Characterization of *Staphylococcus Aureus* of Animal Origin in Nosocomial Infection

*Hamza, I.E.; **Marssel, F.G.; ***Mai, W.E.

* Department of Bacteriology, Immunology, Mycology - Faculty of Veterinary Medicine,   Suez Canal University.
** Department of Food Hygiene, Animal Health Research Institute, Port Said Branch.
*** Department of Infection Prevention and Healthcare Quality, Port Said Military Hospital.

Abstract

This study discussed the prevalence of *S. aureus* in some hospitals in Port-Said governorate and the possibility impact of the *S. aureus* isolates from foods of animal origin on nosocomial infections by genetic relatedness between isolates from foods and infected patients.

The bacteriological examination of 355 samples reveals of *S. aureus* in 28 from 255 total samples taken from hospitals kitchens with percentage 11% and 48 *S. aureus* isolates from 100 swabs of infected patients with percentage 48%, the antibiotic sensitivity test of most isolates revealed multidrug resistant *S. aureus* strains.

Used random amplified polymorphic DNA (RAPD) patterns to determine genetic relatedness among *S. aureus* isolates that revealed genetic relatedness among the isolates and give indication about the possibility to impact of *S. aureus* isolates from foods of animal origin in human infections.

Introduction

Staphylococcus aureus is Gram-positive, coagulase-positive cocci of the family *Staphylococcaceae*, considering an important cause for both human and animal diseases, such as arthritis, mastitis, and urinary tract infection in animals, while in human pneumonia, food poisoning, and nosocomial infections *(John, 2003)*.

Antimicrobial abuse in animal husbandry and other agricultural activities leads to increase antimicrobial resistant bacteria in both animals and human that transported through contact directly with living animals or indirectly by contact or ingestion of food products of animal origin that also can transfer their resistance genes to commensal flora in intestinal tract. *(Kluytmans, 2009)*.

The present study was carried out to investigate the:

1. Characterization of *Staphylococcus aureus* of animal origin in nosocomial infection.

a. Isolation of *Staphylococcus aureus* from different samples in hospitals.

b. Biochemical identification of *Staphylococcus* isolates.

c. Antibiotic sensitivity of *Staphylococcus* isolates.

d. PCR detection of *Staphylococcus aureus*.

2. Determined genetic relatedness among the isolates from foods of animal origins and patients by Random amplified polymorphic DNA (RAPD).

Materials and Methods
Samples:

A total of three hundred and fifty five (355) random samples from the foods of animal origin &swabs from the surfaces where the food were prepared and lesions swabs of hospital acquired infected patients were collected from some hospitals in Port-Said governorate, two hundred and fifty five samples were collected from the hospitals kitchens where 52 swabs were collected from preparing raw meat and chickens surfaces & utensils, 50 samples from processed milk products as cheese, yoghurt, butter and powdered milk, 55 samples from raw meat, 45 samples from raw chicken and 53 samples from raw milk, in addition to 100 swabs taken from lesions of hospital acquired infected patients including 50 swabs from septic burned wounds, 25 from surgical sites infection and 25 from draining pus of peripheral venous catheters

(cannula) associated abscess, the samples were collected aseptically in polyethylene bags and transported to the laboratory in an isothermal box for subsequent analysis and swabs were placed in peptone saline and transported in a cool box with ice packs.

Bacteriological isolation and identification of *Staphylococcus aureus*:

According to ***Quinn et al. (2002) and Koneman et al. (1996);*** samples were cultured onto pepton water for 24 h at 37ºC and then a loopful was taken and cultured onto nutrient agar, blood agar, mannitol salt agar and Baird parker medium. All inoculated plates were incubated at 37ºC for 24-48h, suspected colonies of *S. aureus* were examined morphologically, biochemically according to *(FDA, 2001)* and microscopically according to *(Ryan and Ray, 2004).*

Antimicrobial sensitivity test for *Staphylococcus aureus*:

Disc diffusion test was done according to *(Finegold and Martin, 1982)* for detection of sensitivity degree of *S. aureus* isolates against 14 different antimicrobial discs (Ampicillin, Methicillin, Cephalexin, Cefaclor, Imipenem, Vancomycin, Bacitracin, Amikacin, Erythromycin, Clindamycin, Chloramphenicol, Ciprofloxacin, Norfloxacin and Trimethoprim + Sulphamethoxazole), degree of sensitivity was determined by measuring the clear zone around

each disc and interpreted according to *(Oxoid, 1982).*

Molecular Identification of Isolates:

Samples used for PCR assay:

Sent 17 isolates from foods of animal origin and 19 isolates from patients in the wards that was highly positive reaction for tube coagulase test for PCR assay, missed one sample from animal origin through the transportation, to be the final number of the samples that subjected to PCR assay was 16

isolates from foods of animal origin with total 35 samples.

