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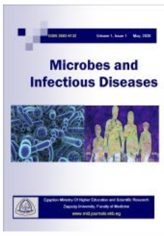

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Original article

Association of tsst-1 gene and phenotypic antibiotic resistance among clinical *Staphylococcus aureus* isolates in a tertiary healthcare center

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A B S T R A C T

Background: The toxic shock syndrome toxin (TSST-1) is important in the pathology of toxic shock syndrome. However, little data exist on its prevalence among clinical isolates of *S. aureus* in Nigeria. Hence, this study was carried out to detect the tsst-1 gene and associate it with phenotypic antibiotic resistance in clinical isolates of *S. aureus***.** **Methods:** *Staphylococcus aureus* isolates were presumptively identified by Gram's staining and conventional biochemical tests while confirmatory identification was through the detection of the thermonuclease (nuc) gene. Antibiotic sensitivity testing was carried out using the modified Kirby-Bauer disc diffusion method while phenotypic detection of methicillin resistance was carried out using the cefoxitin disc sensitivity assay. The tst gene was detected within the genome of the bacterial isolates using Uniplex polymerase chain reaction (PCR). **Results:** Of the 152 *S. aureus* isolates identified in this study, 103 (67.76%) encoded the tst gene. Of these 103 tst-positive isolates, 63 (61.16%) were methicillin-resistant while 40 (38.84%) were methicillin-sensitive. The tst-positive isolates (n=103) were resistant to tetracycline (39.81%), erythromycin (24.27%), gentamicin (22.33%), cotrimoxazole (22.33%), ciprofloxacin (21.36%), fusidic acid (16.5%), fosfomycin (10.68%), and clindamycin (5.82%). Comparatively, tst-negative isolates (n=49) were resistant to tetracycline (69.39%), cotrimoxazole (56.06%), gentamicin (53.06%), ciprofloxacin (51.02%), erythromycin (46.94%), fusidic acid (28.57%), fosfomycin (26.53%), and clindamycin (8.16%). Phenotypic antibiotic resistance is significantly associated with the presence of the tst gene ($p<0.05$) except for clindamycin and fusidic acid ($p>0.05$). **Coclusion:** Hence, the high prevalence of the tst gene and its association with antibiotic resistance in *S. aureus* is a cause for worry.

Introduction

Staphylococcus aureus (S. aureus) is a normal flora of the skin and nasal cavity of healthy immunocompetent individuals [1]. However, the organism causes diseases of immense clinical significance due to its vast array of virulence factors, which includes superantigen toxins [2]. *Staphylococcus aureus* is a clinically significant pathogen whose diseases range from mild superficial infections to severe and life-threatening systemic infections [3,4]. In tropical countries, *S. aureus* remains a significant cause of morbidities and mortalities in clinical settings.

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Pathogenic organisms possess genetic factors that make up their virulence arsenal and enhance their fitness in proliferating host cells and tissues to cause diseases [5]. These virulence factors grade the pathogenicity of microbial pathogens and play important roles in determining the pathology of the vast arrays of microbial diseases. These virulence factors are genetically encoded and translated into proteins under inducible conditions. Toxic shock syndrome toxin (TSST-1) is a virulence factor produced by lysogenic converted strains of *S. aureus* [6].

Toxic shock syndrome toxin induces excessive T cell proliferation and cytokines release by inducing the cross-linking of class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) with the variable region of the β-chain of T cell receptors (TCRs) on naive T lymphocytes [7,8]. This excessive T cell proliferation and cytokine release induce an acute phase of host immune excitation that culminates in a cytokine storm which cripples the host's physiology and leads to death in extreme cases [9]. The TSST-1 is associated with toxic shock syndrome (TSS) and produced by lysogenic converted *S. aureus* strains encoding the *tst* gene, a gene carried on *S. aureus* pathogenicity islands (SaPIs) [6,10].

Toxic shock syndrome is a highly fatal multisystemic disease. It is mostly associated with the usage of tampons by menstruating women (menstrual TSS), although non-menstrual TSS has also been elucidated [11,12]. It is characterized by pathological hallmarks such as rapid fever onset, erythematous skin rash, hypotension, hemodynamic shock, multiorgan failure, and ultimately death [1,7].

