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Phenotypic and genotypic characterization of antibiotic resistance in *Staphylococcus aureus* strains isolated from schoolchildren skin lesions in Bouaké, Centre Côte d'Ivoire

WAYORO Oby Zéphirin[1,2]*, ANOH Etilé Augustin[2], TADET Juste Olivier[2,3], BOKA Irié Sara Ester[2], TUO Marie Nonfra[2], KARI-DIOULA Safiatou[1,2]COULIBALY Baba[4], DIARRASSOUBA Abdoulaye[2,3], TOURÉ Sounan Fidèle[2], GAWA Kokora Junior[2], CISSÉ Amadou[2], MONEMO Pacôme[2,3], N'GUESSAN-OKOUBO Aya Nicaise[1] , AKOUA-Koffi Chantal[2,3]

[1]Laboratoire Biologie Environnement et Santé, UFR Biosciences, Université Félix Houphouët-Boigny, 01 BP V 34 Abidjan, Côte d'Ivoire
[2]Laboratoire Bactériologie-Virologie, Centre Hospitalier et Universitaire de Bouaké
[3]UFR des Sciences Médical, Université Alassane Ouattara, BP 1174 Bouaké
[4]Institut National de la Santé Publique, Institut Pierre Richet, Bouaké

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) skin infections represent a real problem in the therapeutic management of schoolchildren. In this study, we investigated the epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) colonization in schoolchildren skin lesions in Bouaké, Centre Côte d'Ivoire. *S. aureus* isolates were examined for drugs susceptibility and *mecA*, *tetK* and *tetM* genes presence. An analytical cross-sectional study of skin lesions in schoolchildren was conducted from November 2018 to May 2019. Skin lesion swabs were analyzed using conventional bacteriological techniques. Isolated *S. aureus* strains were tested with standard antibiotics using the Kirby-Bauer technique according to CASFM/EUCAST 2019 recommendations. PCR was performed for the detection of *mecA*, *tetK* and *tetM* genes. In total, 199 strains of *S. aureus* were isolated of which 68 (34.17 %) were MRSA and 169 (85 %) were tetracycline resistant. Of the MRSA strains, 40 (58.83%) were *mecA* positive, 29 (42.65 %) harbored the *tetK* gene, 13 (19.12 %) the *tetM* gene and 6 (8.83 %) all three genes. Ninety-nine (58.57 %) of the tetracycline-resistant strains were *tetK* positive, 40 (23.67 %) were *tetM* positive and 23 (13.60 %) had both *tetK* and *tetM* genes. The resistance rate of the *tetK* and *tetM* genes associated with the *mecA* gene was 29.29 % and 30 % respectively. In conclusion, schoolchildren community acquired MRSA isolates suggested that the skin of schoolchildren with lesion may represent a significant reservoir of MRSA colonization in the community. This calls for vigilance of clinicians and microbiologists for the emergence of new epidemics in schoolchildren communities.

Keywords: Skin lesions, MRSA, *mecA*, *tetK* and *tetM* genes, schoolchildren

Résumé

Les infections cutanées à *Staphylococcus aureus* résistant à la méthicilline (SARM) représentent un réel problème dans la prise en charge thérapeutique des écoliers. Dans cette étude, nous avons étudié l'épidémiologie de la colonisation par *S. aureus* résistant à la méthicilline (SARM) dans les lésions cutanées des écoliers à Bouaké, Centre Côte d'Ivoire. Des isolats de *S. aureus* ont été examinés pour la sensibilité aux médicaments et la présence des gènes *mecA*, *tetK* et *tetM*. Une étude transversale analytique des lésions cutanées chez les écoliers a été menée de novembre 2018 à mai 2019. Des écouvillonnages de lésions cutanées ont été analysés à l'aide de techniques bactériologiques conventionnelles. Des souches isolées de *S. aureus* ont été testées avec des antibiotiques standards en utilisant la technique de Kirby-Bauer selon les recommandations CASFM/EUCAST 2019. La PCR a été réalisée pour la détection des gènes *mec*A, *tetK* et *tetM*. Au total, 199 souches de *S. aureus* ont été isolées dont 68 (34,17 %) étaient des SARM et 169 (85 %) étaient résistantes à la tétracycline. Parmi les souches de SARM, 40 (58,83 %) étaient *mecA* positives, 29 (42,65 %) portaient le gène *tetK,* 13 (19,12 %) le gène *tetM* et 6 (8,83 %) les trois gènes. Quatre-vingt-dix-neuf (58,57 %) des souches résistantes à la tétracycline étaient *tetK* positives, 40 (23,67 %) étaient *tetM* positives et 23 (13,60 %) avaient à la fois les gènes *tetK* et *tetM*. Le taux de résistance des gènes *tetK* et *tetM* associés au gène *mecA* était respectivement de 29,29 % et 30 %. En conclusion, les isolats de SARM acquis dans la communauté des écoliers suggèrent que la peau des écoliers présentant une lésion peut représenter un réservoir important de colonisation de SARM dans la communauté. Cela appelle à la vigilance des cliniciens et des microbiologistes face à l'émergence de nouvelles épidémies dans les communautés scolaires.

