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Molecular characterization of clonal lineage and Staphylococcal toxin genes from S. aureus in Southern Nigeria

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Background. The pathogenic role of S. aureus as causative agent of serious infections and food poisoning is on the increase. However, there are few reports on comprehensive analyses of toxins and staphylococcal enterotoxin (SE) genes in S. aureus in Africa. This study analyzed spa types and toxin genes in S. aureus obtained from our previous studies in Southern Nigeria.

Methods. Forty-seven non-duplicate S. aureus isolates were obtained from humans (n = 34) and poultry (n = 13) from previous studies in Southern Nigeria. The strains were analyzed for mecA, selected toxins genes (sea, seb, sec, sed, see, seg, seh, sei, sek, sel, sem, sen, seo, sep, seq, ser, seu), TSST and lukS-PV/lukF-PV by PCR. Population structures of the strains were detected by Staphylococcal protein A (spa) typing.

Results. Twenty three percent of all isolates (47) carried the Panton-Valentine leukocidin (PVI) gene. Two MRSA were detected. Twenty different spa types were obtained, with the highest percentages, 17% belonging to spa type t091 was observed in 4 states from clinical, nasal and poultry samples while t069 is the most prevalent type in poultry. Eighty-nine percent of the all tested isolates harbored at least one staphylococcus enterotoxin. Seo was the most prevalent SE (34%) followed by seg (30%) and sea (21%), while toxic shock syndrome toxin (TSST), seb, sec, see, sej, sel, sem, and ser, seu were absent in all strains. Spa type t355 was associated with the PVI and complete absence of all studied SE. Sea, seq, seb, sek were associated with spa type 069; t127 was associated with sea while sep was associated with spa type t091. There was coexistence of seo/seg and sei/seg.

Conclusions. We detected a high incidence of enterotoxins and PVI encoding genes in these potential staphylococcal reservoir. Specific toxin genes were observed in particular spa types.
Molecular characterization of clonal lineage and staphylococcal toxin genes from S. aureus in Southern Nigeria.

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Abstract

Background: The pathogenic role of S. aureus as causative agent of serious infections and food poisoning is on the increase. However, there are few reports on comprehensive analyses of toxins
and staphylococcal enterotoxin (SE) genes in *S. aureus* in Africa. This study analyzed spa types and toxin genes in *S. aureus* obtained from our previous studies in Southern Nigeria.

**Methods**: Forty-seven non-duplicate *S. aureus* isolates were obtained from humans (n = 34) and poultry (n = 13) from previous studies in Southern Nigeria. The strains were analyzed for *mecA*, selected toxins genes (*sea, seb, sec, sed, see, seg, seh, sei, sek, sel, sem, sen, seo, sep, seq, ser, seu*), TSST and lukS-PV/lukF-PV by PCR. Population structures of the strains were detected by Staphylococcal protein A (*spa*) typing.

**Results**: Twenty three percent of all isolates (47) carried the Panton-Valentine leukocidin (PVl) gene. Two MRSA were detected. Twenty different spa types were obtained, with the highest percentages, 17% belonging to spa type t091 was observed in 4 states from clinical, nasal and poultry samples while t069 is the most prevalent type in poultry. Eighty-nine percent of the all tested isolates harbored at least one staphylococcus enterotoxin. *Sea* was the most prevalent SE (34%) followed by *seg* (30%) and *sea* (21%), while toxic shock syndrome toxin (TSST), *seb, sec, see, seg, sei, sel, sem* and *ser, seu* were absent in all strains. Spa type t355 was associated with the PVl and complete absence of all studied SE. *Sea, seq, seb, sek* were associated with spa type 069; t127 was associated with *sea* while *sep* was associated with spa type t091. There was coexistence of *seo/seg* and *sei/seg*.

**Conclusions**: We detected a high incidence of enterotoxins and PVl encoding genes in these potential staphylococcal reservoir. *Spa* types are associated with specific toxin genes.

