Scientific Research Publishing

Advances in Microbiology, 2025, 15(9), 543-558
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Prevalence of *pvl* and *tsst*1 Virulence Genes in *Staphylococcus aureus* Clinical Isolates from Angré University Hospital in Abidjan, Côte d'Ivoire

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How to cite this paper: Obouayeba, D.N.C., Bahan, G.A.J., Coulibaly, G.E., Kofi, K.A.A., Coulibaly-Diallo, Y.M., Joseph, D.A. and Kacou-Ndouba, A. (2025) Prevalence of *pvl* and *tsst*1 Virulence Genes in *Staphylococcus aureus* Clinical Isolates from Angré University Hospital in Abidjan, Côte d'Ivoire. *Advances in Microbiology*, **15**, 543-558.
https://doi.org/10.4236/aim.2025.159035

Received: August 20, 2025
Accepted: September 22, 2025
Published: September 25, 2025

Abstract

Background: *Staphylococcus aureus* (*S. aureus*) is a major cause of serious human infections and produces multiple virulence factors. In Côte d'Ivoire, local molecular data remain scarce. This study evaluated the prevalence of *pvl* and *tsst*1 genes in clinical *S. aureus* isolates from Abidjan. **Methods**: Two hundred clinical isolates (100 MRSA: methicillin-resistant *S. aureus*; 100 MSSA: methicillin-susceptible *S. aureus*) were collected at Angré University Hospital (CHU Angré) between January 2022 and February 2024. Isolates were identified using phenotypic methods, the VITEK 2 system, and PCR confirmation targeting the *nuc* gene. The *pvl* and *tsst*1 genes were detected using conventional PCR. Virulence profiles were compared according to hospital department, MRSA/MSSA profile, and clinical severity classification. **Results**: The *pvl* gene was detected in 72.0% of MRSA and 60.5% of MSSA isolates. A double-positive profile ($pvl^+/tsst1^+$) was found in 54.6% of the isolates, particularly in critical care units. No significant difference was observed between MRSA and MSSA for *pvl* ($p = 0.344$) or *tsst*1 ($p = 0.286$). However, the presence of the *pvl* gene was strongly associated with clinical severity, with 90.09% of severe cases having the *pvl* gene ($p = 0.0002$). The *tsst*1 gene was more frequently found in non-severe cases (54%) than in severe cases (35%), but the difference was not significant ($p = 0.374$). **Conclusion**: The high prevalence of *pvl* and

*tsst*1 genes, including in MRSA strains, highlights the circulation of highly virulent *S. aureus* lineages in Abidjan. These findings emphasize the need for enhanced molecular surveillance as part of an integrated strategy to control *S. aureus* infections.

Keywords

Methicillin-Resistant *Staphylococcus aureus* (MRSA), Panton-Valentine Leukocidin (*PVL*), Toxic Shock Syndrome Toxin-1 (*TSST*-1), Virulence

1. Introduction

Staphylococcus aureus is a major opportunistic pathogen responsible for a wide spectrum of infections, ranging from mild skin lesions to sepsis and necrotizing pneumonia [1]. Its ability to adapt to antibiotics, particularly the emergence of methicillin-resistant strains (MRSA), makes it a major concern in both healthcare and community settings [2]. In Côte d'Ivoire, internal records from the Bacteriology unit at CHU Angré, one of the country's most recently established hospitals, indicate that *S. aureus* is the most frequently isolated gram-positive bacterium in both inpatient and outpatient samples. A study conducted at Cocody University Hospital (Abidjan) in 2011 reported an MRSA prevalence of 11.8% among clinical *S. aureus* isolates [3]. Since then, the local epidemiology has shifted. At CHU Angré, a higher proportion of MRSA, more than half of the isolates stored, was observed, which suggests a large circulation of resistant strains and increases the need to study the associated virulence profiles.