Despite the enormous clinical significance of TSST-1 as a virulence factor, there is inadequate available data on its prevalence among clinical isolates of *S. aureus* in Nigeria and Africa at large. Furthermore, little research has sought to associate phenotypic antibiotic resistance with superantigen toxin genes such as tst. Hence, this study was carried out to associate phenotypic antibiotic resistance with the possession of the tst gene among clinical isolates of *S. aureus.*

Methods

Study setting and ethical approval

University of Ilorin Teaching Hospital (UITH) is a tertiary healthcare center located at Oke-Oyi, Ilorin East, Kwara State, Nigeria. The services of the hospital are rendered to patients from several states including, but not limited to, Kwara, Oyo, Ekiti, Osun, Lagos, Kogi, Niger, Kebbi, and the Federal Capital Territory (FCT) [13]. Ethical approval for the study was obtained from the Ethical Review Board (ERB) of the UITH.

Study design

The study adopted a laboratory-based cross-sectional design that used clinical isolates of *S. aureus* recovered from clinical specimens submitted to the Department of Medical Microbiology and Parasitology of UITH**.**

Culture, isolation, and identification of *S. aureus*

Clinical specimens, including wound specimens, aspirates, eye swabs, and abscesses were inoculated directly on sheep blood agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) plates. Bact/Alert-positive blood specimens were cultured on sheep blood, chocolate, and MacConkey agar plates. Inoculated plates were incubated aerobically while chocolate agar plates were incubated in a microaerophilic environment in a candle extinction jar. All culture plates were incubated at 37^0C for 18-24 hours. Isolates on culture plates were identified morphologically by Gram's stain reaction and standard biochemical tests that included catalase, coagulase, DNase, and mannitol fermentation tests (Oxoid, UK). Isolates that were Gram-positive cocci in clusters, catalase-positive, coagulase-positive, DNase-positive, and mannitol-fermenters were identified as *S. aureus* [14].

Antibiotic sensitivity test (AST) of *S. aureus*

Antibiotic sensitivity testing was carried out on each *S. aureus* isolate using the modified Kirby-Bauer disc diffusion method. Bacterial inoculum was standardized to 0.5 McFarland standard before inoculating the surface of freshly prepared Mueller-Hinton agar (MHA) plates. The isolates were tested against the following antibiotics (Oxoid, UK); erythromycin (15µg), clindamycin (2µg), tetracycline (30µg), cotrimoxazole (1.25/23.75µg), mupirocin (5µg), linezolid (30µg), tigecycline (15µg), fusidic acid (10µg), fosfomycin (50µg), ciprofloxacin (5 µg), rifampin (5µg), and gentamicin (10µg). *S. aureus* ATCC 25923 was used as a control strain. Diameters of the zone of inhibition were measured with a calibrated ruler and interpretation of each isolate as sensitive, intermediate, or resistant to the antibiotics was done using the Clinical and Laboratory Standards Institute (CLSI) breakpoints [15].

Phenotypic detection of methicillin resistance was carried out using a cefoxitin (30µg) disc diffusion assay. Cefoxitin-impregnated antibiotic disc (Oxoid, UK) was placed on inoculated Mueller-Hinton agar (Oxoid, UK) plates and incubated at 37^0C for 16-18 hours and observed for visible zones of inhibition. *S. aureus* ATCC 43300 was used as a positive control strain for the cefoxitin disc diffusion test. Diameters of the zone of inhibition ≤ 21 mm were classified as methicillin-resistant (MRSA) and those with a diameter ≥ 22 mm as methicillin-sensitive (MSSA) [15].

Molecular detection of *tst* gene

To detect the presence of *tst* (306bp) and *nuc* (279bp) genes among *S. aureus* isolates, DNA extraction was carried out using the conventional boiling method with proteinase K (Inqaba Biotec, Ibadan, Nigeria). The thermonuclease (*nuc*) gene is a target nucleotide sequence that is used to selectively identify and differentiate *S. aureus* from other *Staphylococcus* spp [16]. Uniplex PCR was carried out using the primers *tst*-F: AGCCCTGCTTTTACAAAAGGGGAAAA and *tst*-R: CCAATAACCACCCGTTTTATCGCTTG and *nuc*-F: GCGATTGATGGTGATACGGTT and *nuc*-R: AGCCAAGCCTTGACGAACTAAAGC (Inqaba Biotec, South Africa) and 5X master mix (New England Biolabs Ltd, UK). The PCR conditions implemented for the amplification of the *tst* gene included, DNA template denaturation at 94^0C (60 seconds), annealing at 60^0C (60 seconds), and extension at 72^0C (60 seconds) [17]. The PCR amplification of the *nuc* gene was carried out at 94^0C for 60seconds (denaturation), 56^0C for 2 minutes (annealing), and 72^0C for 2 minutes (extension) [18]. PCR products were thereafter run on gel electrophoresis using 1.2% agarose gel using 100 bp uncut DNA ladder as the standard.

