Diversity of *Staphylococcus aureus* Isolated from Human and Bovine Estimated by PCR - Gene Analysis

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Abstract: The present investigation studied the diversity of 19 *S. aureus* isolates (9 from bovine and 10 from human sources) in comparison with the standard Cowan I strain by conventional methods and by PCR technology. The latter uses primers targeted to species-specific parts of genes encoding coagulase (*coa*), enterotoxin A (*sea*) and B (*seb*), *mec* A gene encoding mecthillin resistant *S. aureus* (MRSA) and *Staphylococcus* protein A (*spa*) gene. *S. aureus* isolates (19) as well as the Cowan 1 strain were tested for antimicrobial sensitivity with 15 antibiotics by disk diffusion method and classified as susceptible, intermediate and resistant. 57.9% of isolates had a relatively high molecular weight plasmid. The *mec A* gene among the chosen MRSA *S. aureus* isolates recovered from human and bovine sources was discussed. Polymorphisms of *coa* and *spa* genes were detected among *S. aureus* isolates. The examined isolates had coagulase gene ranging from 423 bp to 658 bp and the Cowan -1 strain had amplified fragment at 642 bp. All examined *S. aureus* isolates gave an amplified *spa* gene product at approximately from 396-464 bp. The prevalence of enterotoxin genes *sea* and *seb* were determined and the diversity among the chosen isolates was recorded.

[J.El-Jakee, Ata S. Nagwa, Gad El-Said, W.A., Bakry,M.A., Samy, A.A., Khairy E.A., Elgabry, E.A. Diversity of *Staphylococcus aureus* Isolated from Human and Bovine Estimated by PCR - Gene Analysis. Journal of American Science 2010;6(11):487-498]. (ISSN: 1545-1003).

Keywords: *S. aureus,* antibiogram sensitivity, MRSA, Enterotoxins, coagulase gene, *spa* gene.

1. Introduction

Staphylococcus aureus is recognized as causing health care associated and community-acquired infections in every region of the world. Enterotoxigenic *S. aureus* in milk posses a potential health hazard to consumers, the identification of such strains should be used as apart of a risk analysis of milk and milk products (Zouharova and Rysanek, 2008). *S. aureus* is among the most important nosocomial pathogens because of both the diversity and the severity of the infections it causes, including superficial, deep skin and soft-tissue infections, endocarditis, and bacteremia, as well as a variety of toxin-mediated diseases such as gastroenteritis, staphylococcal scalded-skin syndrome, and toxic shock syndrome (Waldvogel, 1995 and Lowy, 1998).

Staphylococcal enterotoxins (SEs) are serologically grouped into five major classical types which are SEA, SEB, SEC, SED and SEE in addition to toxic shock syndrome toxin (TSST-1) which causes toxic shock syndrome in human, SEA and SEB are usually more common in milk and milk products (Chiang *et al.,* 2006). The resistance to antimicrobial agents among staphylococci is an increasing problem; these strains often reveal resistance to various drug classes in addition to beta-lactam resistance (Dizbay *et al.,* 2008).

Results obtained from analysis of enterotoxigenic *S. aureus* biochemically and genotypically by using PCR for encoding genes revealed a strong correlation between each other (Lawrynowicz-Paciorek *et al.*, 2007). A reliable and rapid identification of *S. aureus* colonies from samples is a cornerstone in the control of *S. aureus* infection. Identification of bacterial pathogens still relies mainly on phenotypic criteria. Based on the above it is important to study *S. aureus* using modern differentiating diagnostic techniques like PCR and gene analysis. The goal of the present investigation is to study phenotypic and genotypic characterizes of *S. aureus* recovered from human and bovine sources.

2. Materials and Methods

Samples:

A total of 830 samples were collected from cattle, buffaloes and human for isolation of *Staphylococcus* species. They were collected from milk samples and septic wounds from bovine's dairy farms in Giza, Fayoum and Animal Health Research Institute (AHRI) Dokki. Giza. Egypt. Urine, septic wounds and nasal swabs from cases with respiratory symptoms were obtained from workers in the farms, out patient clinics of Cairo University Hospitals (CUH) and human laboratories (Cairolab, Elborg and Alfa Lab.) were also collected in nine months period from April 2007 to January 2008 as shown in Table (1).

Table (1): Types and numbers of the samples collected.

Source of the isolates	Type of samples	No. of The examined samples
Bovine	- Milk from mastitic cows - Milk from mastitic Buffaloes - Swabs from cow septic wounds	200 150 50
Human	- Urine from infected urinary tract - Swabs from septic wounds - Nasal swabs from cases with respiratory symptoms	150 150 130
Total		830

Identification of staphylococci:

The collected samples were cultured onto nutrient agar "Difco", sheep blood agar and Bacto-Mannitol salt agar "Difco". The inoculated plates were incubated for 24-48 hours at 37°C. The suspected colonies were picked up and propagated in nutrient agar slope for further examinations. Staphylococci were identified according to Quinn *et al*. (2002).