The virulence of *S. aureus* is mediated by several factors (enzymes, adhesins, and toxins). Two determinants are particularly notable: Panton-Valentine leukocidin (*PVL*), encoded by *pvl* genes, which lyses polymorphonuclear leukocytes via pore formation [4], and toxic shock syndrome toxin 1 (*TSST*-1), encoded by *tsst*1, which is involved in potentially fatal systemic inflammatory responses [5]. Globally, the prevalence of *pvl* is variable, with high frequencies in Africa (approximately 50%, with more than 70% reported) and low frequencies in Europe (generally less than 5%) [6] [7]. For *TSST*-1, reported frequencies range across hospitals and settings, with levels described in some MRSA lineages compared to MRSA. In Côte d'Ivoire, published data remain limited and are derived from small Abidjan series (mostly fewer than 35 isolates). *PVL* prevalence reached 45.2% in the most recent study (2020) [8], whereas *TSST*-1, although detected locally, lacks up-to-date estimates; to our knowledge, the only point estimate is ~3% - 4% from 2009 [3]. Moreover, current taxonomy recognizes that *S. aureus* belongs to a complex of related species (*S. argenteus* and *S. schweitzeri*), which are rarely distinguished in routine practice but potentially differ in virulence, supporting the need for broader surveillance [9]. Additionally, several international studies have linked *PVL* and *TSST*-1 to severe clinical presentations, notably among community-associated MRSA [2].

Against this background of limited local data and an apparent increase in MRSA, we investigated the distribution of *pvl* and *tsst*1 genes in an expanded clinical cohort at CHU Angré (Abidjan). Our objectives were to estimate the prevalence of *pvl* and *tsst*1 among clinical isolates of *Staphylococcus aureus* and to analyze their associations with methicillin resistance and clinical severity.

2. Materials and Methods

2.1. Study Site

This study was conducted at the bacteriology unit of CHU Angré.

2.2. Sample Collection and Inclusion Criteria

This study analyzed stored clinical isolates from routine inpatient and outpatient samples collected at the Angré University Hospital between January 2022 and February 2024. All unique, non-duplicate isolates phenotypically identified as *Staphylococcus aureus* in the microbiology laboratory were eligible. Identification was confirmed by PCR targeting the *nuc* gene (and the NRPS marker, when applicable).

The exclusion criteria were screening/colonization specimens, environmental isolates, duplicates (per repository records), uncertain identification, and obvious contaminants. Isolates were preserved either as deep nutrient agar stabs at room temperature or at –80˚C in Brain Heart Infusion (BHI) broth supplemented with 10% glycerol.

2.3. Isolate Selection

Since 2020, the laboratory has archived approximately 100 *S. aureus* isolates per year (with more pronounced losses of viability in 2020). For this study, inclusion was restricted to 2022-2024 because deep nutrient agar preservation from these years was better maintained, yielding higher viability and fewer losses at subculture. Isolates were selected consecutively, without replacement, following the chronological order of archival entry until 200 unique clinical isolates were reached (100 MRSA and 100 MSSA). One isolate per patient/clinical episode was selected. If an isolate was non-viable on subculture or its record was entirely missing, the next one in chronological order was included. Selection was not conditioned by ward, and the distribution of wards among the included isolates broadly mirrored that of the available archives, indicating no notable ward-related bias. A 1:1 (MRSA/MSSA) scheme was retained, consistent with the proportions observed in the laboratory database, where methicillin-resistant and methicillin-susceptible isolates were relatively balanced and available during 2022-2024. The isolates originated from diverse clinical specimens, including wound swabs, blood, pus, urine, and puncture/aspiration fluids (ascitic and pleural).

2.4. Isolation and Identification Procedures

The collected strains were grown on nutritive and Chapman agar and incubated

at 37˚C for 24 h. Preliminary identification of the strains was performed using Gram staining and catalase, DNase, and coagulase tests, supplemented by the VITEK® 2 automated system (bioMérieux, Marcy-l'Étoile, France). Preliminary identification was confirmed by molecular identification targeting the nuc gene (species-specific) and NRPS molecular marker [10] [11] by conventional PCR. Reference strains S. aureus ATCC 43300 (MRSA) and ATCC 25923 (MSSA) were used as controls. Primary culture was performed on nutrient agar and Chapman agar and incubated at 37˚C for 24 h. Initial identification relied on Gram staining and catalase, DNase, and coagulase tests, complemented by the VITEK® 2 automated system (bioMérieux, Marcy-l'Étoile, France). The identification of *S. aureus* was confirmed by conventional PCR targeting the *nuc* gene (species-specific) and the NRPS molecular marker [10] [11]. Reference strains *S. aureus* ATCC 43300 (MRSA) and ATCC 25923 (MSSA) were used as controls.

