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Molecular Analysis of *Staphylococcus aureus* Infections in Trinidad and Tobago

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Authors' contributions

This work was carried out in collaboration between all authors. Author PEA designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors RR and SM managed the analyses of the study including the molecular analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Previous studies regarding *Staphylococcus aureus* in Trinidad and Tobago have so far been conducted mainly on methicillin resistant *S. aureus* (MRSA) isolates. Few reports are available regarding *S. aureus* infections in the country. This study was therefore designed to determine the unique molecular epidemiology and characteristics of *S. aureus* infections both in the community and hospitals in the country.
Materials and Methods: During a 10 month period, 385 persons who had infections caused by *S. aureus* were reviewed. Standardized questionnaires were utilized to obtain demographic data of the infected individuals from three major tertiary hospitals; and 309 *S. aureus* isolates recovered from these individuals were analysed using conventional and molecular microbiological methods including DNA microarray and multi locus sequence typing (MLST).
Results: Skin and soft tissue infections (SSTI) were the most prevalent type of *S. aureus* infections, followed by blood stream, urogenital tract and respiratory tract infections. Results also revealed that surgical, paediatric and medical wards experienced most of the *S. aureus* infections

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in a hospital setting or environment. The most prevalent *S. aureus* clonal complex (CC) associated with infections was CC8, which were methicillin sensitive and also positive for the Panton-Valentine leukocidin (*pvl*) genes - (CC8-MSSA-PVL$^+$). Generally, the *pvl* genes rate among the isolates was observed to be 47% while MRSA now stands at 13.6%. The most prevalent MRSA strains were ST239-MRSA III and ST8-MRSA IV (USA300).

Conclusions: There is a high diversity of *S. aureus* clonal complexes infections in the country and the *pvl* genes which were considered rare are now highly prevalent. Methicillin resistance though slightly higher than previously reported does not represent a significant increase. We propose that surveillance efforts should continue to be directed to monitor *S. aureus* infections in hospitals in the country so as to detect and eliminate any possibility of its outbreak early in the country as currently practiced in other countries.

Keywords: Staphylococcus aureus; MSSA, MRSA; clonal complexes; MLST; PVL; ST239-MRSA III; ST8-MRSA IV; USA300; Trinidad & Tobago.

1. INTRODUCTION

Staphylococcus aureus infection is prevalent worldwide and its distribution is compounded by spread due to local, regional and international travelling. Carriers of *S. aureus* are an essential factor in the epidemiology of the infection; and a much considerable risk factor for spread of hospital-associated and community-associated infections [1].

Molecular typing techniques are widely used in the epidemiological study of both methicillin-sensitive *S. aureus* (MSSA) and MRSA. The spread of certain clones of MRSA is well documented. However, the data on MSSA are not as extensive as that available for MRSA, and therefore limited knowledge is available regarding community setting methicillin-sensitive *S. aureus* clonal structure and epidemiological characteristics [2].

Although data regarding *S. aureus* for different developed countries on MSSA and MRSA infections exists, there is paucity of information in developing countries including Trinidad and Tobago and the Caribbean, for which few reports are available [3]

In Trinidad and Tobago, a few studies on *S. aureus* have been conducted, most of which concentrated on MRSA isolates [4,5,6]. In 2010, Akpaka PE et al reported a case study of a boy aged 13 with *S. aureus* infection who died 48 hours after admission in spite of being cared for at the country's premier intensive care facility. The *S. aureus* involved in this case belonged to a PVL-positive CC8-MSSA-PVL$^+$ strain [7]. Since this report, there has not been any follow up studies to determine the presence and prevalence of *S. aureus* virulence genes or toxins causing infections in the country except one carried out by Monecke S et al. [8].

This present study was carried out to identify the epidemiological risk factors associated with *S. aureus* infections in hospitals and community settings and also use molecular tool to characterize the virulence (toxins and proteins) genes commonly encountered in *S. aureus* isolates involved in both community and hospital associated infections in Trinidad and Tobago.