Statistical analysis

Statistical analyses were computed using IBM SPSS (version 21). Fisher exact test (with Odds ratio and 95% CI) was used to determine the association between methicillin resistance and possession of the *tst* gene as well as between possession of the *tst* gene and antibiotic resistance. *p*-values were computed at a 95% confidence interval to determine the significance of difference. Computed *p*-values less than 0.05 ($p < 0.05$) were considered to be statistically significant.

Results

A total of 152 clinical isolates of *S. aureus* were recovered from clinical specimens. Of these, 103 (67.76%) possessed the *tst* gene. Sixty-three (61.16%) of these *tst*-positive *S. aureus* were methicillin-resistant (MRSA) while 40 (38.84%) were methicillin-sensitive (MSSA). The possession of the *tst* gene was higher among MRSA strains than among MSSA isolates **(Table 1)**. The prevalence of the *tst* gene in different clinical infections is shown in **table (2)**. *Staphylococcus aureus* harboring the *tst* gene was more prevalent in sepsis (29.13%), diabetic foot syndrome (21.36%), and eye infection (10.68%). Conversely, *tst*-negative *S. aureus* was more prevalent in pyomyositis (26.54%), sepsis (18.37%), and otitis media (12.25%). Furthermore, pyomyositis was more associated with *tst*-negative strains of *S. aureus* ($p < 0.05$) while diabetic foot syndrome was more associated with *S. aureus* strains harboring the *tst* gene ($p < 0.05$). For other clinical infections, there was no significant association with the presence of the *tst* gene ($p > 0.05$). All *S. aureus* isolates were sensitive to linezolid, tigecycline, mupirocin, and rifampin. The *tst*-positive MRSA isolates were more resistant to tetracycline (47.62%), erythromycin (31.75%), and cotrimoxazole (26.98%) while *tst*-positive MSSA isolates were more resistant to tetracycline (27.5%), gentamicin (20%), ciprofloxacin (17.5%), and cotrimoxazole (15%). There was no association between methicillin sensitivity among *tst*-positive *S. aureus* and antibiotic resistance except for tetracycline and erythromycin **(Table 3)**. *Staphylococcus aureus* isolates encoding the *tst* gene were more resistant to tetracycline (39.81%), erythromycin (24.27%), and fusidic acid and gentamicin (22.33% each) while *tst*-negative *S. aureus* isolates were more resistant to tetracycline (69.39%), cotrimoxazole and gentamicin (53.06% each), and ciprofloxacin (51.02%). However, *S. aureus* isolates harboring the *tst* gene were significantly more resistant to erythromycin, tetracycline, cotrimoxazole, fosfomycin, ciprofloxacin, and gentamicin than *tst*-negative strains. Hence, a positive association between antibiotic resistance and possession of the *tst* gene was observed against all tested antibiotics, except clindamycin and fusidic acid **(Table 4)**.

Table 1. Prevalence of *tst* gene among *S. aureus* strains.

***S. aureus* strain**	***tst* positive n (%)**	***tst* negative n (%)**	**Total n (%)**	**OR**	**95% CI**	**p-value**
MRSA	63 (61.16)	7 (14.28)	70 (46.05)	9.45	3.87-23.08	<0.0001*
MSSA	40 (38.84)	42 (85.72)	82 (53.95)			
Total	103 (67.76)	49 (32.24)	152 (100.0)			

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; *tst*: toxic shock syndrome toxin gene; OR: odds ratio; CI: confidence interval; n: number of isolates; *: statistically significant.

Table 2. Prevalence of *S. aureus* from clinical infections.