2.5. Methods Definition of Clinical Severity

2.5.1. Data Sources

Severity was operationalized as a binary outcome (severe versus non-severe). An episode was classified as severe if at least one of the following criteria was met: admission or transfer to the ICU or critical care; documented *Staphylococcus aureus* bacteremia or sepsis, defined as explicit mention in records, a positive blood culture associated with initiation of antistaphylococcal therapy, or at least two concordant positive blood cultures (excluding single positive bottles judged as contaminants or not treated and followed by a negative control); evidence of a deep or invasive infection such as pneumonia, endocarditis, osteoarticular infection, deep abscess, necrotizing fasciitis, meningitis, or pyelonephritis; requirement for drainage or surgery related to infection, including abscess evacuation, debridement, or hardware placement or revision; adverse outcomes such as death during the episode or explicit documentation of septic state, severe sepsis, or septic shock; or persistent fever, defined as either explicit mention in records or temperature ≥ 38˚C recorded on at least two occasions over 72 h despite antimicrobial therapy. Episodes that did not meet any of these criteria were classified as non-severe.

2.5.2. Collection of Clinical Data and Related Variables

Models were adjusted for covariates reliably available in the Laboratory Information System (LIS): methicillin-resistance status (MRSA/MSSA), specimen site (grouped as blood vs. other), and an age proxy based on the ward of care, with two categories: pediatrics/neonatology and adult services (internal medicine/geriatrics, surgery/traumatology, emergency/ICU, pulmonology, and other wards). This proxy was chosen because the ward is exhaustively recorded and closely reflects the age distribution at our institution. Information on immune status and prior antibiotic exposure was not captured consistently enough for valid adjustment and was, therefore, excluded.

2.6. Determination of Resistance to Methicillin

Methicillin resistance was assessed using two approaches.

Agar diffusion method: A bacterial suspension equivalent to 0.5 McFarland was spread on a Mueller-Hinton agar and a cefoxitin disc (30 µg) was applied. After incubation at 37˚C for 18 - 24 h, the inhibition diameters were measured and interpreted according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2024). A diameter ≤ 22 mm was interpreted as indicative of an MRSA strain, while a diameter ≥ 22 mm indicated an MSSA strain [12].

Automated method: In parallel, a few isolates were analyzed using the VITEK® system (bioMérieux, Marcy-l'Étoile, France), which provides a sensitivity profile, including methicillin resistance detection.

2.7. Detection of *pvl* and *tsst*1 Virulence Genes

To evaluate the virulence potential of the *Staphylococcus aureus* isolates, conventional PCR targeted the *PVL* locus (*lukS-PV*/*lukF-PV*) and the *tsst*1 gene. *PVL* detection used the primer set (luk-PV-1/luk-PV-2); *tsst*1 was amplified with the corresponding *tsst*1 primer pair. Primer details and expected amplicon sizes are provided in Table 1.

Table 1. Primers sequences used for detection of *pvl* and *tsst*1.

Target gene	foward luk-PV-1 (5'-3')	Reverse luk-PV-2 (5'-3')	Amplicon size	Reference
pvl	ATCATT-AGGTAAAATGTCTGGAC	GCATCAAGTG-TATTGGATAGCA	433 pb	[13]
*tsst*1	CTGGTATAGTAG-TGGGTCTG	AGGTAGTTC-TATTGGAGTAGG	271pb	[14]

2.7.1. Genomic DNA Extraction

Genomic DNA was extracted from fresh cultures grown on nutrient agar using the Biofact ® DNA/RNA Amplification Kit (Biofact, South Korea) according to the manufacturer's instructions. DNA extracts were quantified by spectrophotometry using the A260/A280 ratio and stored at −20˚C for polymerase chain reaction (PCR).

2.7.2. PCR Amplification

The *pvl* and *tsst*1 genes were detected using conventional PCR. Amplifications were run on a CFX96 thermocycler (Bio-Rad) using the HOT FIREPol® Master Mix 5× (Solis BioDyne, Tartu, Estonia), a ready-to-use mixture containing DNA polymerase, dNTPs, Mg^{2+} and reaction buffer. Each reaction was set up in a final volume of 22 µL containing 4 µL of HOT FIREPol® Master Mix 5×, 0.75 µL of each primer (10 µM), 3 µL of template DNA, and 13.5 µL of nuclease-free water.

