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Correlation Between Phage Typing and Toxins Content as an Outbreak Tool in *Staphylococcus aureus*

Manal M. AlKhulaifi, Nagwa M. Aref*, Ali A. AlSalamah and Mohammed. S. Al Shammary

Department of Botany and Microbiology, College of Science, King Saud University, P.O.Box 22452.Riyadh 11495, Kingdom of Saudi Arabia.

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Staphylococcus aureus **is one of the major causes of community and hospital acquired infections. As well as bacteriophage considered as a major risk factor acquires *S. aureus* new virulence genetic elements for it. A total number of 119 *S. aureus* isolates obtained from Riyadh Military Hospital. And were studied for phage typing and the incidence of toxin genes by PCR. Methicillin Resistant *S. aureus* isolates (MRSA) indicated high special prevalence of phage group II with a highly increase for phage type Ø3A compared to MSSA. Phage group II on Methicillin Sensitive *S. aureus* isolates (MSSA) considered an epidemiologic marker with frequent strong reaction compared to group III and phage group I. Phage type Ø75 may play an important role in a combination with Ø80 or/ Ø81 by having PVL toxin to be CMRSA lineages. 68% of *S. aureus* isolates had toxins. The most prevalent toxins were SEO, in 50.8% in MSSA & 25% in MRSA isolates. SEI was detected in 40.3% in MSSA & 29.1% in MRSA isolates. Also, SEA was 28% in MSSA & 33.3% in MRSA isolates. Phenotypic and genotypic variations between MSSA isolates seemed to be horizontally, while in MRSA isolates were vertically. It was obvious that five toxins together were located in more than one isolates in MSSA.**

Key words: *Staphylococcus aureus*, Phage types, Toxins, epidemiologic marker, MSSA, MRSA.

Staphylococcus aureus is one of the major causes of variety of infections; ranging from relatively mild to life threatening infections. It is an important pathogen due to a combination of toxin mediated virulence in vasiveness, and antibiotic resistance (Le Loir *et al*., 2003). It is important to know the strains associated with human infections and their sources in the environment in order to improve our understanding of its epidemiology. It was concluded that there is a complex relationship between various strains of EMRSA and MSSA especially on the skin. This interaction may have an important bearing on colonization of patients with MRSA (Gopal Rao *et al*., 2003). Phage typing is still an internationally recognized method, which has the classical method for detecting epidemic and pandemic strains, and may be a useful tool for rapid research of correlation between MRSA isolates (Wisniewska *et al*., 2012). There is a drift in epidemic phage types within *S. aureus* populations, which appeared to be more pronounced from the year 2000 (Wildemauwe *et al*., 2004).The virulent phage types and host cell were used as a marker enzyme activity depending on the recognition of phage that infects only one bacterial species among mixed population (Neufeld *et al*., 2003).

* To whom all correspondence should be addressed.
Phone: +966-1-4789585 Ext.1439
Fax: +966-1-4768171 & +966-1-4765598
E-mail: narif@ksu.edu.sa, nagwa_aref@hotmail.com

For many years phage typing was the suggested method for typing of *S. aureus* isolates **(**Pantucek *et al*., 2004) and (Van Belkum *et al*., 2007).The toxins content of *S. aureus* stains are associated with mobile genetic elements, such as phages (Sharma, 2000) and (Novick, 2003). It was found that the phage group V *S. aureus* isolates produced enterotoxin B (SEB). A 63% of isolates produced enterotoxin C (SEC), were typed by phage group M. Among phage group III isolates, production of enterotoxin A (SEA) (Marples *et al*., 1993). A close correlation between Toxic Shock Syndrome Toxin (TSST-1) production and susceptibility to phages 29 and/or 52 has been reported (Ejlertsen *et al*., 1994). While between 1959 and 1990; it was shown that 57% MSSA isolates which belonged to phage group I produced TSST-1 toxin (Narita *et al*., 2001). It was revealed that phage conversion of Panton Valentine Leukocidin (PVL) toxins are carried by at least two temperate phages.The phage born exogenous PVL genes were fixed in most recipient *S. aureus* clinical strains like as ØPV83-prophages **(**Zou *et al*., 2000).