2. MATERIALS AND METHODS

This observational cross-sectional study of *S. aureus* infection was carried out over a 10 month period from August 2011 to May 2012 at three regional health authority hospitals located in this twin island country of Trinidad & Tobago. Two of these hospitals (located in the north and south regions of the country) have admission capacities of over 600 beds and serving over 600,000 individuals in the population while the third has an admission capacity of 150 serving over 55,000 individuals in the population.

Three hundred and eighty five (385) suspected *S. aureus* infected cases during the period were reviewed using standardized questionnaires. On final count, only 309 cases were included in the analysis because medical information and the recovered *S. aureus* isolate were unavailable for molecular analysis.

2.1 Bacterial Isolation and Patients' Characteristics

S. aureus were isolated from clinical specimen routinely submitted on behalf of these patients. The specimens were processed using standard microbiological methods [9]. A standardized questionnaire was used to extract demographic data from the medical records of each patient who had confirmed *S. aureus* infections based on clinical and laboratory data. Clinical evidence of inflammatory processes (elevated white cell

count, C-reactive protein and pus in cases of skin and soft tissue infections) and isolation of *S. aureus* from cultured clinical specimens sent to the microbiology laboratory were inclusion criteria used for the cases for the study. Other data extracted included hospital facility, gender, age, any pre-existing condition (such as diabetes, hypertension and heart disease); body site and specimen yielding the bacterial isolate, risk factors for infection (such as prolonged hospitalization, transplant recipient, intra-abdominal surgery and previous antibiotic treatment). Other information included diagnosis, date of onset of symptoms, presenting symptoms as well as treatment outcome, *i.e*., whether the patient recovered, died or was transferred. For ease of reference, the cases of *S. aureus* infections were categorized into hospital or community associated based on the location of the patient as at the time the clinical specimen were submitted to the laboratory. Those patients who were admitted and were being treated in the hospital settings or environment were regarded as hospital associated and the others were community associated if such cases had not been admitted into the hospital within the last 6 or more months.

2.2 DNA Microarray

Molecular analysis of the bacterial isolates were analysed at the Dresden University of Technology, Dresden Germany. The identification as *S. aureus* was confirmed by DNA Microarray using Genotyping kit (Alere Technologies GmbH, Germany). This kit allows DNA-based detection of resistance genes, pathogenicity markers of *S. aureus* and assignment of unknown *S. aureus* isolates to known strains. The target set includes various species markers, toxin-, virulence- and antibiotic resistance genes, microbial surface components recognizing adhesive matrix molecules (MSCRAMMS), enzymes and other types of markers. These procedures were carried out as previously reported [10].

2.3 Multi-Locus Sequence Typing (MLST)

Multi-locus sequence typing for *S. aureus* procedure was performed as developed and reported by Mark Enright [11] using the public database provided at http://saureus.mlst.net/.

2.4 Statistical Analysis

The chi-squared test and Fisher's exact test were used as appropriate to compare data from different groups. The data were descriptive and were reported as comparisons of frequency distributions. *P* values <0.05 were considered statistically significant.

3. RESULTS

Of the 309 *S. aureus* infections analysed, 78% (241) occurred within a hospital setting or environment while 68 (22%) occurred among patients from the community as depicted on Table 1. The difference between the hospital and community associated infections were statistically significant (p = 0.001). Gender distribution of the infections was not statistically different among males and females as 54.4% infections occurred in males and 45.6% in females (p= 0.3). The age distribution of the patients with *S. aureus* infections as shown on Table 1 revealed that pediatric patients under 10 years old experienced the most frequent *S. aureus* infections, accounting for 29.4%.