Thermal cycling comprised an initial denaturation step, followed by cycles of denaturation, annealing, and extension, and a final extension for each target (Table 2). PCR products were separated on 1.5% agarose gels containing SYBR Safe® (Invitrogen®) at 100 V for 30 min, and bands were interpreted according to their expected sizes.

Table 2. Thermal cycling conditions for *pvl* and *tsst*1.

Step	Gene *pvl*	Gene *tsst*1
Initiale denaturation	94˚C, 5 min	94˚C, 5 min
Denaturation (per cycle)	94˚C, 30 s	94˚C, 1 s
Annealing	55˚C, 30 s	54˚C, 2 s
Extension	72˚C, 1 min	72˚C, 1 s
Final extension	72˚C, 10 min	72˚C, 5 min
Number of cycle	30	35

2.8. Statistical Analysis

Data were entered and analysed using Excel and R. Associations between variables were assessed using the chi-squared test, and a p-value < 0.05 was considered statistically significant.

2.9. Ethical Considerations

This study used isolates stored from routine diagnostic samples collected at the Angré University Hospital. No data were collected that could be used to determine the patients' personal data, and all information was processed in an anonymized form. In accordance with current regulations, formal ethical approval was not required.

3. Results

3.1. General Characteristics of the Isolates

A total of 200 isolates were included, with equal representation of MRSA and MSSA strains. Table 3 summarizes the general characteristics of these isolates.

Table 3. General characteristics of *S. aureus* isolates (n = 200).

Variable	Category	N	Percent (%)
Ward	Adults	124	62.0
	Pediatrics	76	38.0
Cefoxitin test	MSSA	100	50.0
	MRSA	100	50.0
Specimen site	Blood	139	69.5
	Other	61	30.5
Clinical severity	Non-severe	168	84.0
	Severe	32	16.0
Age available	Yes	110	57.4
	No	90	42.6
Age	≥15 years	49	42.3
	<15 years	61	57.7
Pvl	Positive	144	72.0
	Negative	56	28.0

Continued

*tsst*1	Positive	121	60.5
	Negative	79	39.5

*Subset computed only for isolates with available age data (n = 110).

The 200 *clinical Staphylococcus aureus isolates were evenly split between MRSA and MSSA, with most originating from adult wards. Blood was the predominant specimen type, and severe presentations were relatively uncommon* (16% *of cases*). *Age data were available for just over half of the isolates; within this subset, cases more often involved patients aged* < 15 *years than* ≥15 *years. Overall, pvl* (72%) *and tsst*1 (60.5%) *were frequent, underscoring the high burden of virulence determinants in this collection.*

3.2. Prevalence of the Virulence Genes *pvl* and *tsst*1 in All Isolates

Detection of virulence genes by PCR (Figure 1) revealed a high prevalence of *pvl* and *tsst*1 genes.

