<u>Original Research Article</u> Molecular analysis of *Staphylococcus aureus* Infections in Trinidad and Tobago

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ABSTRACT

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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 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.

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

10 MRSA III, ST8-MRSA IV, U11 **1. INTRODUCTION**

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13 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

15 essential factor in the epidemiology of the infection; and a much considerable risk factor for

- 16 spread of hospital-associated and community-associated infections [1].
- 17 Molecular typing techniques are widely used in the epidemiological study of both methicillin-
- 18 sensitive *S. aureus* (MSSA) and MRSA. The spread of certain clones of MRSA is well
- 19 documented. However, the data on MSSA are not as extensive as that available for MRSA,
- 20 and therefore limited knowledge is available regarding community setting methicillin-
- 21 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].

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26 In Trinidad and Tobago, a few studies on S. aureus have been conducted, most of which 27 concentrated on MRSA isolates [5, 6]. In 2010, Akpaka PE et al reported a case study of a 28 boy aged 13 with S. aureus infection who died 48 hours after admission in spite of being 29 cared for at the country's premier intensive care facility. The S. aureus involved in this case 30 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 31 genes or toxins causing infections in the country except one carried out by Monecke S et al 32 33 [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.

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40 2. MATERIAL AND METHODS

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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.

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53 Bacterial isolation and Patients' Characteristics

54 S. aureus were isolated from clinical specimen routinely submitted on behalf of these 55 patients. The specimens were processed using standard microbiological methods [9]. A 56 standardized questionnaire was used to extract demographic data from the medical records 57 of each patient who had confirmed S. aureus infections based on clinical and laboratory 58 data. Clinical evidence of inflammatory processes (elevated white cell count, C-reactive 59 protein and pus in cases of skin and soft tissue infections) and isolation of S. aureus from 60 cultured clinical specimens sent to the microbiology laboratory were inclusion criteria used 61 for the cases for the study. Other data extracted included hospital facility, gender, age, any 62 pre-existing condition (such as diabetes, hypertension and heart disease); body site and specimen vielding the bacterial isolate, risk factors for infection (such as prolonged 63 hospitalization, transplant recipient, intra-abdominal surgery and previous antibiotic 64 65 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 66 67 transferred. For ease of reference, the cases of S. aureus infections were categorized into 68 hospital or community associated based on the location of the patient as at the time the 69 clinical specimen were submitted to the laboratory. Those patients who were admitted and 70 were being treated in the hospital settings or environment were regarded as hospital 71 associated and the others were community associated if such cases had not been admitted 72 into the hospital within the last 6 or more months.

74 **DNA Microarray**

75 Molecular analysis of the bacterial isolates were analysed at the Dresden University of

76 Technology, Dresden Germany, The identification as S. aureus was confirmed by DNA

77 Microarray using Genotyping kit (Alere Technologies GmbH, Germany). This kit allows DNA-

78 based detection of resistance genes, pathogenicity markers of S. aureus and assignment of

79 unknown S. aureus isolates to known strains. The target set includes various species

80 markers, toxin-, virulence- and antibiotic resistance genes, microbial surface components 81 recognizing adhesive matrix molecules (MSCRAMMS), enzymes and other types of

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markers. These procedures were carried out as previously reported [10].

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84 Multi-Locus Sequence Typing (MLST)

85 Multi-locus sequence typing for S. aureus procedure was performed as developed and 86 reported by Mark Enright [11] using the public database provided at http://saureus.mlst.net/.

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88 **Statistical analysis**

89 The chi-squared test and Fisher's exact test were used as appropriate to compare data from 90 different groups. The data were descriptive and were reported as comparisons of frequency 91 distributions. P values <0.05 were considered statistically significant.

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93 **Ethical Approval**

94 Ethical approval for this study was granted by the Ethics Committee, The University of the 95 West Indies, St. Augustine and written permissions were also obtained from the health care 96 authorities where the studies were carried out. No consent was needed from patients as 97 there was no contact with any of them and information obtained was not traceable to 98 individuals.

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100 **3. RESULTS**

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102 Of the 309 S. aureus infections analysed, 78% (241) occurred within a hospital setting or 103 environment while 68 (22%) occurred among patients from the community as depicted on 104 Table 1. The difference between the hospital and community associated infections were 105 statistically significant (p = 0.001). Gender distribution of the infections was not statistically 106 different among males and females as 54.4% infections occurred in males and 45.6% in 107 females (p=0.3). The age distribution of the patients with S. aureus infections as shown on 108 Table 1 revealed that pediatric patients under 10 years old experienced the most frequent S. aureus infections, accounting for 29.4%. 109