S. aureus infections that occurred on the surgical ward were most prevalent 43.6% (105/241). This was followed by cases on paediatric wards 27.8% (67/241), medical 19.8% (48/241), intensive care units 5.8% (14/241) and obstetrics and gynaecology being the least with 2.9% (7/241). As depicted on Table 2, skin and soft tissue infections (SSTI) was the most common type of *S. aureus* infections which accounted for 73.8% (228/309) of the cases. Other types included bloodstream infections 11% (34/309), urogenital tract infections 8.7% (27/309), respiratory tract infections 6.2% (19/309) and the last and the least, central nervous system infection 0.3% (1/309) that was a case of meningitis in the intensive care unit (ICU). When these infections types occurring in the hospital settings were compared with the community settings infections, there was a significant difference in skin and soft tissue infections, urogenital tract infections and respiratory tract infection types.

Diabetes mellitus and hypertension were the most prevalent pre-existing diseases observed among patients with *S. aureus* infections in this study. This accounted for 13.6% (42/309) and 5.8% (18/309) cases respectively. Other suspected risk factors involved included prolonged hospitalization 8.7% (27/309), previous antibiotic treatment 8.0% (25/309) and serious illness 4.5% (14/309). There were only 2 cases of death from the *S. aureus* infections, majority recovered and a couple were transferred.

Only less than 26% of all the accessory gene regulator alleles of *S. aureus* strains were involved in the infections but 66% (203/309) of the *agr*I were observed in the infections by the organism. This was followed by *agr*IV (26%), *agr*III (14%) and *agr*II (13%). Six isolates could not be assigned to any of the four *agr* groups. Toxic shock syndrome toxin (*tst*1) was very low; 2.6% (8/309). The enterotoxins such as *seb*, *seq*, *sek* which have been known to form pathogenicity islands as well as to occur individually were all rare. Among the leukocidins however, the *PVL* genes (*lukF/S-PV*) had a prevalence of 47% (144/309). Isolates carrying the *PVL* genes were highly associated with *agr*I and *agr*IV. Other high prevalence rates were seen for the staphylokinase gene *sak,* 86% (265/309), the staphylococcal complement inhibitor gene *scn,* 92% (283/309) and adhesion factor *sas*G, 67% (208/309).

Fifty-four percent (54%) of all isolates carried the *cap*5 gene while the prevalence of *cap*8 was 44% (P >0.05). Six isolates were non-typeable for *cap*1, *cap*5 and *cap*8. These isolates were also non-typeable for *agr* and they were assigned to the *S. argenteus*-lineage. Among the *PVL* positive infections 76% were *cap*5 while 22% were *cap*8. The *agr* groups and capsule types varied highly and thus no distinction was made to any type infections in either the hospital or community settings although the types of infections are well associated to clonal complexes. There was an association of 98% of infections having the same capsule types in strains. The exceptions consisted of one infection in CC5 encoding *cap*8, 1 isolate in CC15-MSSA encoding *cap*5; 3 isolates in CC30-MSSA (PVL^+) encoding *cap*5 and 2 isolates in CC8-MSSA (PVL^+) encoding *cap*8 (data not shown). It is noteworthy that among genes associated with virulence *see* and *etD* were not encountered. The genes present showed much variation between type of *S. aureus* infections and their expected occurrence or prevalence with those occurring 50% and more in hospital settings being *agr*I, *lukF/S-PV*, *sak*, *scn* and *sasG*. These followed the general pattern of greatest frequencies in infections that occurred in surgical, paediatric, medical, ICU and obstetrics and gynaecology wards. Notably no distinct association was seen among *lukF/S-PV* (P >0.05).

Fifty percent of the community setting infections were PVL-positives. Clonal complex and community setting infections were also statistically insignificant.

If compared with their occurrence in MRSA isolates, the MSSA isolates genes associated with virulence (as in Table 3) such as *agrII, agrIII, seb, sed, sej, ser* and *egc* produced statistically significant differences; p-values (P <0.05). With reference to association of genes with virulence in MRSA isolates, the genes *agrI, sea, sek/q* and *sasG* produced significant p-values (P <0.05). Of all 309 isolates tested, 13.6% were *mecA* positive. Very few virulent genes were found including the absence of the PVL gene. They also did not encode the capsule types investigated, *cap5* or *cap8*. Among virulent markers some enterotoxins were found such as the *egc* cluster (*seg* and *sei*) and *seb* were absent.