Characterization of *S.aureus* isolates (Cruickshank *et al.*, 1975):

The *S. aureus* isolates were identified by using the following tests:

Catalase, coagulase, maltose fermentation, urease activity, mannitol fermentation, pigment production onto nutrient agar "Difco", hemolytic activity on sheep and human blood agar, DNase activity on DNase medium "Oxoid", lysozyme activity, gelatinase activity, growth on Baird-Parker Medium "Biomerieux" containing 1% potassium tullerite, lipase activity on egg yolk agar medium, protease activity of *S. aureus* on milk agar medium, fibrinolysin activity on plasma agar medium, Vogues Proskauer test for detection of acetone production, detection of SpA by agglutination using SpA agglutination kits (Wellcome Diagnostics)**.** As well as Crystal violet agar growth type according to Rodgers *et al.* (1999). Crystal violet agar plates were prepared by adding 6 or 8 ug/ml of crystal violet to tryptose agar (Oxoid). Few colonies of the isolates were spot inoculated on plates of both concentrations, incubated at 37°C, and examined after 24 hours. The 6 ug/ml plate was examined if growth was inhibited on the 8 ug/ml plate. Growth of a cream to yellow color with or without violet margins was recorded as growth type A. Growth mainly of blue or violet color was recorded as growth type C and white color was recorded as growth type E.

Susceptibility of *S. aureus* isolates to antibacterial agents:

15 antibacterial disks"Oxoid" were used and the disk diffusion technique was adapted according to Finegold and Martin (1982). After incubation, the degree of sensitivity was determined according to NCCLS (2002) Cheesbrough (2006) and Bannerman and Peacock (2007).

Detection of plasmid.

Plasmid DNA extraction was performed in Biotechnology Centre for Services and Research (BCSR) in Faculty of Veterinary Medicine, Cairo University. Extraction of miniprep performed according to Sambrook and Russel (2001). The extracted plasmid was evaluated as visible bands being sized by DNA molecular marker **(**Hind III digest), that measures molecular weight 81-23000 bp (Gibbco).

PCR procedure (Sambrook and Russel, 2001)

Polymerase chain reaction was performed in Biotechnology Centre for Services and Research (BCSR) in Faculty of Veterinary Medicine, Cairo University. Qiagen extraction kit for DNA extraction from *S. aureus* isolates staphylococcal species was used as described by manufacturer manual of Qiagen, Germany. Primers were synthesized by Metabion Company, Germany as mentioned in Table (2). The presence of specific amplified DNA bands was detected by visualization with UV light at wave length 421 nm and compared with molecular size marker (Ladder) with MW 100 bp and measures MW 100-1500 bp obtained from Amersco Cleveland Ohio, USA. Cowan I strain of *S.aureus* obtained from the Namru 3 in Egypt was used as positive control.

Table (2): Types of Primers, Primers Designs and References.

Primer	Primer Design	Product size bp	Reference
Coagulase gene F	5'-ATAGAGATGCTGGTACAGG-3'	433-638	Hookey *et al*. (1998)
Coagulase gene R	5'-GCTTCCGATTGTTCGATGC-3'		
SAEA-F	5'-CCTTTGGAAACGGTTAAAACG- 3'	127	Becker *et al.* (1998)
SAEA-R	5'-TCTGAACCTTCCCATCAAAAAC- 3'		
SAEB-F	5'-TCGCATCAAACTGACAAACG- 3'	477	
SAEB-R	5'-GCAGGTACTCTATAAGTGCC- 3'		
SPA F	5'-CAAAGATCAACAAAGCGCC- 3'	412	Annemüller and Zschock (1999)
SPA R	5'-CGAAGGATCGTCTTTAAGGC- 3'		
MRSA gene F	5'-GGAGACGAGCACTAAAACC-3'	182	Weller (1999)
MRSA gene R	5'-TCGGACGTTCAGTCATT-3'		

SAEA = *Staphylococcus aureus* enterotoxin A SAEB = *Staphylococcus aureus* enterotoxin B
MRSA = Mecthillin (Oxacillin) resistant *Staphylococcus aureus.* SPA = *Staphylococcus* protein A.

3. Results and Discussion

Analyses of the genotype distributions of *S. aureus* strains of diverse origin demonstrated a certain host specificity. It seems that the occurrence of some staphylococcal lineages is restricted to animals (Sung *et al.,* 2008 and Smyth *et al.,* 2009). Livestock-associated *S. aureus* seems to be an underappreciated source of pathogenic strains (Bystron *et al.,* 2010). Several methicillin resistant *S. aureus* (MRSA) clones have disseminated worldwide (Deurenberg *et al.,* 2007). Although bacterial interaction is a well recognized phenomenon, there has been surprisingly little research with respect to MRSA and MSSA. The mechanism/s responsible for this phenomenon is not readily apparent (Al-Kulaifi *et al.*, 2009).

In this study a total of 830 samples were investigated bacteriologically to detect the occurrence of staphylococci among bovine and humans samples. The isolation rate among human samples was 33% while it was 28.3% in bovine samples. 209 *S. aureus*, 21 *S. intermedius* and 25 *S. hyicus* isolates secured from bovine and humans' origins were identified using the most important conventional biochemical tests as catalase, coagulase and acetone production as shown in Table (3).