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111 S. aureus infections that occurred on the surgical ward were most prevalent 43.6% 112 (105/241). This was followed by cases on paediatric wards 27.8% (67/241), medical 19.8% 113 (48/241), intensive care units 5.8% (14/241) and obstetrics and gynaecology being the least 114 with 2.9% (7/241). As depicted on Table 2, skin and soft tissue infections (SSTI) was the 115 most common type of S. aureus infections which accounted for 73.8% (228/309) of the 116 cases. Other types included bloodstream infections 11% (34/309), urogenital tract infections 117 8.7% (27/309), respiratory tract infections 6.2% (19/309) and the last and the least, central 118 nervous system infection 0.3% (1/309) that was a case of meningitis in the intensive care 119 unit (ICU). When these infections types occurring in the hospital settings were compared 120 with the community settings infections, there was a significant difference in skin and soft 121 tissue infections, urogenital tract infections and respiratory tract infection types 122

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Table 1: Age group distribution of 309 patients with S. aureus infections in Trinidad

and Tobago

	Age Group	N <mark>(%)</mark>	Hospital Associated	Community Associated	p value				
	(in years)		<u>N (%)</u>	<mark>N (%)</mark>					
	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		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=	N=Total number analysed								

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

Type of infection	N <mark>(%)</mark>	Hospital associated <mark>N (%)</mark>	Community associated N (%)	<i>p</i> 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

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.

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

Genes	N <mark>(%)</mark>	BSI	CNSI	RTI	UGTI	SSTI	MSSA	MRSA
<i>agr</i> l	203(65.7)	22	1	15	15	150	162	41
<i>agr</i> ll	38(12.3)	3	0	2	5	28	37	1
agrlll	43(13.9)	6	0	0	4	33	43	0
<i>agr</i> lV	76(24.6)	8	0	8	10	50	62	14
a <i>gr</i> -	2(0.6)	0	0	0	0	2	2	0
tst1	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
etA	12(3.8)	2	0	1	1	8	12	0
etB	8(2.6)	0	0	0	1	7	8	0
etD	0(0)	0	0	0	0	0	0	0
edinA	14(4.5)	1	0	0	0	13	14	0
edinB	17(5.5)	1	0	1	0	15	17	0
edinC	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
sasG	208(67.3)	26	1	13	17	151	165	43
cap5	170(55.0)	16	1	8	13	132	146	23
cap8	138(44.6)	16	0	11	13	98	117	22
cap-	6(1.9)	1	0	0	1	4	6	0

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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*; MSSA = methicillin
 resistant *S. aureus*

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155 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 agrl were observed in the infections by 156 157 the organism. This was followed by agrIV (26%), agrIII (14%) and agrII (13%). Six isolates 158 could not be assigned to any of the four agr groups. Toxic shock syndrome toxin (tst1) was very low; 2.6% (8/309). The enterotoxins such as seb, seq, sek which have been known to 159 160 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). 161 Isolates carrying the PVL genes were highly associated with agrl and agrlV. Other high 162 prevalence rates were seen for the staphylokinase gene sak, 86% (265/309), the 163 staphylococcal complement inhibitor gene scn, 92% (283/309) and adhesion factor sasG, 164 165 67% (208/309).

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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 having the same capsule to capsule types. There was an association of 98% of infections having the same capsule

174 types in strains. The exceptions consisted of one infection in CC5 encoding cap8, 1 isolate in 175 CC15-MSSA encoding cap5; 3 isolates in CC30-MSSA (PVL⁺) encoding cap5 and 2 isolates 176 in CC8-MSSA (PVL⁺) encoding *cap*8 (data not shown). It is noteworthy that among genes 177 associated with virulence see and etD were not encountered. The genes present showed 178 much variation between type of S. aureus infections and their expected occurrence or 179 prevalence with those occurring 50% and more in hospital settings being agrl, lukF/S-PV, 180 sak, scn and sasG. These followed the general pattern of greatest frequencies in infections 181 that occurred in surgical, paediatric, medical, ICU and obstetrics and gynaecology wards. 182 Notably no distinct association was seen among lukF/S-PV (P >0.05). 183 184 Fifty percent of the community setting infections were PVL-positives. Clonal complex and

185 community setting infections were also statistically insignificant. 186 If compared with their occurrence in MRSA isolates, the MSSA isolates genes associated 187 with virulence (as in Table 3) such as agrII, agrIII, seb, sed, sej, ser and egc produced 188 statistically significant differences; p-values (P < 0.05). With reference to association of 189 genes with virulence in MRSA isolates, the genes agrl, sea, sek/g and sasG produced significant p-values (P < 0.05). Of all 309 isolates tested, 13.6% were mecA positive. Very 190 191 few virulent genes were found including the absence of the PVL gene. They also did not 192 encode the capsule types investigated, cap5 or cap8. Among virulent markers some 193 enterotoxins were found such as the egc cluster (seg and sel) and seb were absent. 194

195 4. DISCUSSIONS

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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.