**Keywords** - Staphylococcal enterotoxins, spa type, virulence, Southern Nigeria, PVl

**Background**

*Staphylococcus aureus* is one of the most important human colonizers that can cause infectious diseases. Colonization of human nares by *S. aureus* is a source and risk factor for staphylococcal disease (Wertheim *et al.*, 2004) and invasive staphylococci infection can have its source in strains occurring naturally in the host.

The emergence of MRSA complicated the treatment of infections and increasing the focus on this pathogen. *S. aureus* has a broad spectrum of inherent virulence factors which can enhance infections ranging from mild skin infections to severe sepsis, pneumonia, osteomyelitis and endocarditis (Ayepola *et al.*, 2015). The ability of *S. aureus* to successfully infect man is largely due to the expression of virulence factors which promote adhesion, acquisition of nutrients and evasion of host immunologic responses (Monday and Bohach, 1999) Staphylococcal enterotoxins and toxic shock syndrome toxin (TSST) are produced by *S. aureus* which enhances their status as important food-borne pathogens (Løvseth *et al.*, 2004) because they can cause
food poisoning in humans. The toxins’ genes in *S. aureus* encode different virulence factors which can promote the ability to cause infections in humans. This enhances *S. aureus’* pathogenicity; the toxins produced by the pathogen are responsible for toxin mediated diseases such as toxic shock syndrome and food poisoning. *Spa* typing of *S. aureus* strains provides information which can group isolates in clonal lineages. Clonal analyses can also provide useful insights into the virulence potential and nature of *S. aureus* populations (Kolawole *et al*., 2013).

Shittu *et al*., (2011) reported that *S. aureus* is the main etiological agent of many infections in sub-Saharan Africa and one of the most frequently encountered bacterial species in microbiology laboratories in Nigeria. To establish better infection control, it is important to understand the local epidemiology and clonal lineages of *S. aureus* in Nigeria. Ayepola *et al*., (2015) also stated that some virulence factors are highly prevalent in *S. aureus* isolated from infection but less frequently found in isolates from colonization in Nigeria. PVL can be implicated in skin and soft tissue infections and can also increase *S. aureus’* ability to cause severe infections in humans. Therefore, the objective of this study was to detect selected virulence factors genes and clonal lineages of *S. aureus* previously isolated from seven states of Southern Nigeria.

Methods

**Bacterial isolates**

Forty-seven *S aureus* isolates used in this study were drawn from a larger staphylococcal collection from our previous studies to assess the rate of misidentification of *S. aureus* in 7 states (Oyo, Ogun, Osun, Lagos, Ekiti, Bayelsa, Rivers) of Southwestern Nigeria. Thirty four isolates from 6 states were from humans (previously isolated from nasal carriage in the community and clinical isolates) (Ayeni *et al*., 2014, Ayeni *et al*., 2015, Ayeni *et al*., 2017) while thirteen isolates from one State (Ogun) were co isolated with enterococci in a previous study on poultry (Ayeni and Odumosu, 2016). All *S. aureus* isolates from these previous studies were selected for this study.
Identification of *S. aureus* strains by amplification of *femA* gene

The DNA of all staphylococci isolates was extracted by QuickExtract™ DNA extraction solution (Epicentre, USA) according to the manufacturer’s instructions. One µl of extracted DNA was used in PCR reaction in a total volume of 20 µl with 10 µl of 2-fold concentrated RedTaq Ready Mix (Sigma, Germany), 7 µl PCR grade water, 1 µl of 10 pmol of *femA*-F AACTGTTGCCACTATGA and 1 µl of 10 pmol of *femA*-R CCAGCATTACCTGTAAACTC. After an initial denaturation step (3 min at 92°C), 30 cycles of amplification were performed as follows: denaturation at 92°C for 1 min, annealing at 56°C for 1 min, and DNA extension at 72°C for 1 min with an increment of 2 s per cycle. The reaction was achieved with a final extension at 72°C for 3 min (Vannuffel *et al.*, 1995). The PCR product was analysed on agarose gel and bands corresponding to 686-bp were recorded as positive for *femA*.