A number of different phenotypic and genotypic techniques are available to classified strains for epidemiological investigation in the detection and tracking of outbreaks (Wildemauwe *et al.*, 2010). In veterinary microbiology, many phenotypic methods include (pigment production, hemolytic activities, DNase, etc have been applied for characterization of *S. aureus* strains. As shown in Table (4) it is clear that all isolates were positive for coagulase test, mannitol fermentation, acetone production and show a characteristic growth on Baird parker and crystal violet media which considered being selective media for *S. aureus.* In this concern, Brown and Ngeno (2007) recorded that all positive isolates gave positive reactions in mannitol salt fermentation, in catalase and tube coagulase and latex agglutination tests also, sixteen isolates demonstrated beta hemolysis on horse blood agar while four were not beta hemolytic.

In the present investigation characterization of 19 *S. aureus* isolates (9 from bovine and 10 from human sources) in comparison with the standard Cowan I strain was performed by conventional methods and by PCR technology. Worldwide, the prevalence of multi-resistant *S. aureus* strains has been increased problematically. Increased attention has been focused on plasmid-encoded resistance to antiseptics and disinfectants in antibiotic resistant staphylococci (Bjorland *et al.,* 2003). 57.9% of isolates had a high molecular weight plasmid (more than 18000kbp) as well as Cowan 1 strain as shown in photo (1). Lindsay (2010) recorded that plasmids in *S. aureus* are predominantly of two types, small rolling circle plasmids often encode only one or two resistance genes, such as pT181 (Khan, 2005). The larger plasmids replicate by the theta mechanism and can carry a combination of resistance genes including penicillinase, heavy metals, detergents, trimethoprim and aminoglycosides, some of which are due to integrated small plasmids or transposons (Berg *et al.,*

1998). Some larger plasmids also encode the *tra* genes for conjugative transfer and many strains of *S. aureus* carry one or more plasmids (Lindsay, 2010).

Methicillin (oxacillin) -resistant S. *aureus* (MRSA) was first described in 1961 (Jevons, 1961) and since then has become a significant pathogen in nosocomial infections (Hartman and Tomasz, 1986). For clinicians, the spread of these methicillin-resistant strains has been critical as the therapeutic outcome of infections that result from MRSA is worse that those from methicillin-sensitive strains (MSSA) (Cosgrove *et al.,* 2003). This study aimed to assess the antimicrobial susceptibility patterns and prevalence of methicillin resistance among the chosen *S. aureus* isolated (19 isolates) from human and bovine sources, as well as the Cowan 1 strain as shown in Tables (5-7). High resistance was recorded to methicillin (60%) among the examined *S. aureus* isolates, followed by oxytetracycline (55%) ampicillin & sulphamethoxazole-timethoprim (45% each). Then amoxicillin (40%), ofloxacin (30%), clindamycin & erythromycin (25% each) and amoxicillin clavulanic acid, cefoperazone & cefotaxime (15% each) as shown in Table (5). Meanwhile 95% of the examined *S. aureus* isolates were sensitive to vancomycin, 85% to cefotaxime and 80% to amoxycillin clavulanic acid and cefoperazone. The human isolates were often multidrug resistant, unlike the animal isolates (Lindsay, 2010). 7 out of 10 isolates from human origin were MRSA (70%) and 5 out of 9 *S. aureus* isolates (55.6%) of bovine origin were MRSA, in addition to the Cowan 1 strain as shown in Tables (6 &7).

Interestingly, VRSA are less fit than MRSA in the presence of low concentrations of vancomycin which may be prevalent in hospitals (Foucault *et al.*, 2009). However, in the absence of vancomycin they are fit, yet, no spread of VRSA in hospitals has been reported (Lindsay, 2010). Only one isolate showed an intermediate resistance to vancomycin as shown as in Table (5). Outbreaks of VISA are not reported, and their endemic potential is probably low. Of more concern are fully VRSA strains, first reported in the USA in 2002 (Zhu *et al.,* 2008).

Methicillin-resistant staphylococci carry the *mecA* gene, which encodes a specific low-affinity penicillin-binding protein 2a (PBP_{2a}), this protein is responsible for the methicillin resistance in staphylococci (Hackbart and Chambers, 1989). As shown in Photo (2) all methicillin-resistant *S. aureus* isolates were *mec*A gene positive by PCR among the examined isolates and the standard strain.Polymerase chain reaction and DNA hybridization detection of the *mecA* gene in staphylococci is unaffected by the level of its expression (Mo and Wang, 1997).