MATERIALS AND METHODS

A total numbers of 119 isolates of *S. aureus* from different patients were collected from Microbiology Laboratory in Riyadh Military Hospital (RMH) and were identified by routine work staphylococcal conventional methods in the lab.

Genotypic Detection of Methicillin resistance in *S. aureus* Isolates

Triplex PCR method (Ito *et al*., 2001) and (Al-Shammary; 2005), was performed in the previous published article (Al-Khulaifi *et al*., 2009)**.**

Phage Typing Analysis

The isolates were phage typed by the standard method (Blair *et al*., 1961) and (Parker, 1972), with the International Human Staphylococcal Phage Typing Set (IPS), containing 23 phages were obtained from Central Public Health Laboratory, at Colindale in London. These types were classified in five groups: I (29, 52, 52A, 79, 80) -II (3A, 3C, 55 and 71) -III (6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85) -V (94, 96) -M (81, 95).

Phage Typing Method

It was described in details previously (Al-Khulaifi *et al*., 2009). Phage reactions were recorded by eye according to the phage scales, stated by Public Health Laboratory. Colindale. London.

Multiplex PCR Assay for Toxigenic *S. aureus* isolates

Four rapid reactions multiplex PCR assay were used to detect 16 specific *Staphylococcus* toxin genes including (Toxic Shock Syndrome Toxin (*TSST-1)*, Exfoliative Toxins (*ETA, ETB*), *S. aureus* enterotoxins (*SEA, SEB, SEC, SED, SEE, SEG, SHE, SEI, SEJ, SEM, SEN, SEO*) and Panton Valentine Leukocidin (*PVL*) (Table 1). Specific Primers: Four primer mixes had been used for each group of toxins and were modified (Becker *et al*., 1998) and (Sharma *et al*., 2000) and (Al-Shammary, 2005). Each primer mix got 20µl from each primer and *16SrRNA* as a positive control as the following:
Primer mix 1: *SEA, SEE, SED, SEB &16SrRNA***.**
Primer mix 2: *SEG, SEJ, SEI, SEN & 16SrRNA.*
Primer mix3 :*ETA, ETB, SHE, TSST-1 & 16SrRNA.*
Primer mix 4: *SEO, SEC, SEM, PVL & 16SrRNA.*

PCR Reaction Mix

Four multiplex PCR were done, each reaction was 25µl. 5µl of DNA template was added to Ready-To-GoTM PCR Beads (Amersham). The marker and loading buffer were used by Ready Run (Super ladder-Low kit. Con. 50µg/ml. AB gene Company).

Cycling Protocol

The reaction was carried by using MWG Primes 96 plus Cycler (MWGAG BIOTECH). The cycling protocol consists of one cycle of denaturation for 4min at 95°C, 30 cycles of 95°C for 30s, 55°C for 30s and 72°C for 1min; extension for 5min at 72°C and soaking at 4°C. PCR Product Analysis: 10µl of DNA were resolved in a 2.5% of PFGE agarose gel in 0.5X Tris-Borate-EDTA buffer (Bio-Rad, Hercules and Calif), at 120V for 2h. The gel was stained with ethidium bromide was visualized with UV light.

RESULTS

Molecular detection of MRSA in *S. aureus* isolates

All MSSA isolates were lacked *mecA* gene. However, it was detected in almost all MRSA (Al-Khulaifi *et al*., 2009).

Phage typing analysis

A 74.7% of the total isolates were phage type able by IPS (81.8% in MSSA & 61.9% in