Spa typing of *S aureus* isolates

All *femA* positive isolates were further analysed by *spa* typing. The polymorphic X region of the *spa* gene was amplified in all isolates to a total volume of 20 µl comprising 1 µl of genomic DNA, 10 µl of 2-fold concentrated RedTaq Ready Mix (Sigma, Germany), 7 µl PCR grade water, 1 µl of forward primer 1113F (5’ – TGTTAAACGCAGCCAGTATCCCCTTCGGTGA and 1 µl of reverse primer spa 1514R CAGGAAAACAGCTATGACCAGAGTGGCGTCCGTTTCTT were used in PCR reaction according to protocols previously described (Schmid *et al.*, 2013). PCR products were run on agarose (1 %) gel electrophoresis previously stained with GelRed (BiotiumInc, USA) and run at approximately 40 mAmp for 45 min. The PCR products were purified with EXOSAP-IT (GEHealthcare, UK). Two microliters of the purified amplification products were used for subsequent sequencing using the BigDye 3.1. Terminator sequencing kit (Applied Biosystems, USA) and were finally analyzed on ABI Genetic Analyzer 3500Dx (Applied Biosystems, USA). The chromatograms obtained were analyzed with the Ridom Staph Type software version1.4; (RidomGmbH, Germany http://spa.ridom.de/index.shtml). Spa types were deduced by the differences in number and sequence of spa repeats with BURP algorithm (Ridom GmbH) and the Ridom Spa Server database (Montanaro *et al.*, 2016).

PCR amplification of *mecA/mecC*.

PCR assay was performed for all confirmed *S. aureus* strains to amplify a region of *mecA* gene. Primers were as follows: Fw, 5’TCACCAGGTCCAAC[AAAA] 3’; and Rv, 5’ CCTGAATCW] GCTAATAATATTTC 3 (Garcia-Álvarez *et al.*, 2011). PCR reaction contained 20 µl reaction volume with 1µl of each primer, 10 µl mastermix and 7 µl of PCR grade water. The PCR reaction consisted of an initial denaturation step at 95°C for 5 min; 40 cycles of denaturing at 95°C for 30 seconds; annealing at 55°C for 45 s; extension at 72°C for 45 s; and a final extension at 72°C for 10 min. PCR products were resolved by agarose (1%) gel
electrophoresis previously stained with GelRed (BiotiumInc, USA) and run at approximately 40 mAmp for 45 min.

**Virulence factors detection.**

Exfoliative toxins (sea, seb, sec, sed, see, seg, seh, sei, sej, sek, sel, sem, sen, seo, sep, seq, ser, seu), TSST and the lukS-PV/lukF-PV, encoding the Panton-Valentine leucocidin were detected by single PCRs in previously described protocols (Monday and Bohach, 1999, Jarraud *et al.*, 1999, Orwin *et al.*, 2001, Lina *et al.*, 2003, Løvseth *et al.*, 2004)

**Results**

Forty-seven *S. aureus* isolates (34 from human and 13 from poultry) were identified in this study as confirmed by femA gene amplification. Twenty different spa types were obtained. Most frequent spa type were t091(17%), t355 (17%). t091 was observed in 4 states from clinical, nasal and poultry samples while t069 is the most prevalent type in poultry. (Table 1, Fig 1). Of the tested strains, two were MRSA (Table 2).
No distinctive difference of enterotoxin genes could distinguish between the human and poultry isolates. Eighty-nine percent of all isolates harboured at least one staphylococci enterotoxin and 23% isolates had PVL. Only one of our nasal isolates harbour PVL while the remaining 12 lacked the gene. *Seo* was the most prevalent SE (34%) followed by *seg* (30%) and *sea* (21%). *Ser* was detected in one isolate while *seb*, *sec*, *see*, *sej*, *sel*, *sem*, and *ser*, *seu* were not found in all strains. Several enterotoxin gene combinations were observed including isolates with a combination of two (n = 4, 9%), three (n = 5, 11%), four (n = 13, 28%) and five (n = 2, 4%) different SE genes. There was coexistence of *seo/seg* and *sei/seg* toxins (Table 2, Fig II, Fig III).