Comparable PCR-based systems for identification of *S. aureus* isolates under investigation have been used. The coagulase gene (*coa*) typing (Reinoso *et al.,* 2008) have been used to identify and compare *S. aureus* genotypes. As shown in Photo (3), all isolates examined had coagulase gene. Two different PCR products were detected, one in size ranging from approximately 423 bp to 484 bp and an other at 608 to 658bp. The standard Cowan I strain had an amplified PCR fragment at 642bp (Photo, 3). Length and sequence polymorphisms of the coagulase gene and its use for genotypic characterization of *S. aureus* had been already shown (Stephan *et al.,* 2000 and Su *et al.,* 2000). Studies carried out by other researchers (Kalorey *et al.,* 2007; Reinoso *et al.,* 2008) showed different coagulase gene types. The reason for this polymorphism among *S. aureus* isolates is unclear, but it seems to be because of 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 as a consequence change the *coa* gene size and probably antigenic properties of the coagulase enzyme (Saei *et al.*, 2009). Mobile genetic elements (MGE) are discrete pieces of DNA that encode factors allowing them to mobilise within or between genomes (Lindsay, 2008). In *S. aureus,* the major MGE are bacteriophage, pathogenicity islands (SaPI), plasmids, transposons and staphylococcal cassette chromosomes (SCC). Most MGE show evidence of frequent horizontal transfer and recombination (Lindsay, 2010). The evolution of new human and animal pathogenic strains of *S. aureus* has been due to the accumulation of mobile genetic elements (MGE) encoding methicillin resistance and virulence factors into successful lineages (Lindsay, 2010).

S. aureus is able to produce a number of virulence factors such as protein A or leukocidins (Kerro Dego *et al.,* 2002). Protein A is located in the cell wall and captures antibodies (Foster and McDevitt, 1994). Photo (4) showed agarose gel electrophoresis of *spa* gene amplification products. It is clear that all examined *S. aureus* isolates gave an amplified *spa* gene product at approximately from 396-464 bp. Tang *et al.* (2000) had shown that detection of genetic polymorphisms in the X region of the *spa* gene can be used as a typing method to determine the epidemiologic relatedness of MRSA isolates. Protein A is a component of the *S. aureus* cell wall and is covalently bound to the peptidoglycan. The *spa* gene is approximately 2,150 bp and contains three distinct regions: the Fc portion, the X region, and the C terminus, the polymorphic X region contains various numbers of 24 bp repeats with various sequences had been described by Frénay *et al.* (1996). With the *spa* typing, a great number of

different types were obtained (Wildemauwe *et al.*, 2010). The variability of this gene indicate that sequence analysis of the *spa* gene could be used as an alternative system for the molecular typing of *S. aureus* isolates.

Staphylococcus aureus is one of the most common agents in bacterial food poisoning outbreaks. Its strains produce a spectrum of protein toxins and virulence factors thought to contribute to the pathogenicity of this organism (Adwan *et al.*, 2005). The staphylococcal enterotoxins (SEs) have been classified into many different types. The most common types of these enterotoxins are SEA to SEE. Isolates carrying toxin genes *sea* to *see* are responsible for 95% of staphylococcal food poisoning outbreaks (Bergdoll, 1983). The remaining staphylococcal food-borne disease outbreaks may therefore be associated with other newly identified SEs (MacLauchlin *et al.,* 2000; Omoe *et al.,* 2002 and Rosec & Gigaud, 2002). This study was conducted to determine the prevalence of enterotoxin genes A (*sea*) & B (*seb*) among the chosen *S. aureus* isolates recovered from human and bovine sources. The results in Photo (5) show that bovine strains (33%) were positive for both *sea* and *seb* genes, while 11.1% were positive for *seb* gene only. Among human strains, 20% were positive for *sea* gene and *seb* gene each. The Cowan 1 strain was positive for both *sea* and *seb* genes. Out of the 100 *S. aureus* isolates (milk sheep origin =52: milk cows origin = 48) tested by Adwan *et al.* (2005) for SE-genes, 37 (37%) were positive and the majority of these positive toxin gene isolates, 20 (54.1 %), were *seb*-positive. This result was consistent with previous reports from Japan, Poland and Slovakia, where 64% to 85% of the enterotoxigenic *S. aureus* isolates recovered from raw poultry meat or different food samples and food manufacturers harbored the toxin gene *seb* (Holeckova *et al.,* 2002 and Kitai *et al.,* 2005). In this study 10 out of 20 strains were negative for enterotoxins genes. Also 15 *S. aureus* poultry isolates were found to be non enterotoxigenic by Bystron *et al.* (2010).

It could be concluded that antibiogram clarifying the developed resistance of *S. aureus* strains to commonly used antibiotics ensuring that the right use of antibiotic of choice is very important in line of treatment and control of the infections caused by *S. aureus* especially MRSA strains. Genotyping by PCR is highly effective in detection of *S. aureus* with high sensitivity and specificity especially with polymerization of *coa* and *spa* genes which considered the cornerstone markers for detection and study of *S. aureus*. PCR results of *mec*A gene gave sharp differentiation between many strains which help in determination of the suspected source of infection especially in nosocomial infection cases also in case of repeated infections with the same strain in case of treatment failure or insufficient disinfection.