Clinical infection	***tst* positive (%) (n=103)**	***tst* negative (%) (n=49)**	**Total (%) (n=152)**	**OR**	**95% CI**	***p*-value**
Eye infection	11 (10.68)	5 (10.20)	16 (10.53)	1.052	0.345-3.213	0.464430
Otitis media	8 (7.77)	6 (12.25)	14 (9.21)	0.604	0.197-1.846	0.188022
Wound sepsis	5 (4.85)	3 (6.12)	8 (5.26)	0.782	0.179-3.415	0.372012
Pyomyositis	10 (9.71)	13 (26.54)	23 (15.13)	0.298	0.12-0.74	0.004528*
Surgical site infection	8 (7.77)	5 (10.20)	13 (8.55)	0.741	0.229-2.395	0.308295
Mastitis	4 (3.88)	2 (4.08)	6 (3.95)	0.949	0.168-5.369	0.476624
Sepsis	30 (29.13)	9 (18.37)	39 (25.66)	1.826	0.789-4.226	0.079622
Pneumonia	5 (4.85)	3 (6.12)	8 (5.26)	0.782	0.179-3.415	0.372012
Diabetic foot syndrome	22 (21.36)	3 (6.12)	25 (16.45)	4.164	1.182-14.673	0.013201*

tst: toxic shock syndrome toxin gene; OR: odds ratio; CI: confidence interval; n: number of isolates; *: statistically significant.

Table 3. Analysis of antibiotic resistance among *tst*-encoding *S. aureus* strains.

Antibiotic	**MRSA (%) (n=63)**	**MSSA (%) (n=40)**	**Total (%) (n=103)**	**OR**	**95% CI**	***p*-value**
Erythromycin	20 (31.75)	5 (12.5)	25 (24.27)	3.2558	1.11-9.56	0.026394*
Clindamycin	5 (7.94)	1 (2.50)	6 (5.82)	3.3621	0.38-29.89	0.25093
Tetracycline	30 (47.62)	11 (27.50)	41 (39.81)	2.3967	1.02-5.62	0.042129*
Cotrimoxazole	17 (26.98)	6 (15.0)	23 (22.33)	2.0942	0.75-5.87	0.154221
Fusidic acid	13 (20.63)	4 (10.0)	17 (16.50)	2.34	0.71-7.767	0.156265
Fosfomycin	8 (12.70)	3 (7.50)	11 (10.68)	1.7939	0.45-7.208	0.40511
Ciprofloxacin	15 (23.81)	7 (17.5)	22 (21.36)	1.4732	0.54-4.007	0.446312
Gentamicin	15 (23.81)	8 (20.0)	23 (22.33)	1.25	0.48-3.29	0.654721

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; OR: odds ratio; CI: confidence interval; n: number of isolates; *: statistically significant.

Table 4. Analysis of antibiotic resistance among *tst*-positive and *tst*-negative *S. aureus*

Antibiotic	*tst*-positive (%) (n= 103)	*tst*-negative (%) (n= 49)	Total (%) (n=152)	OR	95% CI	*p*-value
Erythromycin	25 (24.27)	23 (46.94)	48 (31.58)	0.3623	0.176-0.744	0.004943*
Clindamycin	6 (5.82)	4 (8.16)	10 (6.58)	0.6959	0.187-2.588	0.586834
Tetracycline	41 (39.81)	34 (69.39)	75 (49.34)	0.2917	0.141-0.602	0.000652*
Cotrimoxazole	23 (22.33)	26 (53.06)	49 (32.24)	0.2543	0.123-0.527	0.000152*
Fusidic acid	17 (16.50)	14 (28.57)	31 (20.39)	0.4942	0.22-1.11	0.084299
Fosfomycin	11 (10.68)	13 (26.53)	24 (15.79)	0.3311	0.136-0.807	0.01228*
Ciprofloxacin	22 (21.36)	25 (51.02)	47 (30.92)	0.2607	0.125-0.542	0.000217*
Gentamicin	23 (22.33)	26 (53.06)	49 (32.24)	0.2543	0.123-0.527	0.000152*

tst: toxic shock syndrome toxin gene; OR: odds ratio; CI: confidence interval; n: number of isolates; *: statistically significant.

Figure 1. Uniplex PCR gel electrograph for thermonuclease (*nuc*) gene- 279 bp. L: DNA ladder (100bp); P: Positive control; N: Negative control; 1-8: *nuc*-positive samples.