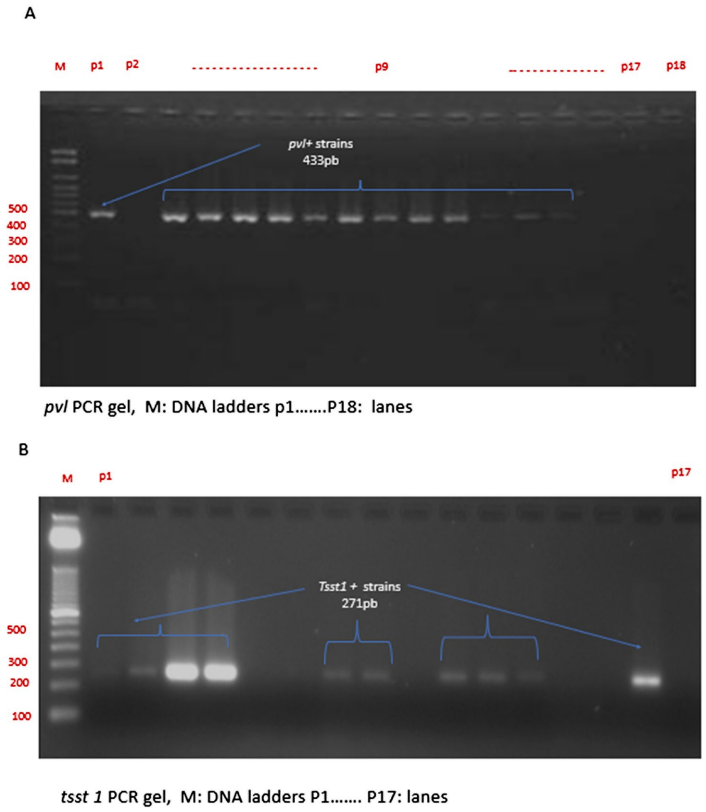

Figure 1. PCR detection of *pvl* and *tsst*1 genes in Staphylococcus aureus isolates. (A) *pvl* amplification showing the expected band at approximately 433 bp in 13/18 samples. (B) *tsst*1 amplification showing the expected band at ~271 bp in 10/17 samples. Amplicons were separated on 1.5% agarose gels and visualized with SYBR Safe®. M, 100 bp molecular weight marker. Braces indicate positive samples, the and absence of a band of the expected size denotes a negative result.

The distribution of the combined patterns was as follows: 54% of isolates (n = 108) were *pvl*$^{+}$/*tsst*1^{+}, 21% (n = 42) *pvl*$^{+}$/*tsst*1^{-}, 14% (n = 28) *pvl*$^{-}$/*tsst*1^{+}, and 11% (n = 22) *pvl*$^{-}$/*tsst*1^{-}.

3.3. Distribution of *pvl* and *tsst*1 by Clinical Service

The isolates were grouped into four clinical categories: pediatrics/neonatology, adult medicine, surgery/traumatology, and critical care (including ICU, pulmonology, and other high-acuity units). Pediatrics/neonatology contributed 38.0% of isolates (76/200), of which 40.8% were *pvl*-positive (31/76) and 48.7% were *tsst*1-positive (*tsst*1^{+}) (37/76). Adult medicine accounted for 26.5% (53/200), with 81.1% *pvl*-positive (*pvl*$^{+}$) (43/53) and 47.2% *tsst*1-positive (25/53). Surgery/traumatology represented 23.5% (47/200), with 89.4% *pvl*-positive (42/47) and 74.5% *tsst*1-positive (35/47). Critical care comprised 12.0% (24/200), and all isolates in this group were double positive (*pvl*$^{+}$/*tsst*1^{+}, 24/24).

3.4. Distribution and Associations of *pvl* and *tsst*1 across Clinical Groups

Isolates were classified into two categories based on the suspected clinical severity of infection at the time of collection, according to the information available on the accompanying sheets (Table 3).

PVL positivity was higher in severe than in non-severe episodes (90.09% [30/32] vs. 30.77% [64/137]; p = 0.0002). It was also more frequent in adults than in pediatric patients (88.9% [48/54] vs. 40.78% [31/76]; p = 0.002). No significant differences were observed in *pvl* rates between specimen types (blood vs. other: 71.2% [99/139] vs. 82.0% [50/61]; p = 0.117). *pvl* co-occurrence with *tsst*1 did not differ meaningfully (*pvl*$^{+}$ among *tsst*1^{+} vs. *tsst*1^{-}: 76.1% [70/92] vs. 68.2% [30/44]; p = 0.406).

For *tsst*1, no significant associations were found with severity (59.1% [19/32] vs. 45.5% [77/168]; p = 0.180), methicillin resistance (71.0% [49/69] vs. 64.2% [43/67]; p = 0.465), ward (adults vs. pediatrics: 64.8% [35/54] vs. 69.5% [57/82]; p = 0.580), or specimen type (67.0% [93/139] vs. 68.9% [42/61]; p = 0.870).

3.5. Distribution and Associations of *pvl* and *tsst*1 by MRSA/MSSA Status

The *pvl* gene was detected in 75% of MRSA (75/100) and 69% of MSSA (69/100) cases, with no significant difference between the groups (p = 0.345). The *tsst*1 gene was found in 64% of MRSA (64/100) and 57% of MSSA (57/100) and was not significant (p = 0.311). Combined *pvl*/*tsst*1 profiles are summarized separately for MRSA and MSSA (Figure 2).

Distribution of virulence gene profiles (*pvl and tsst*1) *among Staphylococcus aureus isolates according to methicillin resistance* (*MRSA vs. MSSA*).