Table (3): Prevalence of *Staphylococcus* species from the collected samples

Source of the isolates	No. of the examined samples	*Staphylococcus* species						Total	
		S. aureus		*S. intermedius*		*S. hyicus*			
		No.	%	No.	%	No.	%	No.	%
Bovine	400	86	21.5	18	4.5	9	2.3	113	28.3
Human	430	123	28.6	3	0.69	16	3.7	142	33
Total	830	209	25.2	21	2.5	25	3	255	30.7

No.: Number of Positive **%:** was calculated according to number of the examined samples

Table (4) Characteristic features of the examined *S. aureus* isolates:

Source of the Isolates	Number of examined samples	Number of *S. aureus* isolates	Colony pigment						Hemolytic activity						DNase activity		Lysozyme activity		Gelatinase activity		Lecithinase activity		Lipase activity	
			White		Creamy		Golden yellow		Sheep blood agar		Human blood agar		Non hemolytic											
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Bovine	400	86	4	4.7	20	23.3	62	72.1	79	91.9	26	30.2	7	8.1	73	84.9	82	95.3	82	95.3	72	83.7	57	66.3
Human	430	123	12	9.8	22	17.9	89	72.4	112	91.1	12	9.8	11	8.9	116	94.3	121	98.4	120	97.6	111	90.2	103	83.7
Total	830	209	16	7.7	42	20.1	151	72.2	191	91.4	38	18.2	18	8.6	189	90.4	203	97.1	202	96.7	183	87.6	160	76.6
Characteristic features of the selected 20 strains for plasmid detection and PCR – gene analysis			0	0	2	10	18	90	20	100	8	40	0	0	19	95	20	100	20	100	20	100	18	90

Table (4): Continue

Protease activity		Tellurite reduction		Fibrinolysin		SpA by agglutination		Crystal violet medium						Mannitol		Novobiocin (S) 30 ug "Biomerieux".		Acetone production		Coagulase test	
								Yellow (A)		Violet (C)		White (E)									
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
69	80.2	81	94.2	61	70.9	54	62.8	26	30.2	47	54.7	13	15.1	86	100	-	-	86	100	86	100
107	87	122	99.2	119	96.7	102	88.9	81	65.9	28	22.8	14	11.4	123	100	-	-	123	100	123	100
176	84.2	203	97.1	180	86.1	156	74.6	107	51.2	75	35.8	27	12.9	209	100	-	-	209	100	209	100
18	90	20	100	19	95	20	100	13	65	6	30	1	5	20	100	20	100	20	100	20	100

No. Positive number % was calculated according to the number of samples S= sensitive

Table (5): Results of chemotherapeutic sensitivity test of the examined *S. aureus* isolates

No.	Antimicrobial agents	Disc potency µg/disc	Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
1	Amoxicillin (AML)	25	8	40	7	35	5	25
2	Amoxicillin / clavulanic (AMC)	20+10	3	15	1	5	16	80
3	Ampicillin (AMP)	10	9	45	5	25	6	30
4	Azithromycin (AZ)	5	4	20	1	5	15	75
5	Cefoperazone (CB)	1	3	15	1	5	16	80
6	Cefotaxime (CX)	30	3	15	0	0	17	85
7	Ciprofloxacin (CF)	10	4	20	5	25	11	55
8	Clindamycin (CD)	2	5	25	2	10	13	65
9	Erythromycin (E)	15	5	25	1	5	14	70
10	Methicillin (Oxacillin) (OX)	5	12	60	1	5	7	35
11	Ofloxacin (ON)	20	6	30	2	10	12	60
12	Oxytetracycline (OT)	30	11	55	4	20	5	25
13	Sulphamethoxazole-Timethoprim (SXT)	23.75+1.25	9	45	5	25	6	30
14	Tobromycin (TN)	20	4	20	2	10	14	70
15	Vancomycin (VN)	3	0	0	1	5	19	95

No. Positive number % was calculated according to the number of samples

Table (6) Analysis of PCR products of MRSA strains from bovine origin

Strains No.	origin	Source	*coa*. gene		Toxins gene		*spa* gene	*mec*. A gene	Plasmid	Most resistant antibiotics	Most sensitive antibiotics
			Mol. wt.		Mol. wt.						
			423 - 484	608 - 658	A (127)	B (477)	Mol. wt. 396-462 bp	Mol. wt. (182)			
1	Bovine strains	Bovine mastitic milk	-ve	+ve 630	-ve	-ve	+ve 396	+ve	+ve	OX, OT, AMC, AML	CX, AZ, CB
2			-ve	+ve 658	-ve	+ve	+ve 418	+ve	+ve	OX, AMP, CX, ON, SXT	AMC, E, TN
3			+ve 423	-ve	+ve	+ve	+ve 464	+ve	-ve	OX, OT,TN, E	CX, AZ, CB
4			-ve	+ve 658	-ve	-ve	+ve 422	-ve	+ve	OX, AMP, CB, AML	AMC, AZ, E, OT
8		Bovine wounds	+ve 432	-ve	-ve	-ve	+ve 452	+ve	-ve	OX, AMP, CF	AMC, CB,ON