Oligonucleotide primers used in PCR and RAPD:

Detection of clumping factor in isolates by clfA gene and Methicillin-resistant isolates by mecA gene by means of PCR, in addition to detection RAPD patterns by two arbitrary oligonucleotide primers M13 and H12 of the isolates.

They have specific sequence and amplify specific products as shown in Table (1).

Table (1): *Oligonucleotide primers encoding for PCR*

Target gene	Primer	Primer sequence (5'-3')	Length Of amplified product (bp)	Reference
*mec*A	*mec*A-1	GTA GAA ATG ACT GAA CGT CCG ATAA	310 bp	McClure *et al.*, 2006
	*mec*A-2	CCA ATT CCA CAT TGT TTC GGT CTAA		
*clf*A	*clf*A-F	GCAAAATCCAGCACAACAGGAAACGA	638 bp	Mason *et al.*, 2001
	*clf*A-R	CTTGATCTCCAGCCATAATTGGTGG		
	M13	GAGGGTGGCGGTTCT	Multiple bands	John, 2003
	H12	ACGCGCATGT	Multiple bands	

2.4.3. Cycling conditions of the primers during cPCR:

Table (2): *Temperature and time conditions of the primers during PCR*

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
*mec*A	94°C 5 min.	94°C 30 sec.	50°C 45 sec.	72°C 45 sec.	35	72°C 10 min.
*clf*A	94°C 5 min.	94°C 30 sec.	55°C 1 min.	72°C 1 min.	35	72°C 10 min.
H12 and M13	94°C 5 min.	94°C 1 min.	35°C 1 min.	72°C 2 min.	35	72°C 12 min.

DNA Molecular weight marker:

Hundred (100) bp DNA markers were used. The ladder was mixed gently by pipetting then 6 µl of the required ladder were directly loaded.

Analysis of Random amplified polymorphic DNA fingerprinting (RAPD) data:

Used GelCompar Π software *(version 6.6; Applied Maths, Belgium, 2013)* for analyzing

banding patterns that generated from each primer by calculate the different band-based similarity coefficient and the average of experiments for both primers to generate a dendrogram by the unweighted pair group method using arithmetic average clustering.

Results and discussion
Among 255 specimens from hospitals kitchens examined in this study, 28 contained *S. aureus* with a percentage of 11%. Prevalence of *S. aureus* from raw meat was 21.8%, from raw chicken was 4.4% and raw milk was 26.4%, while *S. aureus* failed to be detected in the samples from processed milk products as (cheese, yoghurt, butter, and powdered milk) and swabs from preparing raw meat and chickens surfaces and utensils, as shown in table (3).

The detection of *S. aureus* in raw milk and raw meat may be due to unclean worker hands, inferior quality of water supplied for washing utensils and carcasses, unhygienic transportation, bad storage and may be returned to milking process from subclinical mastitic cows and slaughter *S. aureus* positive animals, the present results are in line with the finding of *(Normannoa et al., 2007, Boer et al., 2009, Kluytmans, 2009 and Sarah, 2014).*

In this study, a total of 100 swabs from human origin were examined bacteriologically to reveal 48 isolates of *S. aureus* with incidence of 48%. The isolates were from septic burned wound with percentage 40%, surgical site infection 40% and draining pus of peripheral venous catheters (cannula) associated abscess & cellulites 72%, as shown in table (3), these results were more than the results of *(El-Jakee et al., 2008)* who investigated bacteriologically to detect the occurrence of *S. aureus* in 50 samples from diseased human and found 14 *S. aureus* isolates with percentage 28% but the percentage of *S. aureus* isolates from septic wound was similar to the present results with percentage 45%, The results consider evidence that *S. aureus* responsible for the most health care acquired infection in hospitals.

It was evident from the present work that, there were highly resistance to β-lactam antibiotics as penicillins group (Ampicillin and methicillin) and cephalosporins group (cephalexin and cefaclor) of *S. aureus* isolates from both human and foods of animal origin, while 58% from human isolates were resistant to imipenem unlike the isolates from food of animal origin that were highly sensitive to imipenem with percentage 100%.