Table 1. Age group distribution of 309 patients with *S. aureus* infections in Trinidad and Tobago

Age group (in years)	N (%)	Hospital associated N (%)	Community associated N (%)	p value
0 – 9	91 (29.4)	81 (33.6)	10 (14.7)	0.001
10 – 19	19 (6.1)	16 (6.6)	3 (4.4)	0.3
20 – 29	15 (4.9)	9 (3.7)	6 (8.8)	0.1
30 – 39	32 (10.4)	19 (7.9)	13 (19.1)	0.02
40 – 49	43 (13.9)	32 (13.3)	11 (16.2)	0.5
50 – 59	51 (16.5)	41 (17)	10 (14.7)	0.6
60 – 69	36 (11.7)	26 (10.8)	10 (14.7)	0.4
70 +	22 (7.1)	17 (7.1)	5 (7.4)	1.4
Total	309	241 (88)	68 (22)	0.001

N = Total number analysed

4. DISCUSSION

One of the main foci of this study was identifying the epidemiological risk factors associated with *S. aureus* infections in both hospital and community settings in some major regional hospitals in Trinidad & Tobago. Usually with community settings, colonization incidence is usually investigated by use of nasal swabs, but a relatively small sample size of community setting infections were included as samples were only collected when patients presented themselves to healthcare institutions rather than having obtained samples directly from the community.

In this study, age-group was positively detected as an epidemiological risk factor in *S. aureus* infections that occurred in hospital settings or environment. *S. aureus* infections were most common in the paediatrics age group 0-9. It has been reported that age is a non-essential risk factor for *S. aureus* infection but rather underlying infections and functional debility is to be considered [12]. This high prevalence of *S. aureus* infections in this study among age group 0–9 could be attributed to a lack of staphylococcal components recognition in infections in babies and young children by the body's immune system as reported by Fournier 2005 [13] as well as in inadequately developed immune systems.

Majority of the *S. aureus* infections occurred in the surgical wards and this may be because some of the surgical procedures may end up as nosocomial infections [14] of which *S. aureus* is a main aetiological agent. Although the *S. aureus* infections were not generally observed to be high among individuals from the community settings, but surgery as a risk factor has been highlighted as a means by which *S. aureus* is introduced in homes [15]. It has also been reported that patients undergoing surgical procedures had a 1.5 fold higher risk of MRSA acquisition than those on other wards [16]. Similar to previous report of study conducted by Akpaka et al in the country in 2006, most of the MRSA infections were derived from the surgical ward [5]. Skin and soft tissue infections was the most frequent types of *S. aureus* infections in this current study. Again this is in agreement with previous report in the country by Akpaka et al. [5]. This may be so because the skin are the prime natural habitat of staphylococci and the most frequent site and common carriage and entry to a health institution as well as there is a high incidence of swabbing wounds after medical procedures.

The prevalence of MRSA was evaluated and was found to be 13.6%. This is minimally higher than the 12.8% reported by Akpaka et al. [5], though the increase is not statistically significant. However this rate is considerably lower than the 25% average found in many countries [15]. The MRSA frequency in this study represents a noticeable increase seen from Swanston in 1999, who reported a 4.6% rate [4]. Orrett (2006) conducted a study which resulted in a MRSA prevalence of 20.8% [6]. The study however was carried out over a long period and consisted of a considerable sample size but all its infections were from one study site. This factor may account for the vast difference in MRSA prevalence found within different studies. The majority of MRSA was found to be isolated from the surgical ward infections which is in agreement with that reported by Akpaka et al. [5]. The risk of acquisition of MRSA was more common if the patient was in the surgical or medical wards and being within the age group 50-59. This is in agreement with previous work reported by Akpaka et al which states that surgical MRSA infections have been linked to patients that have spent prolonged periods on ICU [5], which provided the third most MRSA isolates in this study. It has been reported that patients with MRSA tend to be older, would have had more chronic illnesses, history of recent hospitalization [12] as well as history of antibiotic usage [17]. The highly prevalent CC5-MSSA was also observed amongst surgical ward infections. This clonal complex is known to be common and widespread [18]. It has also been reportedly associated with hematogenous infections [19], and surgical procedures can produce a viable channel for transmission which is also highly possible when surgery is being conducted [19].