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205 In this study, age-group was positively detected as an epidemiological risk factor in S. 206 aureus infections that occurred in hospital settings or environment. S. aureus infections were 207 most common in the paediatrics age group 0-9. It has been reported that age is a non-208 essential risk factor for S. aureus infection but rather underlying infections and functional 209 debility is to be considered [12]. This high prevalence of S. aureus infections in this study 210 among age group 0–9 could be attributed to a lack of staphylococcal components 211 recognition in infections in babies and young children by the body's immune system as 212 reported by Fournier 2005 [13] as well as in inadequately developed immune systems.

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214 Majority of the S. aureus infections occurred in the surgical wards and this may be because 215 some of the surgical procedures may end up as nosocomial infections [14] of which S. 216 aureus is a main aetiological agent. Although the S. aureus infections were not generally 217 observed to be high among individuals from the community settings, but surgery as a risk 218 factor has been highlighted as a means by which S. aureus is introduced in homes [15]. It 219 has also been reported that patients undergoing surgical procedures had a 1.5 fold higher 220 risk of MRSA acquisition than those on other wards [16]. Similar to previous report of study 221 conducted by Akpaka et al in the country in 2006, most of the MRSA infections were derived 222 from the surgical ward [5]. Skin and soft tissue infections was the most frequent types of S. 223 aureus infections in this current study. Again this is in agreement with previous report in the 224 country by Akpaka et al [5]. This may be so because the skin are the prime natural habitat of 225 staphylococci and the most frequent site and common carriage and entry to a health 226 institution as well as there is a high incidence of swabbing wounds after medical procedures. 227 The prevalence of MRSA was evaluated and was found to be 13.6%. This is minimally 228 higher than the 12.8% reported by Akpaka et al (2006) [5], though the increase is not 229 statistically significant. However this rate is considerably lower than the 25% average found 230 in many countries [15]. The MRSA frequency in this study represents a noticeable increase 231 seen from Swanston in 1999, who reported a 4.6% rate [4]. Orrett (2006) conducted a study 232 which resulted in a MRSA prevalence of 20.8% [6]. The study however was carried out over 233 a long period and consisted of a considerable sample size but all its infections were from 234 one study site. This factor may account for the vast difference in MRSA prevalence found 235 within different studies. The majority of MRSA was found to be isolated from the surgical 236 ward infections which is in agreement with that reported by Akpaka et al (2006) [5]. The risk 237 of acquisition of MRSA was more common if the patient was in the surgical or medical wards 238 and being within the age group 50-59. This is in agreement with previous work reported by 239 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 240 241 this study. It has been reported that patients with MRSA tend to be older, would have had 242 more chronic illnesses, history of recent hospitalization [12] as well as history of antibiotic 243 usage [18]. The highly prevalent CC5-MSSA was also observed amongst surgical ward 244 infections. This clonal complex is known to be common and widespread [19]. It has also 245 been reportedly associated with hematogenous infections [20], and surgical procedures can 246 produce a viable channel for transmission which is also highly possible when surgery is 247 being conducted [20].

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249 Diabetes was found to be the major pre-existing disease which patients with S. aureus had. 250 It also accounted for 50% of S. aureus cases in patients with multiple pre-existing diseases. 251 This is not surprising because the prevalence of Diabetes has been noted to be high in this 252 country [21] S. aureus infections have been known to be implicated in diabetic foot ulcers 253 [22] and as such would account for it being the most prevalent pre-existing disease encountered. Other risk factors such as prolonged hospitalization, previous antibiotic 254 255 treatment and serious illness have been positively associated with S. aureus infection. Other 256 literatures have previously also cited these factors in the elderly, for which the prevalence of 257 S. aureus is unknown and children (15.3%) [23]. It is noteworthy that 100% of all cases of 258 sepsis were community acquired and can be as a result of untreated infections in the 259 community. Seventy five percent (75%) of these were attributed to MSSA which is similar to 260 findings in different countries as reported by Naber 2009 [24].

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262 Variability of clonal complexes as well as prevalent clonal types in Trinidad and Tobago are 263 comparable to that found in the USA by O'Hara et al 2012 [8, 25]. Clonal complex 8 was the 264 most common clonal type as also found in Germany, Pakistan and most Asian countries [2, 265 26]. CC8-MSSA, PVL⁺ was found to be the most prevalent strain, however this is not in 266 agreement with previously reported results found by Akpaka 2011 whose findings suggests 267 the presence of PVL among CC8-MSSA is rare [7, 8]. This indicates a possible rise in PVL 268 positive strains. The prevalence however of PVL in other Caribbean countries and Latin 269 America is unknown though the toxin has been found in the region [27]. It is therefore not 270 possible to make an association between preponderance in the region and a possible 271 isolated outbreak. However high PVL prevalence have been reported in West and Central 272 Africa [28] and thus its prevalence found in this study can be possibly attributed to the 273 importation of African slaves from primarily West Africa. This is in concordance with our 274 findings of MSSA strains which belong to West African lineages. These included ST72, 275 CC152 and possibly the 'alien' strain belonging to the S. argenteus-lineage and ST1852 for 276 which data is outstanding [29]. It is noteworthy however, that given the time elapsed since 277 this importation has occurred, high PVL prevalence should be attributed to high tourism 278 experienced in the country. 279