Some toxins were associated with particular spa types. 88% of t355 (n = 8) isolates obtained from two different locations had PVL and were characterized by complete absence of SE. *Sea*, *seq*, *seb*, *sek* were associated with spa type 069 (obtained from 2 locations). All t127 isolates carried *sea* while *sep* was associated with spa type t091 and was the only SE gene that all t091 strains except 1 isolate carried.

**Discussion**

We report spa types and virulence factors of *S. aureus* isolates that have been previously collected in Southern Nigeria. This study reports predominant of t091 spa types of *S. aureus* isolates gotten from different locations and sources in Southern Nigeria. Species-confirmation of
the 47 *S. aureus* isolates was done by *femA* gene amplification. Vannuffel *et al.*, (1995) confirmed that *femA* expression is a unique feature of *S. aureus*, allowing its specific detection. O’Malley *et al.*, (2015) reported that MRSA exist in clinical and community settings in Nigeria. We report a relatively low detection of *mecA/mec* in the current study although several authors have reported high phenotypic detection of MRSA in Nigeria (Onanuga *et al.*, 2005, Ayeni *et al.*, 2014). The two detected MRSA isolates were PVI negative. Lack of PVI in Nigerian MRSA strains was also previously reported by Kolawole *et al.*, (2013).

We observed a relatively high rate of PVI positive isolates which is in line with other studies from Africa. Sub-Saharan Africa is observed to be a PVI endemic region showing PVI prevalence among MSSA isolates. O’Malley *et al.* (2015) indicated that 40% (23/57) of MSSA isolates are PVI positive with no PVI positive MRSA therefore they study concluded that PVI positive isolates are most often seen in MSSA. Shittu *et al.*, (2011) also reported high proportion of PVI positive isolates among MSSA (40%) in Nigeria. However, in other region of the world, it was reported that MSSA rarely harbor PVI gene (Becker *et al.*, 2017).

Molecular typing technologies such as *spa* typing provide information which enable the grouping of individual isolates in clonal lineages (Kolawole *et al.*, 2013). Twenty *spa* types were found in this study with the highest percentages belonging to t091. This is different from other studies from Nigeria where t064 have higher prevalence. Kolawole *et al.*, (2013) reported 24 *spa* types with the most frequent *spa* types being t064, t084, t311, and t1931. Also *spa* type t064 is the most common *spa* type among HIV positive patients in Nigeria (Olalekan *et al.*, 2012). Shittu *et al.*, (2011) reported a total of 28 *spa* types with the predominant *spa* type identified as t084 among the MSSA isolates, while t451, t008, t002 and t064 were observed in Southwest Nigeria. These studies, however, were confined to Southwestern Nigeria while a study by O’Malley *et al.*, (2015) which involved nasal carriage from Southwestern and Southeastern Nigeria reported *spa* types t091 and t355, which we also found in our study. Our study locations were also Southwestern and South-South parts of Nigeria and some isolates were from nasal carriage. Therefore, location and site of isolation may be an important factor in types of *spa* found in a study. Interesting, t091 was seen in all three sources of isolates in this study i.e. nasal, clinical and poultry sources. It also spread across widely spaced locations in 4 states of Southern Nigeria and consistently seen even in the small number of isolates used in this study. The predominant *spa* type t091 reported in this study has recently been reported in Germany (Becker *et al.*, 2017) and Poland (Ilczyszyn *et al.*, 2016) while t355 have been recently reported in Uganda (Asiimwe *et al.*, 2017) and Italy (Basanisi *et al.*, 2017).

It has been observed that prevalence of enterotoxin genes differs greatly depending on the geographic affiliation and the population structure tested (Kolawole *et al.*, 2013). In this study, *seo* gene was the most prevalent followed by *seg*.89% of the all tested isolates harbor at least one staphylococcal enterotoxin. This is a high occurrence and has implication in public health. Staphylococcal enterotoxins may induce T-cell stimulation resulting in systemic illness such as toxic shock syndrome and food poisoning. Peck *et al.* (2009) also reported significant differences
and higher prevalence of selected enterotoxin genes in *S. aureus* isolates obtained from blood compared to nasal isolates (7.2% blood vs. 30.5% nasal). The clinical significance of SE cannot be overemphasized. Argudín *et al.*, (2010) stated that staphylococcal food poisoning results from the consumption of foods containing sufficient amounts of preformed enterotoxin and its real incidence is probably underestimated due to misdiagnosis, and improper laboratory examination with the control of social and economic importance.