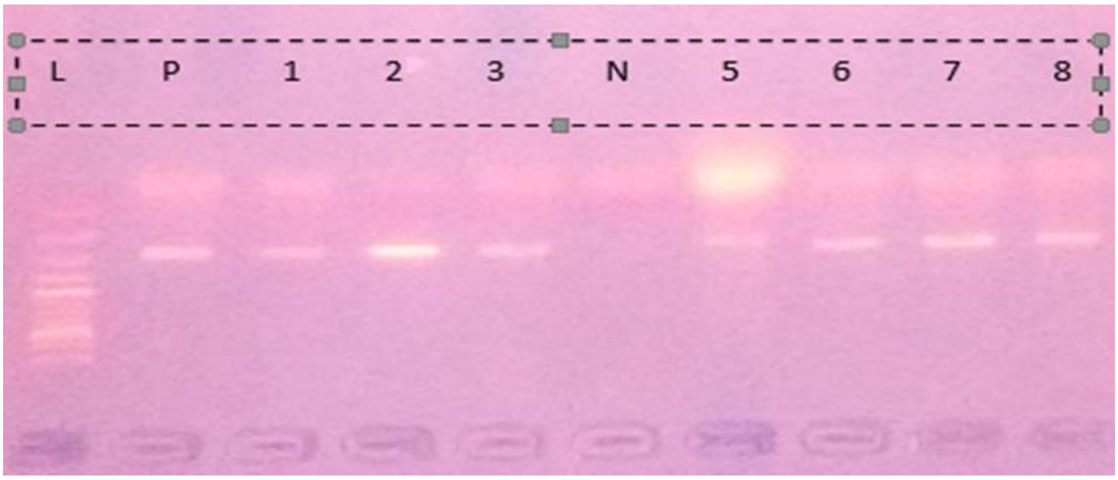
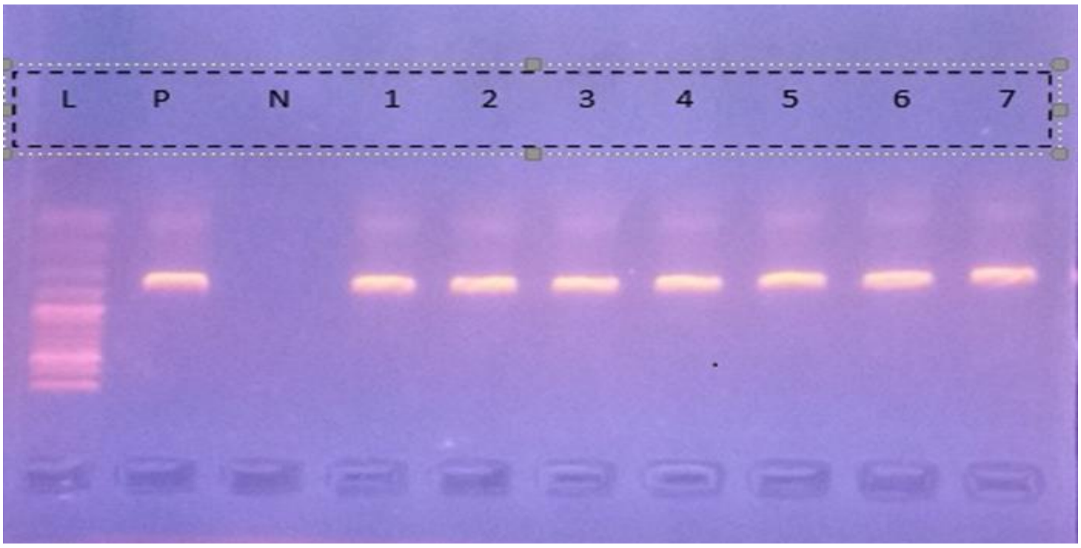


Figure 2. Uniplex PCR gel electrograph for *tst* gene (306 bp). L: DNA ladder (100bp); P: Positive control; N: Negative control; 1-8: *tst*-positive samples.