Diabetes was found to be the major pre-existing disease which patients with *S. aureus* had. It also accounted for 50% of *S. aureus* cases in patients with multiple pre-existing diseases. This is not surprising because the prevalence of Diabetes has been noted to be high in this country [20] *S. aureus* infections have been known to be implicated in diabetic foot ulcers [21] and as such would account for it being the most prevalent pre-existing disease encountered. Other risk factors such as prolonged hospitalization, previous antibiotic treatment and serious illness have been positively associated with *S. aureus* infection. Other literatures have previously also cited these factors in the elderly, for which the prevalence of *S. aureus* is unknown and children (15.3%) [22]. It is noteworthy that 100% of all

cases of sepsis were community acquired and can be as a result of untreated infections in the community. Seventy five percent (75%) of these were attributed to MSSA which is similar to findings in different countries as reported by Naber 2009 [23].

Table 2. Distribution of *S. aureus* types of infections in Trinidad and Tobago (%)

Type of infection	N (%)	Hospital associated N (%)	Community associated N (%)	*p* value
SSTI	228 (73.8)	184 (80.7)	44 (19.3)	0.001
BSI	34 (11.0)	17 (50.0)	17 (50)	1.0
UGTI	27 (8.7)	20 (74.1)	7 (25.9)	0.001
RTI	19 (6.2)	17 (89.5)	2 (10.5)	0.001
CNSI	1 (0.3)	1 (100)	0 (0)	0
Total	309 (100)	239 (77.3)	70 (22.7)	0.001

SSTI = Skin and Soft Tissue Infection; BSI = Bloodstream Infections; UGTI = Urogenital Tract Infections; RTI = Respiratory Tract Infections; CNSI = Central Nervous System Infections

Table 3. Distribution of hospital associated *S. aureus* infections showing virulence genes of the strains, infection types and methicillin susceptibility

Genes	N (%)	BSI	CNSI	RTI	UGTI	SSTI	MSSA	MRSA
*agr*I	203 (65.7)	22	1	15	15	150	162	41
*agr*II	38 (12.3)	3	0	2	5	28	37	1
*agr*III	43 (13.9)	6	0	0	4	33	43	0
*agr*IV	76 (24.6)	8	0	8	10	50	62	14
agr-	2 (0.6)	0	0	0	0	2	2	0
*tst*1	8 (2.6)	1	0	1	0	6	8	0
sea	30 (9.7)	6	0	3	3	18	16	14
sea N315/*sep*	15 (4.8)	I	0	1	5	8	15	0
seb	33 (10.7)	4	0	2	4	23	31	2
sec/sel	22 (7.1)	2	0	1	2	17	21	1
sed	67 (21.7)	7	1	3	1	55	64	3
see	0 (0)	0	0	0	0	0	0	0
seh	10 (3.2)	3	0	0	2	5	10	0
sej	68 (22)	8	1	3	1	55	65	3
sek/q	113 (36.6)	13	1	9	6	84	74	39
ser	67 (21.7)	8	1	3	1	54	65	2
egc	106 (34.3)	2	0	1	2	18	104	2
ORF CM14	19 (6.1)	1	0	2	4	12	19	0
lukF/S-PV	144 (46.6)	12	1	5	5	121	125	19
sak	265 (85.7)	30	1	14	23	197	231	34
chp	184 (59.5)	17	1	10	15	141	163	21
scn	283 (91.5)	31	1	17	23	211	247	36
*et*A	12 (3.8)	2	0	1	1	8	12	0
*et*B	8 (2.6)	0	0	0	1	7	8	0
*et*D	0 (0)	0	0	0	0	0	0	0
*edin*A	14 (4.5)	1	0	0	0	13	14	0
*edin*B	17 (5.5)	1	0	1	0	15	17	0
*edin*C	7 (2.2)	0	0	0	1	6	7	0
ACME	18 (5.8)	1	0	1	2	14	1	17
cna	134 (43.3)	16	0	10	13	95	113	21
*sas*G	208 (67.3)	26	1	13	17	151	165	43
*cap*5	170 (55.0)	16	1	8	13	132	146	23
*cap*8	138 (44.6)	16	0	11	13	98	117	22
cap-	6 (1.9)	1	0	0	1	4	6	0