Keywords: Infections cutanées, SARM, genes *mecA, tetK* and *tetM*, écoliers

Corresponding author E-mail address: Zéphirin Oby Wayoro email: obywayoro@gmail.com

Introduction

Staphylococcus aureus (*S. aureus*) is the most pathogenic bacterial species belonging to the genus Staphylococcus (Effendi et al., 2019). It is estimated that about one-third of healthy population carries *S. aureus* (Kaspar et al., 2015). This bacterium is important in human pathology as it is responsible for community and nosocomial infections with variable degrees of severity (Tattevin, 2011; Zinzendorf et al., 2013). The main risk factors for infection are nasal carriage and any break in the cutaneous mucosal barrier, leading to germ penetration (Masuik et al., 2021). Over the past decade, many authors have increasingly witnessed the emergence of antibiotic-resistant strains, particularly methicillin-resistant Staphylococcus aureus (MRSA) strains (Kengne et al., 2019). The prevalence of MRSA in the world is very heterogeneous geographically. Moreover, the period of study, services and living conditions of the concerned populations, can lead to both increased medical expenses and mortality (Kong et al., 2016; Garoy et al., 2019). The emergence of MRSA infections in the community is often associated with increased morbidity and high children mortality risk (Kateete et al., 2019; Nyasinga et al., 2020). Moreover, MRSA strains can also resist other commonly used antibiotics such as tetracycline and penicillin (Schnellmann et al., 2006; Gurung et al., 2020). Methicillin resistance in *S. aureus* is linked to the synthesis of a penicillin-binding protein, PLP2a (Quincampoix and Mainardi, 2001), which leads to resistance to all betalactam antibiotics (Zhang et al., 2009). The synthesis of this PLP2a is under the control of the mec gene, located on a chromosomal mobile genetic element (Biedendach et al., 2004). Tetracycline is a broad-spectrum antibiotic usually used in the treatment and prevention of bacterial infections (Biedendach et al., 2004). Most tetracycline-resistant bacteria have acquired tetracycline genes. Two main mechanisms of tetracycline resistance have been described in *S. aureus*: active efflux, resulting from the acquisition of the *tetK* and tetL genes located in a plasmid and subsequent ribosomal protection by elongation factor-type proteins that are encoded by *tetM* or *tetO* genes (Emaneini et al., 2013). A spectrum of antibiotics resistant bacteria including MSRA have been reported in Ghana (Krumkamp et al., 2020) and *S. aureus* genotypically identified (Wolters et al., 2020).

In Côte d'Ivoire, cases of skin lesions are reported every year in primary schools, but adequate management is scarcely addressed due to the lack of data on genotyped MSRA from skin lesions. Data on *S. aureus* resistance to antibiotics mainly focused on clinical and/or nosocomial isolates in Côte d'Ivoire. Moreover, data on detection of genes responsible for antibiotic resistance in *S. aureus* responsible for certain skin lesions are still scarce in Côte d'Ivoire. This is especially true in Bouaké, the capital city in Central Côte d'Ivoire where no such data are available in schools. The objective of this study was to establish a phenotypic profile of commonly used antibiotics and genotypically characterize *S. aureus* strains isolated from schoolchildren skin lesions in Bouaké using PCR detection of *tetM*, *tetK* and *mecA* genes.