Discussion

Toxic shock syndrome toxin is a very potent superantigen toxin in the virulence arsenal of *S. aureus* and strains encoding the *tst* gene can cause toxic shock syndrome, a multisystemic disease. From our current study 103 (67.76%) isolates possessed the *tst* gene of the 152 *S. aureus* isolates studied. This prevalence is comparable to 60.6% reported in Egypt [19]. However, **Vandendriessceh et al.** [20] reported 19.4% among blood culture isolates in the Democratic Republic of Congo, **Ezeamagu et al.** [21] reported 14% in Southwest Nigeria, and **Omar et al.** [22] reported 46.7% in Baghdad, Iraq. In Iran, **Derakhshan et al.** [23] reported 22.8%, **Goudarzi et al**. [24] reported 25.43%, **Houri et al.** [25] reported 33.7%, and **Alni et al.** [26] reported 22.5%. Furthermore, **Senon et al.** [27] reported 43.3% in Malaysia, **Bhowmik et al.** [28] reported 50.79% in Southern Assam, India and **Costa et al.** [29] reported 26.31%. Also, the presence of the *tst* gene was significantly associated with diabetic foot syndrome, a significant condition associated with neuropathy, ischemia, and infection [30]. Hence, the high prevalence of the *tst* gene among clinical isolates, as reported in our study is a significant cause of alarm. However, variations in the prevalence of the *tst* gene for other geographical locations can be associated with the fact that the *tst* gene is harbored on *S. aureus* pathogenicity island (SaPIs) whose dissemination is not constant within different ecological niches. Also, as a superantigen, TSST can play a significant role in the pathogenesis of several *S. aureus* diseases including diabetic foot syndrome and sepsis. However, TSST-1 production is a factor of environmental variables such as partial oxygen and carbon dioxide gases, pH, temperature, and iron concentration [25].

Our study reported a higher prevalence of the *tst* gene among MRSA than MSSA. This finding is in tandem with reports made in similar studies where an association was observed between methicillin resistance and the prevalence of the *tst* gene [20,31]. Other similar studies have reported an insignificant association between methicillin sensitivity and prevalence of the *tst* gene [19,22,23]. The positive association between the prevalence of the *tst* gene and phenotypic methicillin resistance is a cause for worry. Hence, it becomes imperative to take caution to curb the spread of *tst*-harboring MRSA strains within hospital and community settings. Furthermore, *tst*-harboring MRSA strains within this center have higher odds of being resistant to tetracycline and erythromycin compared to other antibiotics.

In our present study, the presence of the *tst* gene was associated with resistance to erythromycin and tetracycline while the absence of the *tst* gene was associated with resistance to cotrimoxazole, fosfomycin, ciprofloxacin, and gentamicin. Also, no association was observed between the presence of the *tst* gene and resistance to clindamycin and fusidic acid. **Derakhshan et al.** [23] also reported an association between resistance to ciprofloxacin and the absence of the *tst* gene in Iran. However, **Sultan et al.** [19] and **Omar et al.** [22] did not report any correlation between possession of the *tst* gene and phenotypic antibiotic resistance. All *S. aureus* isolates were susceptible to linezolid, mupirocin, tigecycline, and rifampin. However, caution should be taken to prevent the development of resistant strains due to antibiotic pressure.

Conclusion

Toxic shock syndrome toxin is a superantigen toxin of immense public health importance due to its ability to excite the human immune system, ultimately leading to a cytokine storm that culminates in the pathological signs of TSS. Hence, this study was carried out to determine the prevalence of the *tst* gene in clinical isolates and to also associate the presence of this gene with phenotypic sensitivity to antibiotics. The prevalence of the *tst* gene was 67.76%, with a significantly higher prevalence among MRSA isolates than MSSA isolates. Hence, in the study, the presence of the tst gene was associated with methicillin resistance in *S. aureus.* The study also reported higher resistance to erythromycin and tetracycline among *tst*-harbouring *S. aureus* and higher resistance to cotrimoxazole, fosfomycin, ciprofloxacin, and gentamicin among *tst*-negative *S. aureus* strains. The high prevalence of the *tst* gene among clinical *S. aureus* isolates is a cause for concern in this study area. However, of more concern is the higher prevalence of this gene amongst MRSA isolates and its association with higher resistance rates to antibiotics.

Conflict of interest

We declare that we have no conflict of interest.

Financial disclousure: None.

References

1- **Reddy PN, Srirama K, Dirisala VR.** An update on clinical burden, diagnostic tools and therapeutic options of *Staphylococcus aureus*. Infectious Diseases: Research and Treatment 2017; 10: 1-15.