Four profiles were identified: doubly positive (*pvl*$^{+}$/*tsst*-1^{+}), *pvl*$^{+}$/*tsst*-1^{-}, *pvl*$^{-}$/*tsst*-1^{+} *and doubly negative* (*pvl*$^{-}$/*tsst*-1^{-}). *The pvl*$^{+}$/*tsst*-1^{+} *profile was preva-*

lent in MRSA (64%) *and MSSA* (50%) *isolates, respectively. The pvl*$^{+}$*/tsst*-1^{-} *profile was more common in MSSA* (23%) *than in MRSA* (16%), *whereas pvl*$^{-}$*/tsst*1^{+} *accounted for* 12% *of MRSA and* 16% *of MSSA. The pvl*$^{-}$*/tsst*-1^{-} *profile was the least represented, especially in MRSA* (8%) *compared with MSSA* (11%).

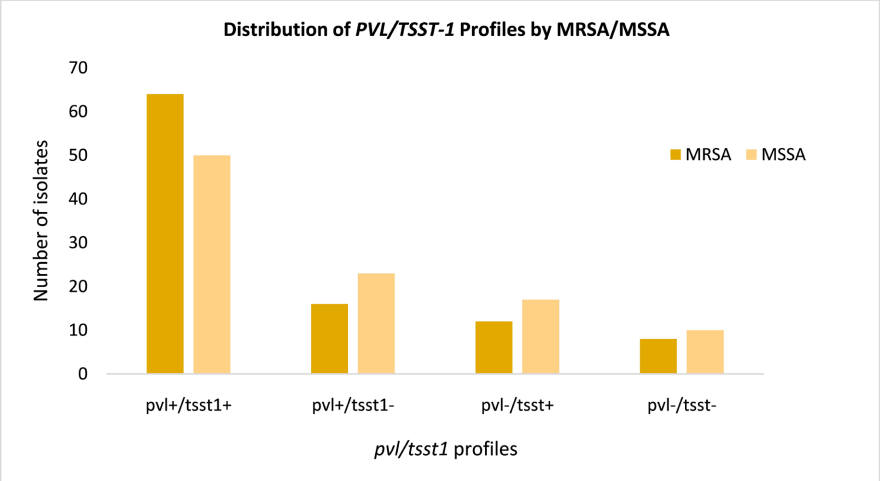

Figure 2. Distribution of *pvl/tsst*-1 combination profiles according to methicillin resistance status.

4. Discussion

4.1. Distribution of Virulence Genes in *Staphylococcus aureus* Clinical Isolates in Abidjan

This study is part of local efforts to molecularly characterize isolates within the *Staphylococcus aureus* complex, highlighting the distribution of *pvl* and *tsst*1 virulence genes in stored strains. We observed a high prevalence (*pvl*: 72.0%; *tsst*1: 60.5%), in agreement with several studies from sub-Saharan Africa, where high frequencies of *pvl* have been reported, for example, in Ghana (75%) [15] and The Gambia (77%) [6], while exceeding the values observed in Senegal (47%) and in several countries included in the multicenter study by Breurec *et al.* We observed high prevalence (*pvl*: 72.0%; *tsst*1: 60.5%), in agreement with several studies from sub-Saharan Africa where high frequencies of *pvl* have been reported, for example in Ghana (75% [15]) and The Gambia (77% [6]), while exceeding the values observed in Senegal (47%) and in several countries included in the multicentre study by Breurec *& al.* (2011) [16]. Conversely, in Europe and Asia, *pvl* frequencies are often lower (≤30%), with marked local variability (Greece: 19% *pvl*$^{+}$ among *S. aureus*; China: 20%) [17] [18]). These discrepancies may reflect, on the one hand, the composition of our sample (inpatients and outpatients), which indicates co-circulation of community and nosocomial strains, and, on the other hand, irregular access to care in our setting, which may favour the spread of strains carrying virulence genes. They also fit within heterogeneous epidemiological contexts, as highlighted by Shallcross *et al.* They also fit within heterogeneous epidemiological contexts, as highlighted by Shallcross *et al.* (2013) [19]. In addition, the circulation

of mobile genetic elements (prophages, pathogenicity islands, and plasmids) can facilitate the acquisition and sometimes co-occurrence of virulence determinants [20] [21]. In fact, 54% of our isolates were *pvl*$^+$/*tsst*1$^+$, an unusual and rare profile internationally but already described in Pakistan [22], suggesting that some local lineages may combine these factors.