Materials and Methods

Type, study population and sampling

This cross-sectional study was held in five primary schools in the city of Bouaké from November 2018 to May 2019. It concerned any schoolchild with skin lesion(s) and under 16 years old, regardless of sex. The skin lesion(s) taken into account were in the form of an open or closed wound with palpable liquid collection (suppurative or serous). From all participating schoolchildren an informed consent of a parent or legal guardian was obtained. Sampling consisted of swabbing of open lesions; in the case of a closed lesion with fluid collection, a syringe puncture was performed to aspirate the fluid. The swabs and syringes were placed in a cooler and sent within 4 hours to the laboratory for bacteriological analysis.

Isolation and phenotypic identification of *S. aureus* strains

The swabs and pus contained in the syringes were grown on Columbia agar (Bio-Rad, Marmes-la Coquette, France) enriched with 5% fresh sheep blood and incubated at 37°C for 18 to 24 hours. Colonies showing typical morphologi

cal characteristics of *S. aureus* were subjected to Gram staining and observed microscopically for confirmation of cocci morphology and catalase testing was performed using 3% hydrogen peroxide (Shahraz et al., 2012). The identification of isolates was performed using the following biochemical tests: oxidation and fermentation on Chapman agar (Bio-Rad, Marmes-la Coquette, France), Dnase tests (Gundogan et al., 2005) and the production of coagulase by the search for free staphylocoagulase with Rabbit plasma (Bio-Rad, Marmes-la Coquette, France) then bound staphylocoagulase with the PASTOREX Staph Kit (Bio-Rad, Marmes-la Coquette, France). The reference strains used for microbiological analysis were *S. aureus* ATCC 29213 sensitive to cefoxitin and *S. aureus* ATCC 43300 resistant to cefoxitin. The bacteriological analyses were carried out at the Bacteriology-Virology Laboratory of the University Teaching Hospital of Bouaké.

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolated *S. aureus* strains was determined by the agar diffusion method. The inhibition diameters were interpreted according to the criteria of the French Commitee for Antibiogram (CASFM/EUCAST V2.0 May 2019). The resistance of staphylococci to methicillin was investigated using a 30 µg cefoxitin disc. Strains with an inhibition diameter < 22 mm were considered methicillin-resistant *Staphylococcus aureus* (MRSA). The resistance profile of *S. aureus* was determined from 21 antibiotic discs: penicillin G (1 µg), cefoxitin (30 µg), gentamicin (30 µg), tobramycin (10 µg), kanamycin (30 µg), erythromycin (15 µg), clindamycin (2 µg), linezolide (10 µg), pristinamycin (15 µg), trimethoprim sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), vancomycin (30 µg), tetracycline (30 µg), minocycline (30 µg), chloramphenicol (30 µg), rifampicin (5 µg), fusidic acid (10 µg), fosfomycin (200 µg).

Genomic DNA extraction

Genomic DNA extraction of selected methicillin and tetracycline resistant strains was done using the heat shock method described by Tuo et al. (2020). Briefly, one to five colonies of the bacterial culture were taken and suspended in an eppendorf tube containing 1 ml sterile distilled water. The suspension was boiled for 30 minutes at 95°C in a water bath. The suspension was then centrifuged at 14,000 rpm for five minutes and the supernatant was transferred to filtration columns. After a final centrifugation at 1200 rpm for five minutes, the supernatant was transferred to a new Eppendorf tube and stored at -20°C until analysis.

PCR detection of *mecA*, *tetK* and *tetM* genes

Molecular detection of the *mecA*, *tetK* and *tetM* genes was performed by simplex PCR using primers specific for each targeted gene (Table 1). PCR amplification was performed in a 25 µL reaction mixture containing 3µl of DNA extract, 6.25 µl of 2x GoTaq® G2 Hot Start Colorless Master Mix (Promega, Madison, USA), 1.25 µL of each forward and reverse primer (10 µM) and 13.25 µL of Nuclease free water. Amplification of all these resistance genes was performed on a thermal cycler (Applied Biosystems, Inc., CA). The cycling parameters is as follows: initial denaturation at 94°C for 3 min followed by 30 amplification cycles at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30s (except for the final cycle, which was 4 min for extension step) (Strommerger et al., 2003). PCR products were analyzed on a 1.5% agarose gel at 120 V for 50 min in TBE 1X containing GelRed® 10,000X nucleic acid stain using a 100 bp DNA ladder (Promega, USA) as a size marker. Visualization of the bands was performed under UV (ultraviolet) in a Bio-Doc Analyse 2.2 of Biometra.