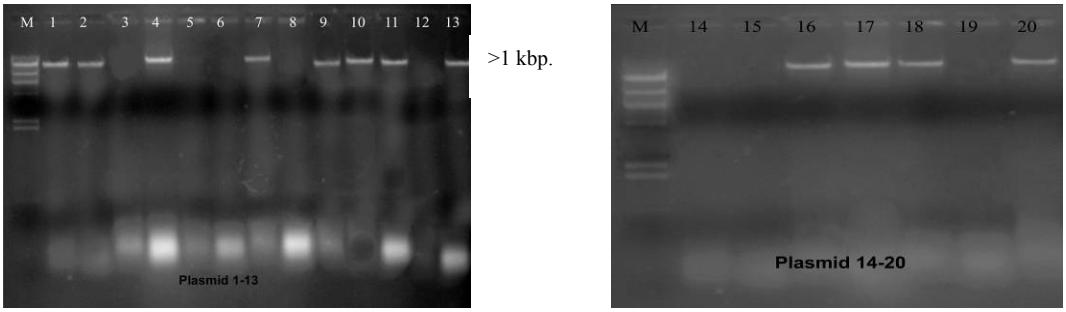
20	**Cowan-1 strain**	-ve	+ve 642	+ve	+ve	+ve 448	+ve	+ve	OX, OT,TN, ON, AML, E	CX, AMC, AZ, CD

-ve= negative, +ve= positive, AML= Amoxicillin – AMC= Amoxicillin / clavulanic – AMP= Ampicillin - AZ =Azithromycin – CF= Ciprofloxacin - CX =Cefotaxime - CB =Cefoperazone – CD= Clindamycin – E= Erythromycin - OX= Methicillin (Oxacillin) – ON= Ofloxacin - OT =Oxytetracycline – SXT=Sulphamethoxazole-Timethoprim – TN= Tobromycin - VN =Vancomycin.

Table (7) Analysis of PCR products of MRSA strains from human origin

Strains No.	**Origin**	**Source**	***coa*. gene**		**Toxins gene**		***spa* gene**	***mec*. A gene**	**Plasmid**	**Most resistant antibiotics**	**Most sensitive antibiotics**
			Mol. wt.		**Mol. wt.**						
			423 - 484	**608 - 658**	**A (127)**	**B (477)**	**Mol. wt. 396-462b.p**	**Mol. wt. (182)bp**			
1	**Human strains**	**Respiratory infection**	-ve	+ve 658	+ve	-ve	+ve 448	+ve	+ve	OX, SXT, TN. AZ	AMC, CB, E, CD, CX
2		**Septic wound**	+ve 448	-	-ve	-ve	+ve 452	+ve	-ve	OX, OT, CD, ON	CX, AZ, CB, CF
3			-ve	+ve 608	-ve	+ve	+ve 452	+ve	+ve	OX, OT, AMP, CF, CX, SXT	AMC, AZ, E, ON, TN
4			+ve 484	-ve	-ve	-ve	+ve 418	+ve	-ve	OX, E, AML, SXT, TN	CX, AMC, CB. ON, CX
5			+ve 484	-ve	-ve	-ve	+ve 448	-ve	-ve	OX, OT, ON, AMP	CX, AZ, E. TN, SXT
6			-ve	+ve 658	-ve	+ve	+ve 462	-ve	+ve	OX, SXT, AZ. AML	AMC, CB, TN, VN, CF
7		**Urinary infection**	-ve	+ve 642	-ve	-ve	+ve 418	+ve	+ve	OX, CD, SXT	AZ, TN,ON, CB, AMC
8	**Cowan-1 Standard strain**		-ve	+ve 642	+ve	+ve	+ve 448	+ve	+ve	OX, OT,TN, AML, E, ON	CX, AMC, AZ, CD

-ve= negative, +ve= positive, AML= Amoxicillin – AMC= Amoxicillin / clavulanic – AMP= Ampicillin - AZ =Azithromycin – CF= Ciprofloxacin - CX =Cefotaxime - CB =Cefoperazone – CD= Clindamycin – E= Erythromycin - OX= Methicillin (Oxacillin) – ON= Ofloxacin - OT =Oxytetracycline – SXT=Sulphamethoxazole-Timethoprim – TN= Tobromycin - VN =Vancomycin.

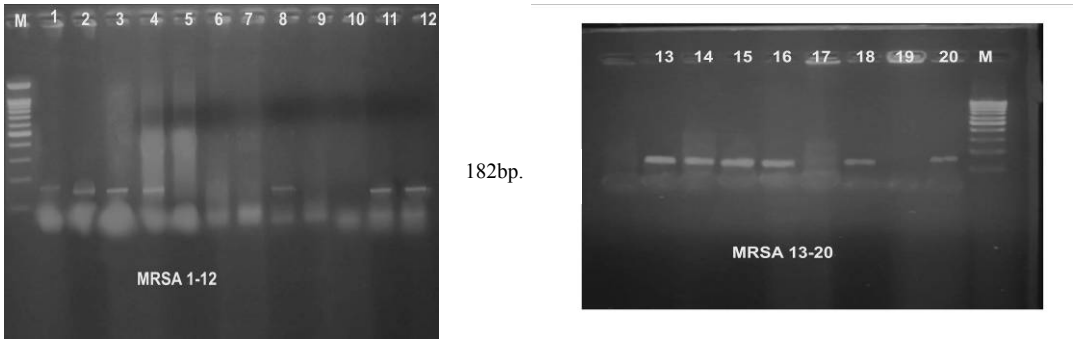


A B

Photo (1): Agarose gel electrophoresis showing plasmid profile in *S. aureus* isolated strains