Distribution by clinical ward showed an over-representation of *pvl*$^+$/*tsst*1$^+$ profiles in critical care units, where this profile reached 100% of isolates, as well as high rates in surgery/trauma wards (89.3%). This concentration in high-risk areas is consistent with the role of *S. aureus* toxins in severe forms (deep abscesses, necrotizing pneumonia, complicated infections), while recognizing that clinical virulence results from a set of bacterial and host factors [2] [4]. In adult medicine, the *pvl* gene was present in 81.1% of isolates, indicating substantial circulation of virulent strains outside surgical departments, whereas *tsst*1 was less frequent (47.1%), possibly reflecting greater lineage diversity or lower selective pressure for this gene in that context. In pediatrics and neonatology, which accounted for most samples, the rates were more moderate for *PVL* (40%) and *tsst*1 (48.6%); the relatively high prevalence of *tsst*1 in this young population with fragile immune systems raises questions about superantigen-specific interactions, even without proven toxic shock [5]. Overall, the critical care, surgery, and adult medicine departments concentrated *pvl*$^+$/*tsst*1$^+$ profiles, reinforcing the hypothesis that particularly virulent strains contribute to severe *S. aureus* infections [23] [24], whereas the more balanced distribution observed in pediatrics suggests greater genetic diversity of strains and variability in pathogenic potential according to department and patient profile.

4.2. Association between Virulence Genes (*pvl, tsst*1), Methicillin Resistance, and Clinical Severity

Our results revealed that a significant proportion of *Staphylococcus aureus* strains carrying the *pvl* gene were present among both susceptible (MSSA) and resistant (MRSA) isolates. This contrasts with data reported in Abidjan by Kacou-N'Douba *et al.* (2011), where, although *pvl* was present in more than 60% of samples, no MRSA strain carried *pvl* [3]. The current co-circulation of *pvl*$^+$ MRSA observed in our study may reflect local epidemiological evolution, possibly related to the introduction and/or selection of more virulent clones. This aligns with patterns documented elsewhere, including the introduction of *pvl*$^+$ isolates into hospitals with subsequent local dissemination [25], and the establishment of *pvl*$^+$ community lineages in West Africa [26]. Recent molecular epidemiology studies from the region support this observation: in Ghana, a multicenter whole-genome sequencing study reported a high *pvl* prevalence (65% overall, 84% in MRSA), largely dominated by ST152 [27], while in Nigeria, *pvl* genes were detected in 12.5% of isolates from soft-tissue infections, in both MRSA and MSSA, with recurrent infection independently associated with *pvl* carriage [28].

In our cohort, *pvl* and *tsst*1 remained common regardless of MSSA/MRSA sta-

tus; although they were slightly higher in MRSA, the differences were not statistically significant. This lack of a formal association suggests that, in our context, the dissemination of these virulence genes does not directly depend on methicillin resistance. Internationally, the link between *pvl* and resistance is heterogeneous and varies by region. In Nigeria, *pvl* is mainly carried by MSSA, although *pvl*$^+$ MRSA are also present [29]. In Gaza (Palestine), *pvl* was detected in both groups at comparable frequencies (MRSA, 30.5%; MSSA, 28.2%) [30].

The differences between studies can be explained, at least in part, by local epidemiological factors and the circulation of lineages combining resistance and virulence. Without typing (*spa*/MLST) in our study, we remain cautious and do not propose clonal attribution. As a hypothesis supported by African literature, a recent synthesis reported a continent-wide increase in the distribution of ST1, ST22, and ST152 clones [26], the latter of which is frequently *pvl*$^+$ [31]. This hypothesis is further reinforced by the Ghanaian findings, where ST152 accounted for the majority of *pvl*-positive MRSA, suggesting the regional expansion of this epidemic lineage [27]. The absence of differences across specimen types suggests that *pvl* functions mainly as a general virulence determinant, acting across multiple infection sites, and is more closely linked to the clonal background than to the site of isolation [19].