Table 1: Primers used for PCR

Genes	Primers	Séquence of primers (5'-3')	Size of amplicons (bp)	GenBank accession no.	Reference
MecA	*mecA1*	AAAATCGATGGTAAAGGTTGGC	532	Y00688, bp 1282–130	**Strommerger *et al*., 2003**
	mecA2	AGTTCTGCAGTACCGGATTTGC		Y00688, bp 1814–1793	
Tetk	*tetk1*	GTAGCGACAATAGGTAATAGT	360	S67449, bp 871–891	
	tetk2	GTAGTGACAATAAACCTCCTA		S67449, bp 1231–1211	
TetM	*tetM1*	AGTGGAGCGATTACAGAA	158	X56353, bp 301–318	
	tetM2	CATATGTCCTGGCGTGTCTA		X56353, bp 459–440	

Statistical analyses of the data

The socio-demographic and microbiological data of the schoolchildren were recorded in a database and analysed using Epi-Info7 version 7.1.5.2 (2015); quantitative variables were presented as means and standard deviations and qualitative variables as proportions. Values of $p<0.05$ were considered statistically significant.

Results and discussion

In this study, we describe the epidemiology of *S. aureus* isolated from skin lesions of schoolchildren in five schools in Bouaké, central Côte d'Ivoire. From a total of 3174 schoolchildren surveyed, 1220 had skin lesions out of which 325 were enrolled in the study. Three hundred and forty biological samples (skin lesion swabs and syringe pus punctures) were collected and 199 strains of *S. aureus* were isolated. Out of the 199 *S. aureus* strains, 68 (34.17 %) were resistant to methicillin and 169 (84.83 %) to tetracycline. The distribution of MRSA by school showed a predominance of strains in the TSF 22/68 (32.36 %) school group, followed by Khankro 16/68 (23.53 %). N'Dakro, Minankro and West Bouaké schools showed 13/68 (19.12 %), 9/68 (13.24 %), 7/68 (10.30 %) respectively. All *S. aureus* strains were resistant to most of the antibiotics used (Table 2). Of the 169 S. *aureus* strains resistant to tetracycline, 65 (38.47 %) were MRSA and 56 (33.14 %) were resistant to minocycline. *S. aureus* strains were generally susceptible to fosfomycin (98%), trimethoprim-sulfamethoxazole (97 %), gentamycin (83 %), pristinamycin (67 %) and minocycline (57 %). Of the 199 *S. aureus* strains isolated, 34.17 % were found to be MRSA by the cefoxitin disc diffusion method. This result is significantly similar to that obtained by Adhikari et al. (2017) with a prevalence of 35, 50 %. In Ghana, the MRSA rate recorded in a recent study of chronic wounds in a rural area was slightly lower at 29 % (Krumkamp et al,.2020). However, lower prevalence have been reported in some community studies. For example, in schoolchildren aged 5 to 15 years old in three schools in the Republic of Fiji in the tropical West Pacific, *S. aureus* strains isolated from impetigo lesions included 7 % (20/299) MRSA (Jenney et al., 2014). Furthermore, in eastern Uganda, out of 742 healthy children, 140 *S. aureus* strains (18.9 %, 140/742) were isolated and identified, of which 5.7 % (42/742) were MRSA. Almost all MRSA isolates (95.2 %, 40/42) were multi-drug resistant (Kateete et al., 2019). Some authors have reported slightly higher prevalence of 42.3% and 91.7 % respectively (Alli et al., 2015; Adegboyega et al., 2019). In some regions of Africa MRSA prevalence is highly variable. In general, the average prevalence in Western Africa is 16 % in Senegal and Niger (Beurec et al., 2011), 20-47 % in Nigeria (Ghebremethin et al., 2009; Akerele et al., 2014), 36 % in Benin (Ahoyo et al., 2006), and 35.7 % in Togo (Kambate et al., 2011). But a lower frequency (less than 10 %) has been reported in Maghreb countries (Elouennass et al., 2008). These rates across African countries are alarming and represent a real threat to the public health of populations in resource-limited countries. Since data on the epidemiology of MSRA on schoolchildren skin lesions is limited in West Africa, the 34.17 % prevalence of MRSA observed in this study can be considered highest within this region. This MRSA rate among schoolchildren may be explained by the inaccessibility to safe drinking water and good hygiene conditions, unfavorable economic conditions of parents associated with malnutrition (Alsan et al., 2015), overcrowding of students in classrooms, the frequency of superinfections of wounds and skin lesions, and the inappropriate application of broad-spectrum antibiotic therapies to skin infections. In addition, the lack of health centers, infirmaries or dispensaries in schools resulting in immediate inaccessibility to diagnostic tools and adequate health care could contribute to the spread of MRSA among school children. Poverty does not allow some students living in outlying areas of large cities to have access to quality antibiotics. Indeed, a Ugandan study showed that poverty was responsible for children stopping treatment early or sharing a