On other hand, all isolates from foods of animal origin were resistant to vancomycin while 62.5% only from human isolates were resistant to it. Moreover, resistance of *S. aureus* isolates against chloramphenicol and amikacin was slightly low in foods

of animal origin isolates with percentage 18% & 39.3% respectively, while was high in human isolates with percentage 93.7% & 83.3% respectively, as shown in figures (1&2).

The present finding indicate to high prevalence of multidrug resistant *S. aureus* in both foods and patients isolates and what we are very close to dangerous zone where cannot find suitable antibiotics for these organisms that agree with those mentioned by *(WHO, 2015)* who recorded spread of antibiotic resistant bacteria in countries where antibiotics can be bought without a prescription or haven't standard treatment guidelines and warned from rising of resistance to dangerously high levels where minor injuries can once again kill and without urgent action, we are heading for a post-antibiotic era.

Genetic relatedness among the isolates ranged from 90% to 100% classified to 17 patterns according to the similarity values that exceed 95% in combined H12 and M13 primers, as shown in figure (3).

These revealed high genetic similarity among some isolates from foods of animal origin such as 2A, 3A, 4A, 5A and 6A isolates that possessed RAPD pattern type P2 with 96.3% genetic similarity and 12A & 11A that possessed RAPD pattern type P4 with 96.8% genetic similarity in addition to 8A, 9A and 10A that possessed RAPD pattern type P5 with 96.5% genetic similarity. Also, the same in some

isolates from infected patients such as 15H, 17H and 19H that possessed RAPD pattern type P10 with 95.1% genetic similarity and 9H & 13H that possessed RAPD pattern type P11 with 95.9% genetic similarity, in addition to 1H and 2H that possessed RAPD pattern type P13 with 95.8% genetic similarity, moreover 3H, 4H and 7H that possessed RAPD pattern type P14 with 97.4% genetic similarity, these results have clear reason that all of these isolates came from the same origin.

While found genetic relatedness among one isolate from raw meat 1A and another one from surgical site infection 11H that possessed RAPD pattern type P1 with 95% genetic similarity, in addition to genetic relatedness between 13A & 14A & 16A isolates from raw milk and 12H isolate from surgical site infection that possessed RAPD pattern type P3 with 95.8% genetic similarity that indicate possibility to impact of *S. aureus* isolates from foods of animal origin in human infections.

This result agree with *(John, 2003)* who found identical RAPD patterns between some isolates from food animals and certain isolates from humans that reveals the possibility of transmission the infection from contaminated food product from infected animals to humans, *(WHO, 2015)* mentioned 1 in 5 resistant infections in humans are caused by germs from food and animals, *(CDC, 2013)* recorded 22% of

antibiotic-resistant illness in humans is in fact linked to food and *(Scott, 2010)* who mentioned that contaminated food with MRSA is plausible and has role in human infection.

(CDC, 2013 and WHO, 2015) attributed this phenomenon to antibiotics that given to animals, most bacteria are killed but resistant bacteria can survive, multiply and spread through animal products or contaminated foods from environment to people who get sick with resistant infections and lead to mild illness or severe illness and may lead to death.

Table (3): *Prevalence of S. aureus that isolated from some hospitals in Port-Said governorate:*