Table 1. Primers Used in the Multiplex PCR for Detection of S. aureus Toxins

Toxin	Primer	Oligonucleotide sequence (5'-3')	BASE Pairs	QC Organisms
	Sa16SfSa16Sr	5’ GTAGGTGGCAAGCGTTATCC3’CGCACTCAGCGTCAG3	228	Positive Control
SEA	SEA-3SEA-4	5,CCTTTGGAAACGGTTAAAACG3’TCTGAACCTTCCCATCAAAAAC	127	NCTC 10652
SEB	SEB-1SEB-4	5’TCGCATCAAACTGACAAACG3’GCAGGTACTCTATAAGTGCCTGC	447	NCTC 10654
SEC	SEC-3SEC-4	5’CTCAAGAACTAGACATAAAAGCTAGG3’TCAAAATCGGATTAACATTATCC	271	NCTC 10655
SED	SED-3SED-4	5’CTAGTTTGGTAATATCTCCTTTAAACG3’TTAATGCTATATCTTATAGGGTAAACATC	319	NCTC 10656
SEE	SEE-3SEE-2	5’CAGTACCTATAGATAAAGTTAAAACAAGC3,TAACTTACCGTGGACCCTTC	178	FRI 578
SEG	SEG-fSEG-r	5,CGTCTCCACCTGTTGAAGG3’CCAAGTGATTGTCTATTGTCG	327	FRI 578
SEH	SHE-fSHE-r	5’CAACTGCTGATTTAGCTCAG3’GTCGAATGAGTAATCTCTAGG	360	ATCC 51811
SEI	SEI-FSEI-R	5’CAACTCGAATTTTCAACAGGTAC3’CAGGCAGTCCATCTCCTG	465	FRI 578
SEJ	SEJ-FSEJ-R	5’CATCAGAACTGTTGTTCCGCTAG3’CTGAATTTTACCATCAAAGGTAC	142	NCTC 10652
SEM	SEM1SEM2	5’CTATTAATCTTTGGGTTAATGGAGAAC3’TTCAGTTTCGACAGTTTTGTTGTCAT	300	NCTC 13142
SEN	SEN1SEN2	5’ATGAGATTGTTCTACATAGCTGCAAT3’AACTCTGCTCCCACTGAAC	680	NCTC 13142
SEO	SEO1SEO2	5’AGTTTGTGTAAGAAGTCAAGTGTAGA3’ATCTTTAAATTCAGCAGATATTCCATCTAAC	180	NCTC 13142
TSST-1	TST-3TST-6	5’AAGCCCTTTGTTGCTTGCG3’ATCGAACTTTGGCCCATACTT	446	NCTC 11693
ETA	ETA-3ETA-4	5’CTAGTGCATTTGTTATTCAAGACG3’TGCATTGACACCATAGTACTTATTC	119	eta
ETB	ETB-3ETB-4	5’ACGGCTATATACATTCAATTCAATG3/AAAGTTATTCATTTAATGCACTGTCTC	262	etb
PVL	PVL1PVL2	5’ATCATTAGGTAAAATGTCTGGACATGATCCA3’GCATCAASTGTATTGGATAGCAAAAGC	433	NCTC 13300

Table 2. Toxin Profiles among MSSA & MRSA Isolates

Toxin Profiles	MSSA Isolates (%)	MRSA Isolates (%)
SEI	(17.5%)	(12.5%)
SEA	(8.7%)	(16.6%)
SEG	(10.5%)	-
ETA 2	(3.5%)	(12.5%)
SEA/SEO	4 (7%)	(4.1%)
SEA/SEI	(5.2%)	(8.3%)
SEO	(7%)	-
SEG/SEI/SEN/SEO/SEM/PVL	(7%)	-
PVL	(1.7%)	(12.5%)
SEO/SEG/TSST-1	(1.7%)	(12.5%)
SEI/PVL	-	(8.3%)
SEI/ETA/SEM/SEG/SEO	(3.5%)	-
SEO/SEM	(3.5%)	-
SEG/TSST-1/SEO/SEM	-	(8.3%)
SEG/SEO	(1.7%)	-
SEG/SEI/SEN/SEO/SEM	(1.7%)	-
SEG/SEO/SEM/SEI	(1.7%)	-
SEA/TSST-1/SEO	(1.7%)	-
SEA/SEG/SEI/SEN/SEO/PVL	(1.7%)	-
SEA/SEG/SEI/TSST-1/SEO/SEM	(1.7%)	-
SEG/SEO/SEM	(1.7%)	-
SEO/SEC/SEM	(1.7%)	-
SEG/SEI/SEB/SEM/SEO	(1.7%)	-
SEO/PVL	(1.7%)	-
TSST-1/SEO/SEC/SEM	(1.7%)	-
SEA/ETB	-	(4.1%)
SEA/SEH	(1.7%)	-