(A) M: DNA molecular weight marker adapted by (Hind III digest). Lane 1: Cows milk (Positive for plasmid). Lane 2: Cows milk (Positive for plasmid). Lane 3: Cows milk (Negative for plasmid). Lane 4: Cows milk (Positive for plasmid). Lane 5: Cows milk (Negative for plasmid). Lane 6: Buffaloes milk (Negative for plasmid). Lane 7: Buffaloes milk (Positive for plasmid). Lane 8: Bovine septic wounds (Negative for plasmid). Lane 9: Bovine septic wounds (Positive for plasmid). Lane 10: Human respiratory infection (Positive for plasmid). Lane 11: Human respiratory infection (Positive for plasmid). Lane 12: Human septic wounds (Negative for plasmid). Lane 13: Human septic wounds (Positive for plasmid).

(B) M: DNA molecular weight marker adapted by (Hind III digest). Lane 14: Human septic wounds (Negative for plasmid). Lane 15: Human septic wounds (Negative for plasmid). Lane 16: Human septic wounds (Positive for plasmid). Lane 17: Human septic wounds (Positive for plasmid). Lane 18: Human infected urinary tracts (Positive for plasmid). Lane 19: Human infected urinary tracts (Negative for plasmid). Lane 20: Cowan-1 standard strain (Positive for plasmid)

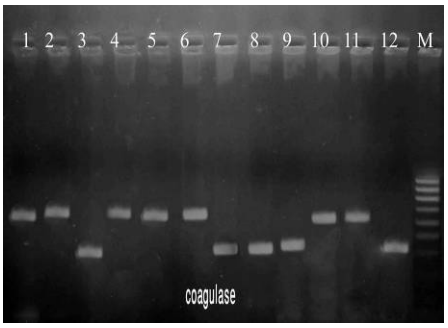


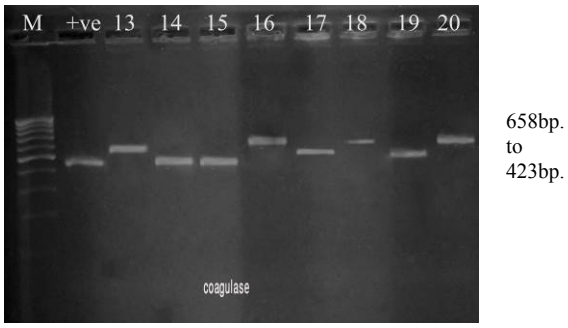
A B

Photo (2): Agarose gel electrophoresis showing the result of amplification of *mec*. A gene (182 bp)

(A) M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (Positive for *mec*. A gene). Lane 2: Cows milk (Positive for *mec*. A gene). Lane 3: Cows milk (Positive for *mec*. A gene). Lane 4: Cows milk (Positive for *mec*. A gene). Lane 5: Cows milk (Negative for *mec*. A gene). Lane 6: Buffaloes milk (Negative for *mec*. A gene). Lane 7: Buffaloes milk (Negative for *mec*. A gene). Lane 8: Bovine septic wounds (Positive for *mec*. A gene). Lane 9: Bovine septic wounds (Negative for *mec*. A gene). Lane 10: Human respiratory infection (Negative for *mec*. A gene). Lane 11: Human respiratory infection (Positive for *mec*. A gene). Lane 12: Human septic wounds (Positive for *mec*. A gene)

(B) M: DNA molecular weight marker (100 b.p. ladder). Lane 13: Human septic wounds (Positive for *mec*. A gene). Lane 14: Human septic wounds (Positive for *mec*. A gene). Lane 15: Human septic wounds (Positive for *mec*. A gene). Lane 16: Human septic wounds (Positive for *mec*. A gene). Lane 17: Human septic wounds (Negative for *mec*. A gene). Lane 18: Human infected urinary tracts (Positive for *mec*. A gene). Lane 19: Human infected urinary tracts (Negative for *mec*. A gene). Lane 20: Cowan-1 standard strain (Positive for *mec*. A gene).



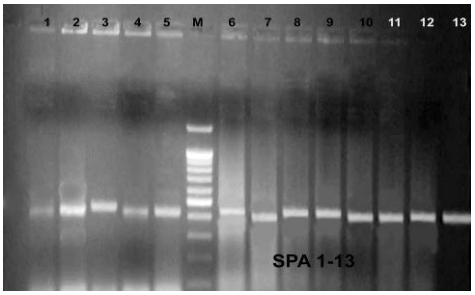


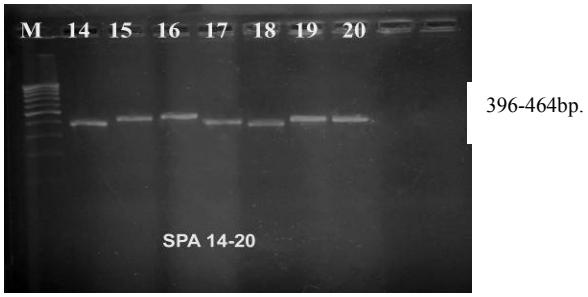
A B

Photo (3): Agarose gel electrophoresis showing the result of amplification of coagulase gene polymorphisms of the gene encoding staphylococcal coagulase

(A) M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (630 bp). Lane 2: Cows milk (658 bp). Lane 3: Cows milk (423 bp). Lane 4: Cows milk (658 bp). Lane 5: Cows milk (658 bp). Lane 6: Buffaloes milk (658 bp). Lane 7: Buffaloes milk (428 bp). Lane 8: Bovine septic wounds (432 bp). Lane 9: Bovine septic wounds (456 bp). Lane 10: Human respiratory infection (658 bp). Lane 11: Human respiratory infection (658 bp). Lane 12: Human septic wounds (448 bp).