Table 2: Antibiotic resistance profile of *S. aureus* strains isolated from skin lesions

	Number of isolated *S. aureus* per primary school					
	TSF-4(n=61)	N'Dakro (n=38)	Khankro (n=37)	Bouaké-ouest (n=34)	Minankro (n=29)	Total(n=199)
	R%	R%	R%	R%	R%	R%
Antibiotics						
PNG	61(100)	32(84.21)	35(94.59)	32(94.11)	29(100)	189(95)
FOX	22(36.06)	13(21.31)	16(43.24)	8(23.52)	9(31.03)	68(34)
KNM	6(9.83)	4(10.52)	14(37.83)	8(23.52)	4(13.79)	36(18)
GMN	4(6.55)	5(13.15)	7(18.91)	8(23.52)	10(34.48)	34(17)
TMN	5(8.19)	3(4.91)	11(29.72)	7(20.58)	12(41.37)	38(19)
ERY	7(11.47)	14(36.84)	24(64.86)	19(55.88)	22(75.86)	86(43)
CD	4(6.55)	12(31.57)	27(71.05)	15(44.11)	18(62.06)	76(38)
LZD	4(6.55)	15(39.47)	25(67.56)	8(23.52)	22(75.86)	74(34)
PTN	1(1.63)	20(32.78)	12(32.43)	18(52.94)	15(5172)	66(33)
SXT	0	0	2(5.40)	4(11.76)	0	6(3)
CHL	6(9.83)	5(13.15)	8(21.62)	0	6(20.68)	25(13)
CIP	45(73.77)	25(65.78)	19(51.35)	25(73.52)	21(72.41)	135(68)
LVX	41(67.21)	24(63.15)	19(51.35)	20(58.82)	19(65.51)	123(62)
NXN	38(62.29)	30(78.94)	25(67.56)	32(94.11)	28(96.55)	153(77)
OFX	44(72.13)	26(68.42)	22(59.45)	31(91.17)	20(68.96)	143(72)
VAN	0	4(10.52)	6(16.21)	12(35.29)	8(27.58)	30(15)
TET	51(83.60)	33(86.84)	35(94.59)	27(79.41)	23(79.31)	169(85)
MNO	26(42.62)	19(50)	11(29.72)	12(35.29)	17(58.62)	85(43)
RIF	7(11.47)	1(2.63)	1(2.70)	0	1(3.44)	10(5)
FOS	0	2(5.26)	0	2(5.88)	0	4(2)
FAD	2(3.27)	1(2.63)	5(13.71)	0	0	8(4)

PNG : penicillin G , FOX : cefoxitin , KMN : kanamycin, GMN : gentamycin, TMN : tobramycin, ERY : erythromycin , CD : clindamycin, LZD : linozolid, PTN : pristinamycin, SXT : trimethoprim-sulfamethoxazole, CHL : chloramphenicol, CIP: ciprofloxacin, LVX: levofloxacin, NXN: norfloxacin, OFX: ofloxacin, VAN: vancomycin, TET: tetracycline, MNO: minocycline, RIF: rifampicin, FOS: fosfomycin, FAD: fusidic acid

single dose of treatment with an entire family (Byarugaba, 2004). A literature review assessing the burden of MRSA in Africa also suggested socio-economic conditions, communicable and non-communicable diseases, and selection pressure due to overuse of antibiotics as factors influencing the variable prevalence of MRSA in different localities (Falagas et al., 2013). According to Ouédraogo et al. these factors are the compelling causes of the spread and emergence of multidrug-resistant *S. aureus* infections (Ouédraogo et al., 2017). In this study, the cyclin antibiotic susceptibility test showed strong resistance.