N=Total number identified; BSI = Bloodstream infections; CNSI = Central Nervous System infections; RTI = Respiratory tract infections; UGTI = Urogenital tract infections; SSTI = Skin and soft tissue infection; MSSA = methicillin susceptible S. aureus; MRSA = methicillin resistant S. aureus

Variability of clonal complexes as well as prevalent clonal types in Trinidad and Tobago are comparable to that found in the USA by O'Hara et al. [8,24]. Clonal complex 8 was the most common clonal type as also found in Germany, Pakistan and most Asian countries [2,25]. CC8-MSSA, PVL^+ was found to be the most prevalent strain, however this is not in agreement with previously reported results found by Akpaka 2011 whose findings suggests the presence of PVL among CC8-MSSA is rare [7,8]. This indicates a possible rise in PVL positive strains. The prevalence however of PVL in other Caribbean countries and Latin America is unknown though the toxin has been found in the region [26]. It is therefore not possible to make an association between preponderance in the region and a possible isolated outbreak. However high PVL prevalence have been reported in West and Central Africa [27] and thus its prevalence found in this study can be possibly attributed to the importation of African slaves from primarily West Africa. This is in concordance with our findings of MSSA strains which belong to West African lineages. These included ST72, CC152 and possibly the 'alien' strain belonging to the *S. argenteus*-lineage and ST1852 for which data is outstanding [28]. It is noteworthy however, that given the time elapsed since this importation has occurred, high *PVL* prevalence should be attributed to high tourism experienced in the country.

MRSA strains encountered in this study belonged only to a small number of distinct clones. This was in agreement with Yun Cha et al 2005 who reported findings of phylogenetic studies that suggests the global occurrence of MRSA infection are due to a few epidemic MRSA clones [29]. Notably many of the well-known pandemic strains were not found in Trinidad and Tobago such as the Paediatric clone (ST5-MRSA IV), New York/Japan clone (ST5-MRSA II), Iberian (ST247-MRSA IA) and Chilean clone (ST5-MRSA I). However, the second most frequent MRSA strains was ST8-MRSA IV (USA300), 40% of which harbored the ACME cluster. This strain was, however, the most common among CA-MRSA isolates. Chroboczek et al 2013 found among a few MRSA isolates studied from Trinidad and Tobago, USA300 also was the second MRSA strain most encountered. These were also detected in Jamaica and Martinique, while Hispanics/Latin Americans have another CC8-MRSA-IV that also is *PVL* positive but ACME negative (and another subtype of SCC*mec*IV) [30].

Presently, the prevalence of USA300 in Trinidad and Tobago appears to be increasing as in a previous study 3.75% (3/80) MRSA infections belonged to that strain [24]; while in this current study we found 40.5% (17/42) of MRSA infections. Local high prevalence of USA300 (CC8-MRSA-IV with PVL+ and ACME) is definitely from North America with direct transfer from North American visitors. Previous reports have indicated that most of Trinidad and Tobago's tourists are from the USA and to a lesser extent only from other Caribbean counties [30]. Trinidad and Tobago is known to attract large numbers of tourists each year and this factor may account for the high diversity among *S. aureus* strains.