| locations	sources of the samples	number of examined samples	Bacteriological finding	
			No. of positive samples	%
From the kitchens	surfaces and utensils used for raw meat and chickens	52	0	0
	processed milk products (cheese , yoghurt , butter and powdered milk)	50	0	0
	raw meat	55	12	21.8
	raw chicken	45	2	4.4
	raw milk	53	14	26.4
Total		255	28	11
From the wards	Septic burned wounds	50	20	40
	surgical sites infection	25	10	40
	draining pus (abscess & cellulites)	25	18	72
Total		100	48	48
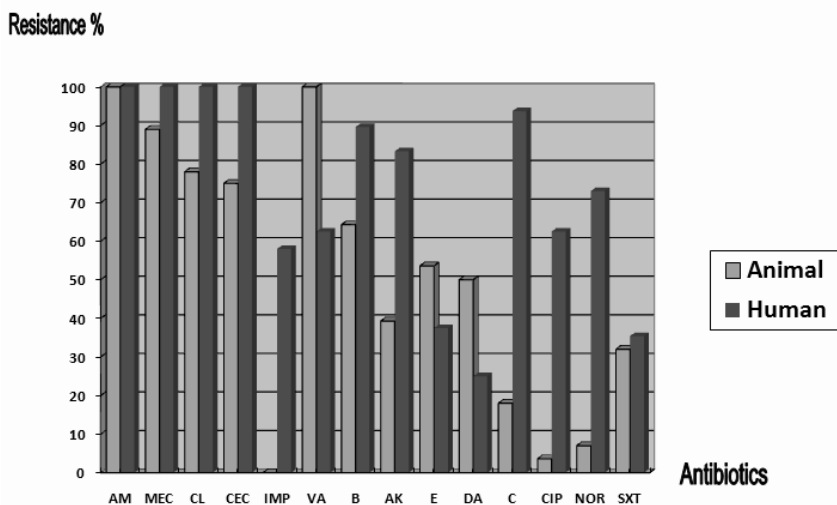
Fig (1): *Resistance rate of S. aureus strains isolated from both animal products in kitchens and patients in wards against various antibiotics.*
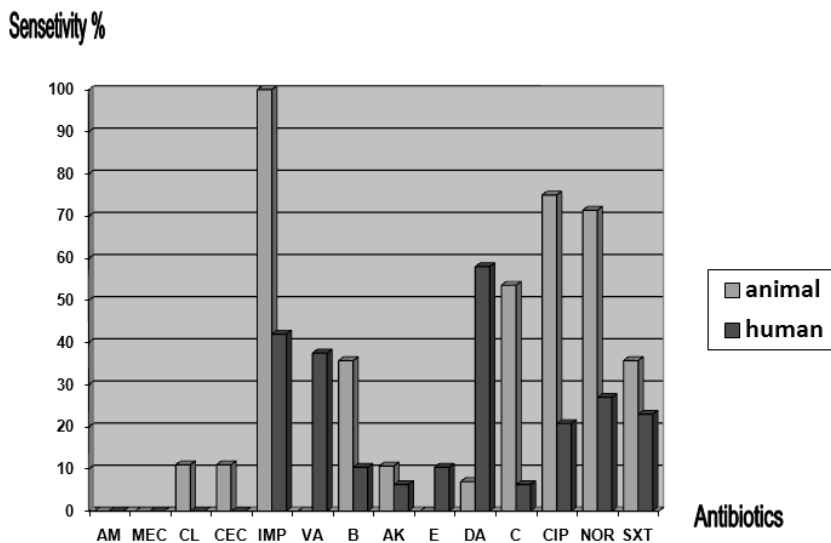

Fig (2): *Sensitivity rate of S. aureus strains isolated from both animal products in kitchens and patients in wards against various antibiotics.*
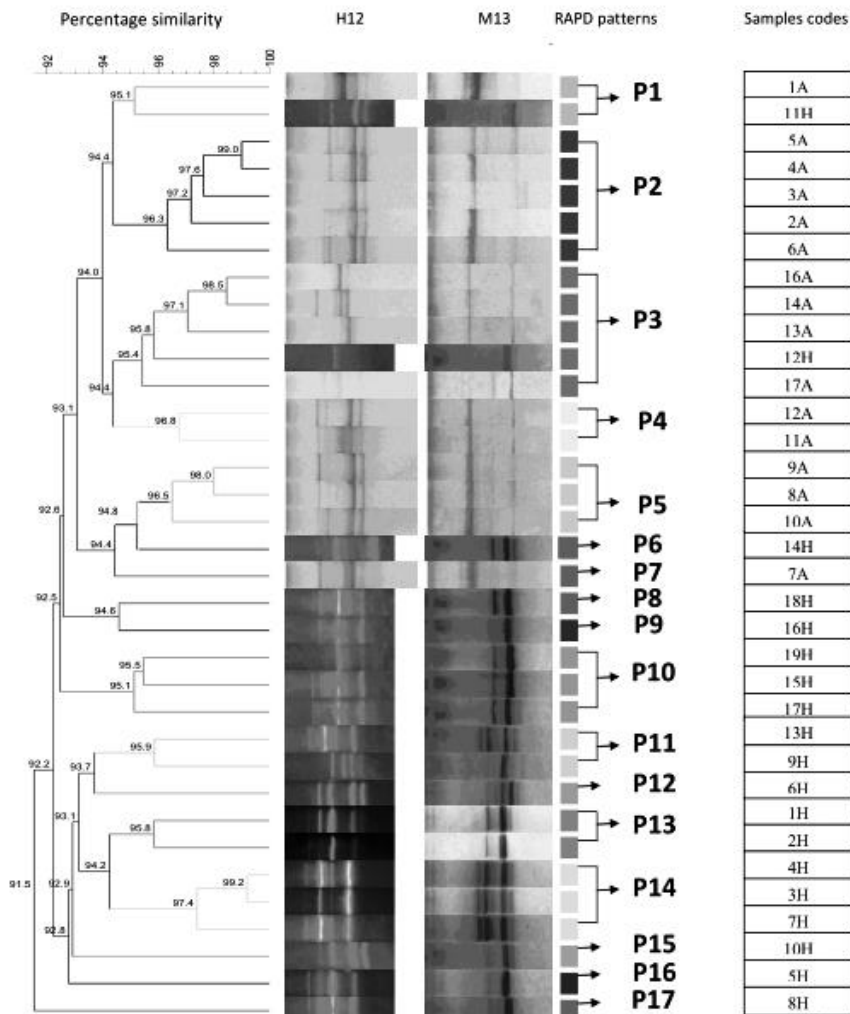
Figure (3): *Dendrogram showing the levels of similarity between RAPD patterns of 35 S. aureus isolates based on the combination between M13 & H12 results.*

