gene in these isolates which could happen due to point mutations in the repeated region of the *coa* gene abolishing the *Alu* I restriction site (**Dastmalchi Saei *et al.*, 2009**). In contrast, all the isolates were digested by the *Cfo* I enzyme although several of these isolates produced only two fragments. Similar results were reported by **Dastmalchi Saei *et al.*, (2009)** who reported that 35 out of 58 coagulase positive *S. aureus* isolates were not digested by the *Alu* I enzyme, thus highlighting the inability of this enzyme to be used alone for the coagulase typing of *S. aureus*. To overcome this problem, the restriction results of both enzymes were combined together to encompass all the isolates as described by **Goh *et al.* (1992)**. When the restriction data of the two enzymes were combined together, the RFLP of the coagulase gene revealed 19 distinct patterns (Figure 2). The number of RFLP patterns appeared to be small compared to a study reported by **da Silva and da Silva, (2005)** who reported 49 RFLP patterns for only 64 isolates from bovine mastitis in Brazil indicating a very high variation among their isolates. Nevertheless, other researchers reported results similar to our results when using the *Alu* I to generate RFLP patterns of *S. aureus*. **Goh *et al.* (1992)** reported that 19 RFLP patterns were observed among 69 clinical *S. aureus* isolates when digested with *Alu* I enzyme. In contrast, **Karahan and Cetinkaya, (2007),** reported only 23 different restriction patterns for digestion of coagulase gene of 161 *S. aureus* using the *Alu* restriction enzyme. Similarly, **Morandi *et al.* (2010)** reported 30 RFLP patterns for 130 *S. aureus* isolates from dairy products. These studies indicate slightly less heterogeneity among these isolates owing to the low number of patterns compared to the high number of the isolates. As indicated above, the variations among RFLP of the coagulase gene may be due to variation in the sequence of coagulase gene among different isolates leading to different restriction sites. The variation in these restriction sites is utilized to differentiate isolates from different regions, sources or hosts.

Normally camels are grazed in wide open areas far from urbanization, with very little consumption of their meat and milk. They encounter little contact with humans and other animals; thus it would be expected that they keep relatively homogenous populations of coagulase positive *S. aureus*. Whereas those obtained from bovine samples showed a higher heterogeneity which probably could be due to movement of *S. aureus* from humans to cows and between other domestic species (**Reinoso *et al.*, 2004**). Nevertheless, when the camel isolates were analyzed by the UMPGA cluster analysis, the dendrogram revealed that the 48 classified isolates were genetically diverse and comprised a heterogeneous population with 14 genotypes at a 44.4% similarity level ranging between 0.0-1.0 (Figure 3). This means that the isolates shared only 44.4% of their RFLP fragments indicating a high level of DNA polymorphism among *S. aureus* isolates. The moderate level of similarity was not expected as isolates were drawn from one animal species and from one geographical location in Jordan in which we expected a higher level of similarity. The reason behind these results could be due to cross contamination of the camel carcass from the handlers or the processing utensils and might not reflect the true heterogeneity among the *S. aureus* populations in these camels. Similarly, results on *S. aureus* isolated from cows demonstrated heterogeneity of isolates from one herd and even one cow (**Kapur *et al.*, 1995**).

Six of the *S. aureus* isolates from swabs were typed in cluster II (4, 12, 15, 25, 120, 134) while isolate 143 was typed in Cluster III. Further, there was no meaningful correlation between isolates with *mec* A gene and a particular cluster. Interestingly, we have observed clusters with identical similarity indices (similarity index 1.0) containing both *mec* A sensitive and *mec* A resistant isolates indicating the inability of the RFLP typing system to differentiate between these two types of the isolates and the lack of any relation between the two virulence factors i.e. the methicillin and the coagulase. The identification of *mec* A resistant strains again emphasizes the fact that animals and their products may form important potential reservoirs of MRSA. The predominance of MRSA in animals and their products may suggests that animal or meat become colonized through contact with infected or colonized people and that meat could in turn pass MRSA back to humans (**Leonard and Markey, 2008**). Studies on *S. aureus* prevalence among Jordanian human nasal carriage and clinical isolates revealed large proportion of 25 and 50% MRSA, respectively (**Al zubi *et al.*, 2004; Borg *et al.*, 2007**). It is hard to draw any comparison between reported types and other animal, food or other reported isolates because of different typing techniques used in these studies.

CONCLUSION

This report demonstrates that *S. aureus* are common inhabitants of camels and their dressed carcasses. Traditional culturing and biochemical bio-typing methods used in this study fail short to identify and trace back the origin of *S. aureus* isolates. Therefore molecular techniques are suggested to be practiced in testing potential risk for *S. aureus* in food safety evaluation. In addition, it appeared that *S. aureus* isolates were heterogeneous although isolated from camels which normally graze in open areas separated from other animals highlighting the possibility of cross-contamination during processing. Furthermore, the detection of high percentages of *Staphylococcus* positive for *mec* A gene among the isolates indicates that animals and their products may form a reservoir of MRSA which could fire an alarm on the spread and transmission of MRSA among different hosts and food handlers as well.

Acknowledgments: The authors would like to acknowledge the Deanship of Research at the Jordan University of Science and Technology for the financial assistance of this project (Grant Number 159/2007).

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