2- **Wanner S, Schade J, Keinhorster D, Weller N, George SE, Kull L, et al**. Wall teichoic acids mediate increased virulence in *Staphylococcus aureus*. Nature Microbiology 2017; 2: 16257.

3- **Sato'o Y, Omoe K, Naito I, Ono HK, Nakane A, Sugai M, et al.** Molecular epidemiology and identification of a *Staphylococcus aureus* clone causing food poisoning outbreaks in Japan. Journal of Clinical Microbiology 2014; 52(7): 2637-40.

4- Osiyemi JA, Ade TI, Akinduti PA, Osiyemi EO, Sunmola NO, Awoyemi OA. Regional burden of methicillin-resistant *Staphylococcus aureus* (MRSA) in South-West, Nigeria: A systematic review. Tropical Journal of Health Sciences 2020; 27(1): 1-6.

5- Carroll KC, Morse SA, Mietzner T, Miller S. Jawetz, Melnick and Adelberg's Medical Microbiology (27th ed.). New York: McGraw Hill Education; 2016:153-65.

6- **Salgado-Pabon W, Herrera A, Vu BG, Stach CS, Merriman JA, Spaulding AR, et al.** *Staphylococcus aureus* β-toxin production is common in strains with the β-toxin gene inactivated by bacteriophage. Journal of Infectious Diseases 2014; 210(5): 784-92.

7- **Andrey DO, Renzoni A, Monod A, Lew DP, Cheung AL, Kelley WL.** Control of the *Staphylococcus aureus* toxic shock *tst* promoter by the global regulator *Sar*A. Journal of Bacteriology 2010; 192(22): 6077-85.

8- **Petersson K, Forsberg G, Walse B.** Interplay between superantigens and immunoreceptors. Scandinavian Journal of Immunology 2004; 59: 345-55.

9- **Tuffs SW, Haeryfar SMM, McCormick JK.** Manipulation of innate and adaptive immunity by staphylococcal superantigens. Pathogens 2018; 7: 53.

10- **Sharma H, Smith D, Turner CE, Game L, Pichon B, Hope R, et al**. Clinical and molecular epidemiology of staphylococcal toxic shock syndrome in the United Kingdom. Emerging Infectious Diseases 2018; 24(2): 258-66.

11- **Nguyen AT, Tallent SM.** From commensal to consumer: *Staphylococcus aureus* toxins, diseases, and detection methods. Journal of AOAC International 2018; 101(1): 1127-34.

12- **Shams-Abadi MS, Halaji M, Hoseini-Alfatemi SM, Gholipour A, Mojtahedi A, Ebrahim-Saraie HS**. Epidemiology of toxic shock syndrome toxin-1 harboring *Staphylococcus aureus* obtained from clinical samples in Iran: A systematic review and meta-analysis. Ann Ig 2018; 30: 391-400.

13- **Ogah SA, Ologe FE, Dunmade AD, Lawal IA.** Facial index as seen in University of Ilorin Teaching Hospital (UITH), Ilorin Nigeria. Asian Journal of Multidisciplinary Studies 2014; 2(5): 20-22.

14- **Ade TI, Osiyemi JA, Aso RE, Akinduti PA, Sunmola NO.** Prevalence of macrolide-lincosamide-streptogramin-B resistance among clinical *Staphylococcus aureus* isolates in University of Ilorin Teaching Hospital, Ilorin, Nigeria. African Journal of Clinical and Experimental Microbiology 2022; 23(2): 168-173.

15- **Clinical Laboratory Standards Institute.** Performance standards for antimicrobial susceptibility testing (M100; 31st ed.). USA: Clinical Laboratory Standards Institute; 2021.

16-**Pinto B, Chenoll E, Aznar R.** Identification and typing of food-borne *Staphylococcus aureus* by PCR-based techniques. Systematic and Applied Microbiology 2005; 28(4): 340-352.

17-**Yu F, Liu Y, Lv J, Qi X, Lu C, Ding Y, et al.** Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of *Staphylococcus aureus* isolates associated with skin and soft tissue infections. Brazilian Journal of Infectious Diseases 2015; 19(6): 614-622.

18-**Costa AM, Kay I, Palladino S.** Rapid detection of *mec*A and *nuc* genes in staphylococci by real-time multiplex polymerase chain reaction. Diagnostic Microbiology and Infectious Disease 2005; 51: 13-17.