One hundred and sixty-nine (85 %) of the strains were resistant to tetracycline and 65 (38.46 %) had concomitant resistance to methicillin. Our results diverge considerably from those of some authors who reported higher rates of concomitant methicillin resistance to tetracycline in the UK (57.1 %) and lower rates in Ireland (1.5 %) (Schmitz et al., 2001; Reynolds et al., 2004). The incidence of tetracycline resistance in North America was 11.9 % and 2.5% for MRSA isolates. In Eastern Europe and South Africa the incidence was 16. 1 % and 6.1 % respectively (Fluit et al., 2000). The high rate of tetracycline resistance observed in the present study could be due to the abusive use of this antibiotic. Indeed, the uncontrolled use of this molecule in pig, cattle and chicken farms due to its broad spectrum of action (Chopra and Robert 2001; Levy, 2001) may have led to strain resistance and consequent infection emergence (Abba et al., 2017). Tetracycline misuse can also allow resistant strain selection pressure. Such resistant bacteria are likely to exchange multiple resistance genes with other bacteria, sometimes leading to the emergence of multi-drug resistant bacteria (Koné et al., 2019). In addition, tetracycline is one of the relatively inexpensive antibiotics in some countries, and is most frequently prescribed after penicillin for the treatment of a number of bacterial infections, including those caused by staphylococci (Trzcinski et al., 2000). In the United States, tetracycline is used in the treatment of many human infections, including acne and the prophylaxis and treatment of protozoan and periodontal diseases (Roberts, 2003). Direct comparison of the occurrence of tetracycline and MRSA is poorly documented in sub-Saharan Africa and lacking in Côte d'Ivoire.

Of the 68 methicillin-resistant strains (MRSA), 40 (58.83 %) were positive for the *mecA* gene, while 29 (42.65 %) harbored the *tetK* gene, 13 (19.12 %) the *tetM* gene and 6 (8.83 %) all three genes (Figure 1). The *mecA* gene was detected in all five schools with 37.5 % of cases in TSF-4 followed by Khankro and Bouaké - West schools with 17.5 % of cases each. Of the 169 tetracycline-resistant strains, 99 (58.57 %) carried the *tetK* gene, 40 (23.67 %) carried the *tetM* gene and 23 (13.60 %) had both the *tetK* and *tetM* genes. The distribution of strains carrying *mecA*, *tetK* and *tetM* genes according to the different schools is presented in Table 3.

Table 3: Distribution of resistance genes according to school location using PCR

	Resistance gene		
	mecA **n/N(%)**	***tetK*** **n/N(%)**	***tetM*** **n/N(%)**
Primary school			
TSF-4	15/22(68.19)	30/51(58.83)	14/51(27.45)
N'Dakro	5/13(38.47)	14/30(46.66)	1/30(3.34)
Khankro	7/16(43.75)	14/35(40)	11/35(31.43)
Bouaké- ouest	7/8(87.5)	27/27(100)	9/27(33.34)
Minankro	6/9(66.67)	14/23(68.87)	5/23(21.74)
Total	40/68(58.83)	99/169(58.58)	40/169(23.67)

mecA: methicillin resistance gene. *tetK* and *tetM:* tetracycline resistance genes. N: number of antibiotic-resistant *S. aureus* strains tested by PCR in each primary school. n: number of strains positive for the resistance genes tested. (%): percentage of PCR-positive genes