Table 3. The Relationship between Toxins and Phage Groups in *S. aureus* Isolates

Toxin	Toxin produced by MSSA isolates	Frequency of Phage Groups						Toxin produced by MRSA isolates	Frequency of Phage Groups					
		I	II	III	M	V	Phage Nonty-peable		I	II	III	M	V	Phage Nonty-peable
SEA	16	8	5	11	2	1	4	8	3	1	2	1	1	4
SEB	2	-	-	1	-	1	1	-	-	-	-	-	-	-
SEH	1	1	-	1	-	-	-	-	-	-	-	-	-	-
SEC	2	-	-	-	-	-	2	-	-	-	-	-	-	-
SEG	21	8	9	9	8	5	4	5	1	-	1	-	-	3
SEI	23	9	11	12	4	3	5	7	2	2	2	2	-	4
SEM	16	5	4	5	3	5	5	2	1	-	-	-	-	1
SEN	6	1	2	2	3	1	2	-	-	-	-	-	-	-
SEO	29	10	7	14	9	8	7	6	1	-	2	-	-	3
ETA	4	2	4	2	1	1	-	3	-	1	2	1	-	1
ETB	-	-	-	-	-	-	-	1	1	-	-	-	-	-
TSST-1	4	1	-	2	-	1	2	5	1	-	1	-	-	3
PVL	7	2	3	3	3	-	2	5	-	-	3	-	-	2

MRSA). There were 55 different phage typing patterns among MSSA isolates, while 21 various patterns of phage typeable MRSA isolates, were obtained. Distribution of different *S.aureus* isolates into various phage groups were analyzed in Fig. 1. The predominant phage group in the study was belonging to the mixed group followed by phage group III.

Phage group III: In MSSA isolates, 43 of the isolates were typed by phage group III alone or in combination with the other groups. In MRSA isolates, 17 isolates were typed by phage group III alone or with other groups. Phage group I**:** MSSA, were present with a prevalence of 30 isolates. Where as, MRSA isolates had 17 isolates that typed by group I. Phage group II, 29 of MSSA isolates were typed, where as MRSA isolates had 11 isolates were typed. Phage group M:20 of MSSA isolates were typed. While MRSA isolates had 10 isolates; were typed by group M. Phage group V,19 of MSSA isolates were typed by it. Where as four isolates of MRSA were typed by group V which mixed with others (Fig.1).

Some isolates showed identical phage pattern. The numbers of repetitions of them were high in MRSA rather than MSSA isolates. The prevalence of the same phage typing patterns for isolates were remarkable in MRSA isolates. It was observed identical phage typing patterns between MSSA & MRSA isolates: Ø81 & Ø54/85. Interestingly, it was observed a predominant two phage marker types Ø54 (35.1%) and/or Ø85 (28.5%) in *S. aureus* alone or in combination with other phage types. For MSSA isolates; had a phage typing marker with a prevalence of Ø54 (38%) and for MRSA the phage marker was Ø85 (34.6%) alone or in combination with other types. There was variation between sort of phage types in both MSSA & MRSA isolates. Phage types Ø79 & Ø47, Ø53 & Ø96 increased in MSSA isolates compared