*Seo* and *sei* were found in association with *seg* in this study. Previous studies have reported associations of *seg* and *sei*. Kolawole *et al.*, (2013) reported that the most frequent SE genes detected were *seg/sei* (41.0%) while Loncarevic *et al.*, (2005) stated that 27.9% of 215 isolates harbored *seg* and *sei*. Rosec and Gigaud (2002) reported that 80.6% of 155 isolates harbored *seg* and *sei*. Kim *et al.*, (2011) reported that *sec, seg, sei, sel, sen, seo*, were associated with genomic islands thereby probably responsible for their observed combined occurrence. Some *S. aureus* strains in this study also had several enterotoxin gene combinations, from a combination of two to a combination of five different SE genes.

Some toxins were observed in specific spa types. Most t355 spa types had PV1 genes in contrast to other spa types where there was complete absence of PV1 genes. t355 is also characterized by complete absence of all investigated SE. *Sea, seq, seb, sek* were observed in spa type 069. All t127 carried *sea* gene while *sep* gene was seen only in spa type t091 and that is the only SE gene that all t091 strains carried, except 1 isolate. These *S. aureus* strains were isolated from different locations across Nigeria, yet the spa types consistently displayed the presence or absence of a particular virulence gene. This information could be useful in predicting virulence toxins a particular strain of *S. aureus* likely carries once the spa type is known. However, further representative studies with larger sample sizes are needed to confirm this. Shittu *et al.*, [6] also reported association of some toxin genes (*seh* and *etd*) with a sequence type (ST25).

**Conclusions**

This study reports predominance of t355 and t091 spa types of *S. aureus* from different locations in Nigeria. A relatively high rate of PV1-positive isolates was found. In this study, *seo* was the most prevalent followed by *seg* while 89% of the all tested isolates harbour at least one staphylococci enterotoxin. *Seo* and *Sei* are found in association with *Seg* in this study. Interestingly, some toxins were seen only in specific spa types.

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Table 1 (on next page)

Prevalence of spa types in different locations in Southern Nigeria
### Table 1: Prevalence of spa types in different locations in Southern Nigeria

| State    | Region        | Source  | Number | spa type (no)     | Predominant spa type |
|----------|---------------|---------|--------|-------------------|----------------------|
| Bayelsa  | South-South   | Nasal   | 13     | t084 (1)          | t091 (3)             |
|          |               |         |        |                   | t1045 (2)            |
|          |               |         |        |                   | t127 (3)             |
|          |               |         |        |                   | t939 (1)             |
|          |               |         |        |                   | t311 (1)             |
|          |               |         |        |                   | t786 (1)             |
|          |               |         |        |                   | t1154 (1)            |
|          |               |         |        |                   | t127 (3)             |
|          |               |         |        |                   | t084 (1)             |
|          |               |         |        |                   | t091 (3)             |
|          |               |         |        |                   | t1045 (2)            |
|          |               |         |        |                   | t127 (3)             |
|          |               |         |        |                   | t939 (1)             |
|          |               |         |        |                   | t311 (1)             |
|          |               |         |        |                   | t786 (1)             |
|          |               |         |        |                   | t1154 (1)            |
|          |               |         |        |                   | t127 (3)             |
| Oyo      | South-West    | Clinical| 4      |                   | t091 (2)             |
|          |               |         |        |                   | t127 (1)             |
|          |               |         |        |                   | t008 (1)             |
| Rivers   | South-South   | Clinical| 1      |                   | t127 (1)             |
| Osun     | South-West    | Clinical| 4      |                   | t355 (2)             |
|          |               |         |        |                   | t537 (1)             |
|          |               |         |        |                   | t091 (1)             |
| Ekiti    | South-West    | Clinical| 7      |                   | t355 (6)             |
|          |               |         |        |                   | t1931 (1)            |
| Lagos    | South-West    | Clinical| 5      |                   | t1095 (2)            |
|          |               |         |        |                   | t069 (1)             |
|          |               |         |        |                   | t1045 (1)            |
|          |               |         |        |                   | t021 (1)             |
| Ogun     | South-West    | Poultry | 13     |                   | t069 (4)             |
|          |               |         |        |                   | t091 (2)             |
|          |               |         |        |                   | t14223 (1)           |
|          |               |         |        |                   | t905 (1)             |
|          |               |         |        |                   | t292 (1)             |
|          |               |         |        |                   | t939 (1)             |
|          |               |         |        |                   | t318 (1)             |
|          |               |         |        |                   | t050 (1)             |
|          |               |         |        |                   | t1171 (1)            |
Table 2 (on next page)