19-**Sultan AM, Nabiel Y.** Association of *tsst-1* and *pvl* with *mec*A genes among clinical *Staphylococcus aureus* isolates from a tertiary care hospital. Journal of Applied Microbiology 2019; 13(2): 855-864.

20-**Vandendriessche S, De Boeck H, Deplano A, Phoba M-F, Lunguya O, Falay D, et al.** Characterisation of *Staphylococcus aureus* isolates from bloodstream infections, Democratic Republic of the Congo. European Journal of Clinical Microbiology and Infectious Diseases 2017; 36: 1163-71.

21-**Ezeamagu C, Imanatue I, Dosunmu M, Odeseye A, Baysah G, Aina D, et al.** Detection of methicillin resistant and toxin-associated genes in *Staphylococcus aureus*. Beni-Suef University Journal of Basic and Applied Sciences 2018; 7: 92-97.

22-**Omar NN, Mohammed RK.** A molecular study of toxic shock syndrome toxin gene (*tsst*-1) in B-lactam resistant *Staphylococcus aureus* clinical isolates. Iraqi Journal of Science 2021; 62(3): 825-37.

23-**Derakhshan S, Navidinia M, Haghi F.** Antibiotic susceptibility of human-associated *Staphylococcus aureus* and its relation to *agr* typing, virulence genes, and biofilm formation. BMC Infectious Diseases 2021; 21: 627.

24-**Goudarzi M, Razeghi M, Chirani AS, Fazeli M, Tayebi Z, Pouriran R.** Characteristics of methicillin-resistant *Staphylococcus aureus* carrying the toxic shock syndrome toxin gene: High prevalence of clonal complex 22 strains and the emergence of new spa types t223 and t605 in Iran. New Microbes and New Infections 2020; 36: 100695.

25-**Houri H, Samadpanah M, Tayebi Z, Norouzzadeh R, Malekabad ES, Dadashi A-R.** Investigating the toxin profiles and clinically relevant antibiotic resistance genes among *Staphylococcus aureus* isolates using multiplex-PCR assay in Tehran, Iran. Gene Reports 2020; 19: 100660.

26-**Alni RH, Mohammadzadeh A, Mahmoodi P, Yousef M.** Detection of toxic shock syndrome toxin (*tsst*) gene among *Staphylococcus aureus* isolated from patients and healthy carriers. Avicenna Journal of Clinical Microbiology and Infection 2018; 5(1): e14249.

27-**Senon N, Al-Talib H, Adnan A.** Detection of toxins and antibiotic resistance genes profile among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates and their types of infection in a tertiary hospital in Malaysia. International Journal of Medical Research and Health Science 2021; 10(2): 151-159.

28-**Bhowmik D, Chetri S, Das BJ, Chanda DD, Bhattacharjee A.** Distribution of virulence genes and SCC*mec* types among methicillin-resistant *Staphylococcus aureus* of clinical and environmental origin: A study from a community of Assam, India. BMC Research Notes 2021; 14: 58.

29-**Costa FN, Belo NO, Costa EA, Andrade GI, Pereira LS, Carvalho IA, et al.** Frequency of enterotoxins, toxic shock syndrome toxin-1, and biofilm formation genes in *Staphylococcus aureus* isolates from cows with mastitis in the Northeast of Brazil. Tropical Animal Health and Production 2018; 50: 1089-97.

30-**Tuttolomondo A, Maida C, Pinto A.** Diabetic foot syndrome: Immune-inflammatory features as possible cardiovascular markers in diabetes. World Journal of Orthopedics 2015; 6(1): 62-76

31-**Gergova RT, Tsitou VS, Gergova II, Muhtarova AA, Mitov IG.** Correlation of methicillin resistance and virulence genes of *Staphylococcus aureus* with infection types and mode of acquisition in Sofia, Bulgaria. African Journal of Clinical and Experimental Microbiology 2019; 20(4): 280-288.

Ade TI, Odewale G, Daji M, Ohirhian J, Ojedele RO. Association of tsst-1 gene and phenotypic antibiotic resistance among clinical *Staphylococcus aureus* isolates in a tertiary healthcare center. Microbes Infect Dis 2023; 4(2): 350-358.