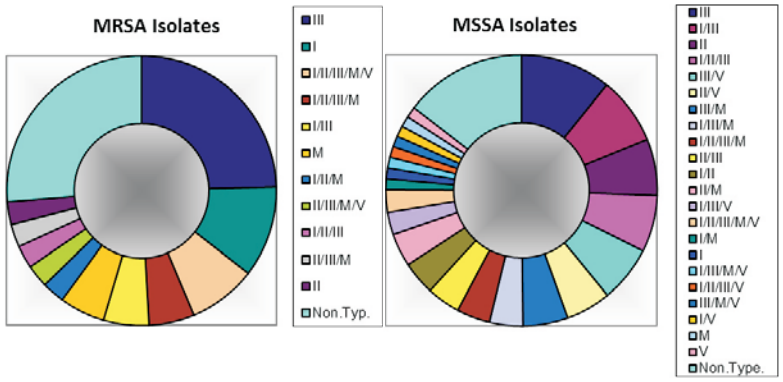

Fig. 1. Phage Typing Pattern of MRSA and MSSA Isolates

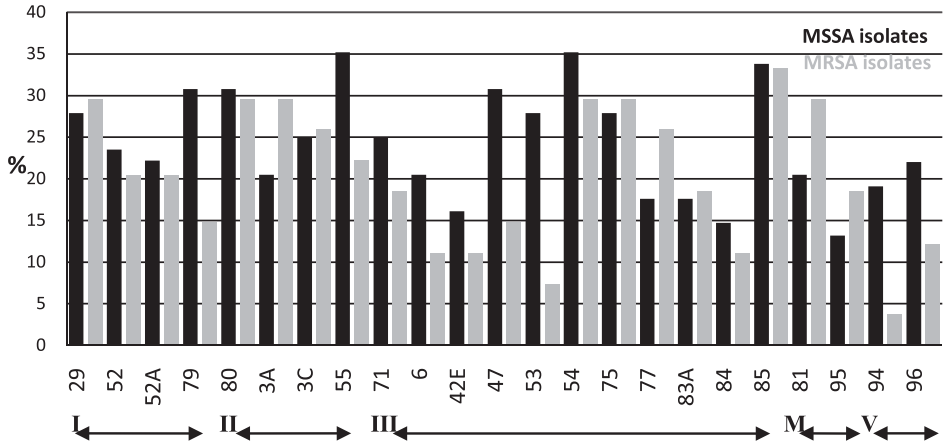

Fig. 2. Prevalence of Phage Types among MSSA and MRSA Isolates

to MRSA isolates which had low percentage as shown in Fig. 2. The predominant mixed phage group patterns were grouped as follows: In MSSA isolates were (I/III), (III/V), (I/II/III). (III/M). In MRSA were (I/II/III/M/V) and (I/II/III/M). Most of MRSA isolates were typed by individual phage groups.

Toxins content among *S. aureus* Isolates

68% of *S. aureus* isolates had toxins (74% in MSSA and 57.1% in MRSA isolates). It was observed that the different prevalence of enterotoxins between MSSA and MRSA isolates. The most prevalent enterotoxins were SEO (50.8% in MSSA and25% in MRSA isolates). SEI toxin was detected 40.3% in MSSA & 29.1% in MRSA isolates. Also, SEA toxin was 28% in MSSA and 33.3% in MRSA isolates. The SEO toxin was the most prevalence in MSSA isolates while SEA was the common in MRSA isolates. The SEB, SEC, SEH and SEN toxins were only observed in MSSA