In this study type of *S. aureus* infections was observed to be associated with certain strains most of which were CC8-MSSA, PVL^+. These included blood, respiratory tract and SSTI. This was typical since this strain was the most prevalent strain found. Urogenital tract however differed and the greatest incidences were observed among CC5-MSSA, CC8-MSSA and CC12-MSSA. Considering the fact that urogenital tract infections was small, it is unclear whether a well-defined divergence from the norm is being exhibited or whether urogenital tract isolates are particularly associated with different strains than those most commonly found. Most studies on clonal complexes and their accompanying strains have been conducted on MRSA isolates, therefore comparison of the body site of the infection and isolates from previous findings was not possible.

Among genders, CC15-MSSA and CC152-MSSA, prevalence was detected as being greater in males than females. It has been reported though that in males the general carriage and colonization by *S. aureus* is higher than in females [31,32,33]. Ruimy et al 2008 also reported similar findings in Mali in which the two aforementioned strains contributed 52.3% of isolates from nasal *S. aureus* isolates [34], thereby accounting for the observed differences among genders with regard to CC15-MSSA and CC152-MSSA, PVL^+.

The characterization of genes and proteins found in diabetic patient infections were also analysed to assess a possible association between *S. aureus* and the most common pre-existing disease, diabetes. Though the sample size was small, among resistant genes, *mecA* and *sat* genes were considerably higher in this cohort

when compared to findings of the total study. The higher occurrence of *mecA* among these may not be typical among diabetes although isolated cases or situation in a previous study conducted on MRSA by Monecke et al. [35], the *sat* gene was found to have a high prevalence among a group of 100 infections in which those derived from diabetic foot ulcers were greatest in number. Among virulent genes, high prevalence in genes were observed as in the entire study. However, *agrI* which was equally prevalent was not encountered in infections as expected while *pvl* was also considerably lower. The clonal complexes observed among diabetic patient with *S. aureus* infections were spread among 5 lineages, the most common or prevalent was CC8 and ST239-MRSA III.

There was a high diversity of clonal complexes most of which belonged to CC8. The most prevalent strain found was CC8-MSSA, PVL^+. PVL which was previously thought to be rare but now present at a rate of 47% and bears no significant association with MRSA or MSSA infection. There was a prevalence of 13.6% of infections harbouring the *mecA* gene, most of which again belonged to CC8. The most prevalent MRSA strains were the Brazilian clone, ST239-MRSA III and its variants as well as PVL/ACME + ST8-MRSA IV, otherwise known as USA300, and its variants.

5. CONCLUSIONS

Among virulence genes, *sak*, *scn* and *sasG* had high prevalences. The distribution of *cap5* and *cap8* was found to be almost homogenous as 54% of infections encoded the *cap5* gene and 44% encoded *cap8*. The most common virulent genes among *S. aureus* infections in both the hospital or community settings were *agrI*, *lukF/S-PV*, *sak, scn* and *sasG*.

Surgical, paediatric and medical wards provided the majority of the *S. aureus* infections in the hospital settings as well as SSTI in both MRSA and MSSA infections. Diabetes mellitus was found to be the most common pre-existing disease patients in *S. aureus* infections together with prolonged hospitalization and previous antibiotic treatment.

Multi locus sequence typing was conducted on unique *S. aureus* strains for which most of the *S. aureus* bore similarities to ST2250/2277 that has now been assigned to *S. argenteus*. Virulence markers were found including enterotoxins such as the *egc* cluster (e.g. *seg, sei, sem, sen, seo, seu*). PVL however was absent. These isolates did not harbour any of the capsule types investigated and they also did not yield signals for *agr* probes. It was remarkable to identify these lineages here, as not much is known on their provenance and geographic distribution in our region. They are known mainly from Australia, South East Asia and Central / West Africa. All these diversity of factors associated with *S. aureus* infections in the Trinidad and Tobago means that increased surveillance efforts should continue to be focused at hospital settings to monitor *S. aureus* infections in order to detect and eliminate any possibility of its outbreak in the country as currently practiced in other countries.

ETHICAL APPROVAL

Ethical approval for this study was granted by the Ethics Committee, The University of the West Indies, St. Augustine and written permissions were also obtained from the health care authorities where the studies were carried out. No consent was needed from patients as there was no contact with any of them and information obtained was not traceable to individuals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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