Clinically, we observed a significant association between *pvl* and disease severity. This finding is consistent with reports linking *pvl* to deep-seated skin and soft tissue infections (dSSTIs) and necrotizing pneumonia [13] [32] [33]. However, *pvl*-positive isolates are also found in mild infections, underscoring that severity depends on multiple host- and site-related factors [1] [19]. The higher *pvl* frequency in adults than in pediatric patients should be interpreted with caution, since the ward (adults vs. pediatrics) was used as a proxy for age due to missing data. This approach may lead to misclassification, which usually attenuates, rather than generates, associations. Therefore, the observed signal is plausible; however, confirmation using actual age data is required. In contrast, *tsst*1 was not associated with disease severity in the present study population. This aligns with the notion that *tsst*1 is mainly a marker of a specific syndrome (toxic shock) rather than a consistent predictor of severity across all *S. aureus* infections [24] [34]. Finally, the co-occurrence of *pvl* and *tsst*1 alone is not sufficient to establish a systematic link with methicillin resistance or severity outside the targeted syndromes.

4.3. Limitations and Public Health Implications

This study had some limitations. First, the isolates were obtained from a single hospital, which limits the generalizability of the findings to the entire country. Second, some clinical data linked to the isolates were incomplete, constraining the assessment of risk factors (prior antibiotic exposure, immune status, and comprehensive resistance profiles). Finally, our analysis focused on two major virulence genes (*pvl* and *tsst*1) without in-depth molecular typing namely MLST (multilocus sequence typing of seven housekeeping genes to assign sequence types and

clonal complexes), *spa* typing (sequencing the polymorphic X region of the *spa* gene to derive t-types), and SCCmec characterization (defining the staphylococcal cassette chromosome mec that carries *mecA/mecC* and *ccr* genes) which would have refined the characterization of circulating lineages and their epidemiological potential.

Despite these constraints, the high prevalence of virulence determinants suggests an elevated risk of invasive disease and severe complications in the future. In settings with limited diagnostic and therapeutic resources, the circulation of virulent and potentially methicillin-resistant strains poses major challenges for patient care and safety.

From a public health perspective in Côte d'Ivoire, these findings highlight the need for strengthened microbiological surveillance of *S. aureus* that integrates both resistance and virulence markers. Priorities include reinforcing hospital laboratory capacity (basic molecular biology), establishing a small sentinel network to monitor MRSA and virulence genes with regular reporting, incorporating *S. aureus* indicators into infection prevention programs, and providing targeted training on early diagnosis, isolation, and antimicrobial stewardship. Finally, a One Health approach should be considered, taking into account community reservoirs and possible zoonotic sources of infection.

5. Conclusion

This study highlights the high prevalence of the virulence genes *pvl* and *tsst*1 among clinical isolates of *Staphylococcus aureus* in Abidjan, with a high proportion of double-positive strains, particularly in departments that manage severe cases of infection. The absence of a significant association with methicillin resistance suggests independent dissemination of resistance, possibly driven by specific lineages. The association between *pvl* and severe cases supports its involvement in virulence, although its presence alone does not systematically predict clinical severity. These findings underscore the importance of strengthening surveillance by combining virulence-gene detection with molecular typing (*spa*, MLST, SCCmec) to identify circulating clones, better understand their epidemiological dynamics, and anticipate the risks associated with hypervirulent strains.

6. Thanks

This project did not receive any funding.

We would like to thank all those who contributed to this study. We thank CHU Angré (Medical Biology Laboratory, Bacteriology Unit) for their support, equipment, and biological materials. We would like to thank Dr. Pakora Alex, Prof. Koffi Stéphane and Prof. Gonedele Seri from the bacteriology-virology laboratories of the Medical Sciences Department, Central Laboratory (Bacteriology and Molecular Biology Unit of the Treichville University Hospital) and the genetics laboratory of the Biosciences Department of Technology for providing valuable field infrastructure.

Authors' Contributions

ODNC was involved in all stages of the research (study design, data collection, data analysis, and data interpretation) and wrote the manuscript. BGA and CDYM participated in sample collection and associated data. CGAE contributed to the molecular analysis and manuscript revision. KKAA participated in the statistical analysis and interpretation of the data. KNA and DAJ supervised the conceptualization and study design, critically revised the manuscript, and contributed to the final version of the manuscript.

All authors have read and approved the final manuscript.

Abbreviations

All abbreviations are defined at their first mention in the text.

Conflicts of Interest

The authors declare no conflicts of interest.

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