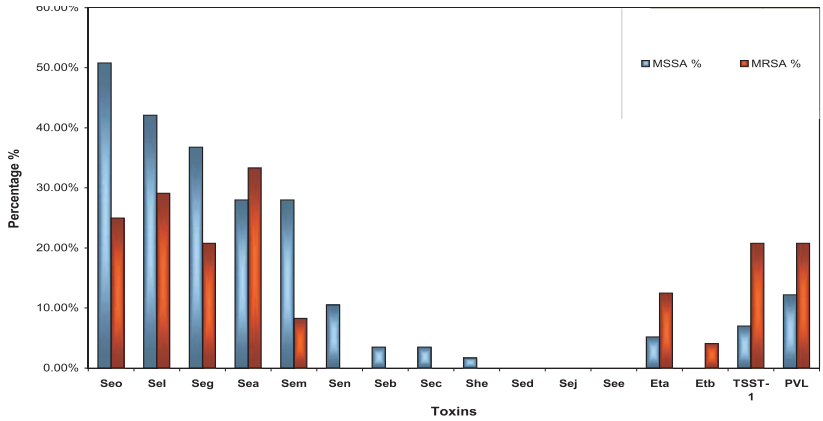

Fig. 3. Toxins Produced by MSSA & MRSA Isolates

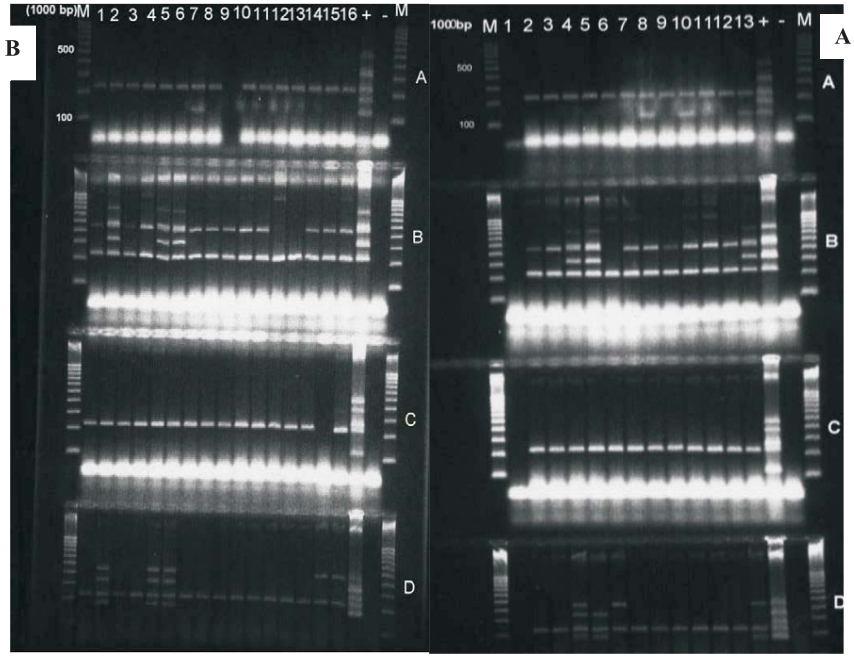

Fig. 4. Two examples of Toxigenic *S. aureus* Isolates illustrated for 16 Toxins: 1000bp molecular size DNA marker. (A) 1: Negative isolate. 2-3-7-9-11-12: SEI.4:SEG/SEI/SEM/SEN/SEO/PVL. 5: SEG/SEI/SEM/SEN/SEO. 6*:* PVL. 8-10: SEA/SEI. 13: SEA/SEG/SEI/SEN/SEO/PVL. (B) 1-3-4-8-9-10-11-14: SEI. 2-5-6: SEG/SEI/SEM/SEN/SEO/PVL. 7: SEA/SEI 12- 13: Negative isolates. 15- 16: SEI/PVL. (+): Positive control. (-): Negative control

isolates. No isolate of *S. aureus* produced the SED or SEJ toxins as shown in Fig.3. Whereas, Exfoliative toxins (ET) were found in 7% in MSSA and 12.5% in MRSA isolates were produced ETA and only one MRSA isolate was produced ETB toxin. Toxic Shock syndrome toxin (TSST-1) was found in 7% in MSSA & 20.8% in MRSA isolates. Panton Valentine Leukocidin (PVL) toxin was produced by 12.2% in MSSA and 20.8% in MRSA isolates. The toxin profiles were illustrated in (Table 2 and Fig. 4). A slightly higher proportion of MRSA isolates produced enterotoxins but MRSA isolates had a higher proportion with ET, TSST-1 and PVL toxins.

Results indicated the relationship between the toxins and phages among isolates. In MSSA, phage group III were the common among the isolates which produced SEA, SEI and SEO toxins. While Phage groups I, II, III and M were the common with SEG toxin isolates. Within isolates which produced ETA toxin, the common phage group were II. PVL toxin isolates were distributed among the phage groups except group V. One out of two isolates which had SEB toxin was typed by phage groups III &V. Also, two isolates had SEC toxin were phage nontypeable. All MSSA nontypeable isolates were produced various types of toxins except ETA & SEH. Whereas in MRSA isolates, SEA toxin isolates were distributed among all phage groups, but the most were I and III. SEI toxin isolates typed by I, II, III and M. One of two SEM toxin isolates was typed by group I alone. Group III were the common with ETA and PVL toxins. The isolate which produce ETB toxin was typed by I alone. Most phage nontypeable MRSA isolates were contained SEA. Two isolates were produced PVL, three isolate were contain SEG, SEO and TSST-1 toxins.