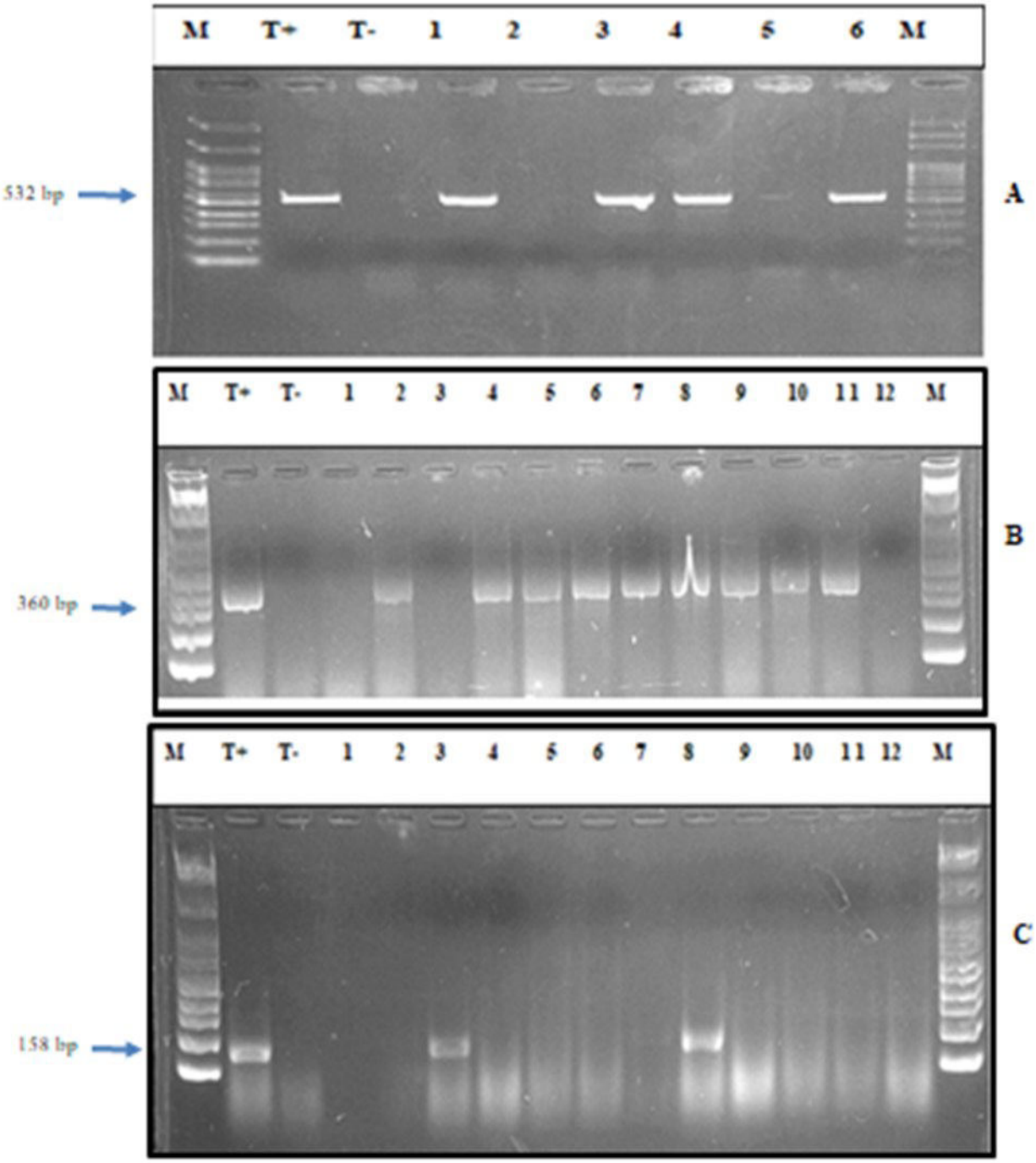

Figure 1: Agarose gel (1.5 %) electrophoresis of PCR amplified *mecA*, *tetK* and *tetM* genes from *S. aureus* strains.
M: DNA marker (100-1500 bp), T+: positive control, T-: negative control, M: DNA marker (100-1500 bp).
A) *mecA* gene amplification of positive strains (1, 3, 4 and 6) and negative strains (2 and 5)
B) *tetK* gene amplification of positive *S. aureus* strains (2, 4, 5, 6, 7, 8, 9, 10, 11) and negative strains (1, 3, 12).
C) *tetM* gene amplification of positive *S. aureus* strains (3 and 8) and negative strains (1, 2, 4, 5, 6, 7, 9, 10, 11 12).