(B) M: DNA molecular weight marker (100 bp. ladder). Lane 13: Human septic wounds (608 bp). Lane 14: Human septic wounds (484 bp). Lane 15: Human septic wounds (484 bp). Lane 16: Human septic wounds (658 bp). Lane 17: Human septic wounds (428 bp). Lane 18: Human infected urinary tracts (642 bp). Lane 19: Human infected urinary tracts (518 bp). Lane 20: Cowan-1 standard strain (642 bp). +ve: Positive control



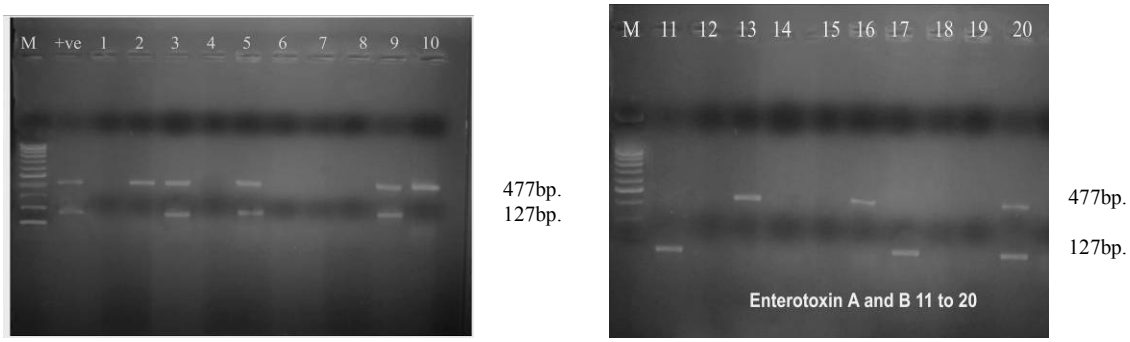


A B

Photo (4): Agarose gel electrophoresis showing the result of amplification of *spa* gene

(A) M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (396). Lane 2: Cows milk (418 bp). Lane 3: Cows milk (464 bp). Lane 4: Cows milk (422 bp). Lane 5: Cows milk (430 bp). Lane 6: Buffaloes milk (452 bp). Lane 7: Buffaloes milk (428 bp). Lane 8: Bovine septic wounds (452 bp). Lane 9: Bovine septic wounds (452 bp). Lane 10: Human respiratory infection (448 bp). Lane 11: Human respiratory infection (448 bp). Lane 12: Human septic wounds (452 bp). Lane 13: Human septic wounds (452 bp).

(B) M: DNA molecular weight marker (100 bp. ladder). Lane 14: Human septic wounds (418 bp). Lane 15: Human septic wounds (448 bp). Lane 16: Human septic wounds (462 bp). Lane 17: Human septic wounds (452 bp). Lane 18: Human infected urinary tracts (418 bp). Lane 19: Human infected urinary tracts (448 bp). Lane 20: Cowan-1 standard strain (448 bp).



A B

Photo (5): Agarose gel electrophoresis showing the result of multiplex PCR for detection of enterotoxin genes from *S. aureus* strains

(A) *sea*: *S. aureus* enterotoxin A (127 bp). *seb*: *S. aureus* enterotoxin B (477bp). M: DNA molecular weight marker (100 bp. ladder). Lane 1: Cows milk (negative). Lane 2: Cows milk (*seb* gene). Lane 3: Cows milk (both *sea* and *seb* genes). Lane 4: Cows milk (negative). Lane 5: Cows milk (both *sea* and *seb* genes). Lane 6: Buffaloes milk (negative). Lane 7: Buffaloes milk (negative). Lane 8: Bovine septic wounds (negative). Lane 9: Bovine septic wounds (both *sea* and *seb* genes).

sea: *S. aureus* enterotoxin A (127 bp). *seb*: *S. aureus* enterotoxin B (477bp). M: DNA molecular weight marker (100 b.p. ladder). Lane 11: Human respiratory infection (*sea* gene). Lane 12: Human septic wounds (negative). Lane 13: Human septic wounds (*seb* gene). Lane 14: Human septic wounds (negative). Lane 15: Human septic wounds (negative). Lane 16: Human septic wounds (*seb* gene). Lane 17: Human septic wounds (*sea* gene). Lane 18: Human infected urinary tracts (negative). Lane 19: Human infected urinary tracts (negative). Lane 20: Cowan-1 standard strain (both *sea* and *seb* genes)

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9/1/2010