DISCUSSION

The distribution of various phage types could be considered as an indicator for the outbreak of infection associated with a predominant phage type. This could demonstrate the Spread of MRSA in Saudi hospitals. Phage typing data on MRSA isolates indicated high special prevalence of phage group II with a highly increase for phage type Ø3 A compared to MSSA isolates, Fig. 2. Phage group II on MSSA may considered as an epidemiologic marker with frequent strong reaction type ability (59%) individually or mixed with other phage groups compared to group III (66.6%) and phage group I (51.3%). It was observes that phage group III resembled the highest type ability for *S. aureus* followed by group I that started to appear. Remarkably, phage groupII did not exist mostly in that study (Al-Digs, 2004 and Aref & Al-Digs, 2009). Phage group III were predominant amongst MRSA strains isolated from the hospital acquired infections but the predominant phage amongst MSSA strains from the community was phage type 81 (Mehndiratta *et al*., 2010).

Depending on a phage marker Ø85 which was predominant in MRSA isolates. It was clear to discriminative MRSA into three groups by this phage indicator. That had (21.4%) prevalence of this marker, while the second group had 45.3% to be typed by other and the third was phage nontypeable (33.3%). 88.8% of *S. aureus* MRSA isolates submitted from Riyadh Military Hospital in Al-Riyadh from the previous phage groups, were typed by phage type Ø85 (Moore *et al*., 2001).We assessed the previous five isolates produced PVL toxin, typed only by phage group III individually (that are frequently found among the hospital strains) except twoisolate, which was phage non typeable.

It was noticed also, that phage type Ø75 was the only predominant phage for three isolates typed by it with a week reaction. This phage type could play an important role in this *S. aureus* population in a combination with other two important phage types 80/81, which have multilocus sequence type in MSSA population (Robinson *et al*., 2005). MSSA strains could acquire SCC *mec* type IV directly or via phage type 80/81 by having PVL toxinto be CAMRSA line ages. This acquisition could promote the spread of *S. aureus* clone in hospital and the community (Robinson *et al*., 2005).

In the present era, analysis of genome sequences of bacterial pathogens can expeditiously reveal whether virulence factors are associated with phage-like DNA sequences regardless of whether they are transmissible (Wagner and Walder, 2002). Depending on the obtained data, it could be considered the three isolates of MRSA isolates with *mecA* and PVL CAMRSA isolates were characterized as the

following: (i) (Phage group III)-(Toxin: PVL), (ii) (phage nontypeable)- (Toxins: SEI/PVL) and (iii) (phage group III) -(Toxin: PVL).

Similar seven correlated MSSA isolates having PVL toxin reflected the re-emergence of different clones building up. Remarkably, five MSSA isolates were typed by phage group III with a high percentage of phage types in another mixed group of M/orII/ or and I as shown in Table (3). These phage types resembled Ø80 or Ø81/75or Ø80/85 or Ø81/71. The strong reactions of phage typing for each were high, from 50- 80% contrarily that detected for MRSA- PVL toxin isolates with a very low reaction. It means some genetic elements have been gained, and could possess a serious public health challenge in coming years especially for that outpatient isolates. These five MSSA phage typeable isolates were:

(i) (phage groups I/III) (Toxins: SEO/PVL), (ii) (phage groups II/III) (Toxin: PVL), (iii)(phage groups II/M) (Toxins: SEG/SEI/SEM/SEN/SEO/ PVL), (iiii)(phage groups I/III/M) (Toxins: SEA/ SEG/SEI/SEN/SEO/PVL) and(iiiii)(phage groups II/ M)(Toxins: SEG/SEI/SEM/SEN/SEO/PVL).

Whereas the phage nontypeable MSSA isolates were:(Toxins: SEG/SEI/SEM/SEN/SEO/ PVL).