280 MRSA strains encountered in this study belonged only to a small number of distinct clones. 281 This was in agreement with Yun Cha et al 2005 who reported findings of phylogenetic 282 studies that suggests the global occurrence of MRSA infection are due to a few epidemic 283 MRSA clones [30]. Notably many of the well-known pandemic strains were not found in 284 Trinidad and Tobago such as the Paediatric clone (ST5-MRSA IV), New York/Japan clone 285 (ST5-MRSA II), Iberian (ST247-MRSA IA) and Chilean clone (ST5-MRSA I). However, the 286 second most frequent MRSA strains was ST8-MRSA IV (USA300), 40% of which harbored 287 the ACME cluster. This strain was, however, the most common among CA-MRSA isolates. 288 Chroboczek et al 2013 found among a few MRSA isolates studied from Trinidad and 289 Tobago, USA300 also was the second MRSA strain most encountered. These were also 290 detected in Jamaica and Martinique, while Hispanics/Latin Americans have another CC8-291 MRSA-IV that also is PVL positive but ACME negative (and another subtype of SCCmecIV) 292 [31]. 293

294 Presently, the prevalence of USA300 in Trinidad and Tobago appears to be increasing as in 295 a previous study 3.75% (3/80) MRSA infections belonged to that strain [25]; while in this 296 current study we found 40.5% (17/42) of MRSA infections. Local high prevalence of USA300 297 (CC8-MRSA-IV with PVL+ and ACME) is definitely from North America with direct transfer 298 from North American visitors. Previous reports have indicated that most of Trinidad and 299 Tobago's tourists are from the USA and to a lesser extent only from other Caribbean 300 counties [31]. Trinidad and Tobago is known to attract large numbers of tourists each year 301 and this factor may account for the high diversity among S. aureus strains. 302 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. 303 304 This was typical since this strain was the most prevalent strain found. Urogenital tract 305 however differed and the greatest incidences were observed among CC5-MSSA, CC8-306 MSSA and CC12-MSSA. Considering the fact that urogenital tract infections was small, it is 307 unclear whether a well-defined divergence from the norm is being exhibited or whether 308 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 [32; 33; 34]. 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 [35], thereby accounting for the observed differences among genders with regard to CC15-MSSA and CC152-MSSA, PVL⁺.

318 The characterization of genes and proteins found in diabetic patient infections were also 319 analysed to assess a possible association between S. aureus and the most common pre-320 existing disease, diabetes. Though the sample size was small, among resistant genes, 321 mecA and sat genes were considerably higher in this cohort when compared to findings of 322 the total study. The higher occurrence of *mecA* among these may not be typical among 323 diabetes although isolated cases or situation in a previous study conducted on MRSA by 324 Monecke et al 2005, the sat gene was found to have a high prevalence among a group of 325 100 infections in which those derived from diabetic foot ulcers were greatest in number [36]. 326 Among virulent genes, high prevalence in genes were observed as in the entire study. 327 However, agrl which was equally prevalent was not encountered in infections as expected 328 while pvl was also considerably lower. The clonal complexes observed among diabetic 329 patient with S. aureus infections were spread among 5 lineages, the most common or 330 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.

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340 5. CONCLUSIONS341

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 both the hospital or community settings were *agrl*, *lukF/S-PV*, *sak*, *scn* and *sasG*.

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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.

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352 Multi locus sequence typing was conducted on unique S. aureus strains for which most of 353 the S. aureus bore similarities to ST2250/2277 that has now been assigned to S. argenteus. 354 Virulence markers were found including enterotoxins such as the egc cluster (e.g. seg, sei, 355 sem, sen, seo, seu). PVL however was absent. These isolates did not harbour any of the 356 capsule types investigated and they also did not yield signals for agr probes. It was 357 remarkable to identify these lineages here, as not much is known on their provenance and 358 geographic distribution in our region. They are known mainly from Australia, South East Asia 359 and Central / West Africa. All these diversity of factors associated with S. aureus infections 360 in the Trinidad and Tobago means that increased surveillance efforts should continue to be 361 focused at hospital settings to monitor S. aureus infections in order to detect and eliminate 362 any possibility of its outbreak in the country as currently practiced in other countries. 363

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