Conclusion and recommendation

It can be concluded that high prevalence of multi drug resistant *S. aureus* in both isolates from foods of animal origin and infected patients in addition to transmission *S. aureus* from contaminated food products of animal origin to humans is very plausible.

It can emphasize on *(WHO, 2015)* recommendation for global action plan as following:

1. Improve awareness of the impact of antimicrobial resistance.

2. Support research works and antimicrobial resistance surveillances.

3. Activate the infection prevention measures.

4. Promote the appropriate use of antimicrobial medicines.

5. Apply action plan for countering antimicrobial resistance.

The agricultural sector can help by:

1. Ensure that antibiotics given to animals are only used to treat infectious diseases and under veterinary supervision.

2. Reduce the need for antibiotics by vaccinating animals.

3. Strengthen the good practices during processing of foods that originated from animal.

4. Handling of animals under hygienic condition.

5. Apply international standards that set out by OIE, FAO and WHO.

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توصيف المكور العنقودي الذهبي ذو المصدر الحيواني في عدوى المستشفيات

*حمزه إبراهيم عيد ** مارسيل فخري جلاب *** مي وفيق النكلاوي

*قسم البكتريولوجي و المناعة و الفطريات ـ كلية الطب البيطري- جامعة قناة السويس

**قسم صحة الأغذية ـ معهد بحوث صحة الحيوان ـ فرع بورسعيد

*** قسم الوقاية من العدوى وجودة الرعاية الصحية ـ مستشفى بورسعيد العسكري

هذه الدراسة ناقشت انتشار بكتريا المكورات العنقودية في بعض المستشفيات بمحافظة بورسعيد واحتمالية تأثير بكتريا المكورات العنقودية الذهبية المعزولة من الأغذية ذات المنشأ الحيواني علي عدوى المستشفيات عن طريق الارتباط الجيني بين العترات المأخوذة من الأغذية والمرضي.

وقد أسفر الفحص البكتيري (355) عينة عن وجود المكورات العنقودية في 28 عينة من أصل 255 عينة تم عزلها من مطابخ المستشفيات بنسبة 11% و 48 عترة من المكورات العنقودية من إجمالي 100 مسحة أخذت من المرضى بنسبة 48%.

وقد تم التعرف علي كل العترات المعزولة و التأكد من إنها خاصة لبكتريا المكورات العنقودية عن طريق المزارع البكتيرية, الاختبارات الكيميائية الحيوية و الكشف عن جين clfA عن طريق استخدام تقنية تفاعل إنزيم البلمرة المتسلسل PCR, بالإضافة إلي التعرف على حساسية كل العترات المعزولة للمكورات العنقودية الذهبية لـ14 نوع مختلف من المضادات الحيوية في المعمل وكشفت النتائج عن مقاومة المكورات العنقودية للعديد منها.

وقد تم استخدام تقنية تضخيم الحمض النووي متعدد الأشكال RAPD لمعرفة الارتباط الجيني للعترات المعزولة من المطابخ و عنابر المستشفيات و وجد ارتباط جيني في بعض العترات المعزولة من كلاهما.

أسفرت النتائج عن احتمالية انتقال العدوى إلى الإنسان عن طريق أطعمة من أصل حيواني ملوثة بالمكور العنقودي الذهبي.