The decrease number of V phage group typing of MRSA isolates individually, and the relative increase of new introducing mixed phage typing with groups M and V may resembled the role of these two phage groups in transduction.The investigations revealed that,PVL toxin was found in14.8% in all studied *S. aureus* isolates. While others were found that the PVL toxin only 2-3% of *S. aureus* strains (Said-Salim *et al*., 2003). The percentage of PVL toxin was increased in the present study for MRSA isolates (20.8%) than that was found in other study (10.7%) (Al-Shammary; 2005).CMRSA strains were distinguished depending on some features (having *mecA* III- Carry PVL genes) (Campbell *et al*., 2002). Thus, it could be that four isolates were may speculated as CMRSA. Three of them were noticed to be typed by phage Ø75 alone for the two formers and with phage type Ø6 for the later having weak reaction and the fourth one was phage non typeable as shown in Table (2).

These clones isolates thought to be sister member of the same descendants of phage types 80/81 have acquired methicillin resistance that were re-emerging as a community-acquired MRSA clones termed by phage type Ø75, because the occurrence of PVLtoxin was noticed to be correlated with the only clone termed phage types 80/81/75 or phage non typeable in the studied populations.This phage type Ø75 was increased lately from (6.8%) since1995 (Al-Salamah, 1995), and in our study which were 29.2%.The increasing in CMRSA continued to be higher up to 33%, over the years in Saudi Arabia and that was noticed (Bukharie *et al*., 2001) and (Al-Shammary; 2005).

In addition, the major reservoir for *S. aureus* in the hospitals is colonized or infected patients and medical staff. The exact pathogenicity of the carrier state is not sufficiently clear, but it is known that the adherence to the human nasal mucosa is a complex process mediated by multiple bacterial adhesions and host receptors which probably are responsible for a long-time persisten ceof special connections between a given staphylococcal strains and a given host (Kluytmans *et al* ., 1997).

It was explained the secret of the extreme flexibility of this organism is inscribed in its genome sequence, which contains several putative alien genes that, although *S. aureus* presents us with ever-challenging infections we should never stop appreciating the *S. aureus* evolutionary 'plot' that is unfolding before us (Hiramatsu *et al* ., 2001).

Phenotypic and genotypic analysis revealed diversity in the epidemiologic between MSSA/MRSA studied isolates. Heterogeneity inside MSSA isolates for having virulent factors was so wide, while it was homogenized for MRSA isolates, *i.e.* the variations between MSSA isolates seemed to be horizontally, while in MRSA isolates were vertically.

There was a heterogeneity characters inside MSSA population. A 61.71% of the phage typeable, were genetically typed resulting one type with one genetic difference. Also the same population exhibited phenotypic phage groups; I and III. Similar phage types were used as a marker for many researchers as a prevalence of *S. aureus*.

Phage type Ø75 could play an important rolein combination with other two important phage types Ø80/81 by having PVL toxin to be CMRSA line ages. We could conceder the three out patient isolates which were defined as CMRSA. It was

obvious that five toxins together were located in more than one isolates in MSSA.

The changes in the comparative phage typing patterns and the percentage of type ability in MSSA/MRSA isolates showed high deviation caused by the introduction and spread of many strains. Phage type Ø95 may appear to be a new, strong and stable colonizer. The phage marker Ø85 or/and / Ø54 was common in most isolates.

MRSA isolates reflected somehow homogeneity concerning phage typing.The indication of the important comparative study of MSSA isolates versus MRSA isolates in the same population appeared speculation of the EMSSA/ CMRSA. In the present study, inside 119 *S. aureus* isolates; 29 MSSA isolates may segregated as EMSSA. Half of them typed with phages Ø75/ Ø80, and half of these had PVL toxin. Similarly, five MRSA isolates segregated with PVL toxin/ phages Ø75/Ø80/Ø81 which could be acquired *mecA*gene (CMRSA) by having PVL gene. This study could be an indication to combine MSSA/MRSA isolates in different comparative studies for evaluating data in different communities in Saudi Arabia. Also; a remarkable combined phage typing cocktail of powerful local phages including two temperate phages had a wonderful biomedicine results in five days as a therapy for thirty MRSA diabetic foot infections, wounds, burns and abscess cases. These were isolated from local hospital for typing MRSA in Saudi Arabia (Aref &Al-Digs, 2009).

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