Spa types and enterotoxin gene profiles of S. aureus isolates from Southern Nigeria.
| Isolate          | spa  | pvl | A | O | M | Q | N | K | P | L | B | G | R | U | I | H | Total |
|------------------|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|------|
| *S. aureus* FA01 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA02 | t357 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA03 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 0    |
| *S. aureus* FA04 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA05 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA06 | t1931 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA07 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA08 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA09 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA10 | t355 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA11 | t045 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 3    |
| *S. aureus* FA12 | t021 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA13 | t069 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA14*| t095 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 3    |
| *S. aureus* FA15 | t095 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 2    |
| *S. aureus* FA16 | t095 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA17*| t069 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA18*| t069 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA19*| t14223 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 0    |
| *S. aureus* FA20*| t095 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA21*| t095 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA22*| t095 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| *S. aureus* FA23*| t095 | +   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1    |
| *S. aureus* FA24*| t292 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 5    |
| *S. aureus* FA25*| t939 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 3    |
| *S. aureus* FA26*| t318 | +   | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 4    |
| S. aureus FA027* | t069 | + | + | + | + | 4 |
| S. aureus FA028* | t050 | + | + | + | | 3 |
| S. aureus FA029* | t1171 | | | | | 0 |
| S. aureus FA031 | t091 | + | | | | 1 |
| S. aureus FA034 | t084 | + | | | | 0 |
| S. aureus FA035 | t091 | + | + | | | 2 |
| S. aureus FA036 | t1045 | + | + | + | | 3 |
| S. aureus FA037 | t1045 | + | + | + | + | 4 |
| S. aureus FA039 | t127 | + | + | + | + | + | 5 |
| S. aureus FA040 | t939 | + | + | + | + | | 4 |
| S. aureus FA041 | t311 | + | + | + | + | | 4 |
| S. aureus FA043 | t127 | + | | | | + | 2 |
| S. aureus FA044** | t786 | | | | | | 0 |
| S. aureus FA045 | t091 | + | | | | 1 |
| S. aureus FA046 | t091 | + | | | | 1 |
| S. aureus FA047 | t127 | + | | | | + | 2 |
| S. aureus FA048 | t091 | + | | | | 1 |
| S. aureus FA049 | t127 | + | | | | 1 |
| S. aureus FA050 | t1154 | + | + | + | + | | 4 |
| S. aureus FA051 | t127 | + | | | | | 1 |
| S. aureus FA052 | t008 | + | | | | 1 |
| S. aureus FA053 | t091 | + | | | | 1 |

| No | 11 | 10 | 16 | 8 | 6 | 4 | 6 | 9 | 2 | 6 | 14 | 1 | 2 | 5 | 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % | 23 | 21 | 34 | 17 | 13 | 9 | 13 | 19 | 4 | 13 | 30 | 2 | 4 | 11 | 6 |

2 Note: *=Poultry Isolates

3 **=MRSA

4 %= % occurrence of each SE and PV1
Figure 1 (on next page)

Frequency of Spa Types in 47 S. aureus Isolates
Fig 1: Frequency of spa types occurrence in *47 S. aureus* isolates
Figure 2 (on next page)

Prevalence of Staphylococci Enterotoxins in Studied Isolates
Fig II: Prevalence of staphylococcal enterotoxins in studied isolates
Figure 3 (on next page)

Association of Enterotoxins with Spa Types
Fig III: Association of enterotoxins with spa types