PCR detection of *mecA tetK*, *tetM* genes confirmed the presence of these common genetic determinants of methicillin resistance as well as classical tetracycline resistance in these isolates. However, 28 of the 68 MRSA strains found by the diffusion method did not show a *mecA* gene in the PCR test. In this study, there were discrepancies in the frequencies of phenotypically characterized MRSA and *mecA* gene detection by PCR, as has been reported elsewhere (Davoodi et al., 2012; Pounajaf et al., 2014). Indeed, the diffusion test using cefoxitin discs can show false negatives, especially with strains with heterogeneous resistance (Anand et al., 2009; Pillai et al., 2012). Furthermore, the determination of methicillin resistance in *S. aureus* by conventional bacteriology is subject to variations, including inoculum size, diameter of inhibition, pH and salt concentration of the medium, incubation time, etc. One of the possible reasons for methicillin resistance in the absence of the *mecA* gene may be due to the hyperproduction of β - lactamase (Pounajaf et al., 2014). To this end, the use of PCR for the detection of the *mecA* gene is very useful and accurate. Moreover, in a study on methicillin resistance in *S. aureus*, it was revealed that the mecC gene is a homolog of the *mecA* gene. Thus, the mecC gene was found to confer methicillin resistance in *S. aureus* in which the *mecA* gene was absent (Ballhausen et al., 2014). Although further research is needed to understand this genotypic phenomenon, questions can be raised if *mecA* is considered the only genetic marker for methicillin resistance. In our study, we could not verify the presence of mecC as a possible reason for the phenotypic expression of methicillin resistance in the absence of the *mecA* gene. This study was entirely dependent on the detection of *mecA* on chromosomal DNA. The *mecA*-encoded plasmid may have contributed to methicillin resistance in the phenotypic tests. Therefore, all genotypic possibilities should be analysed for phenotypic expression of methicillin resistance in *S. aureus* in order to discover the appropriate epidemiological marker for methicillin resistance as envisaged by some authors (Chen et al., 2012).

Moreover, the prevalence of the *mecA* gene on the African and European continent varies regionally from 6.5 % to more than 50 %. (Shittu et al., 2012; Ibadin et al., 2017). The detection of the *mecA* gene among *S. aureus* isolates is explained by the fact that this bacterium has a strong adaptive capacity and has developed different mechanisms of resistance to antistaphylococci (Lakhundi et Zhang, 2018). In addition, most strains produce penicillinase, which leads to cross-resistance between penicillins and other Betalactam antibiotics through the production of a protein, PLP2a. (Dumitrescu et al., 2010).

The prevalence of *tetK* (58.57 %) and *tetM* (23.67 %) resistance genes observed in *S. aureus* isolates can be explained by their usual genetic locations. Indeed, the presence of the *tetK* gene on small multicopy plasmids and *tetM* on conjugative transposons contributes to the spread of these determinants (Abdolmaleki et al., 2019). The coexistence of *tetM* and *tetK* genes among MRSA isolates was also detected in this study. Data on the prevalence of tetracycline-encoding genes are limited in Africa. Our results are in accordance with those indicating the presence of *tetK* and *tetM* genes in *S. aureus* isolates (Trzcinski et al., 2000; Emaneini et al., 2013). However, the presence of the tetracycline resistance determinants *tetK* and *tetM* among MRSA isolates also varied by region, ranging from 11.9 % to 46.2 % among the isolates tested. (Biedendach et al., 2004; Fluit et al., 2005). The difference in prevalence observed between tetracycline resistance by the phenotypic method using the tetracycline disc (30 µg) and the PCR test is due first, to the difference of sensitivity of the two methods use and secondary to tetracycline-dependent energy efflux or protection of bacterial ribosomes from tetracycline action. The genes encoding this resistance are normally acquired via plasmids and/or transferable transposons.

The presence of the *tetM* and *tetK* determinants in Gram-positive and Gram-negative bacteria significantly reduces the efficacy of tetracycline treatment (Robert, 2005). The reliability of phenotypic tests can be inconsistent, depending on the quality of the sample, the size of the initial inoculum and variations in laboratory culture conditions that can alter the phenotypic expression of resistance (Billy, 2003). The detection of the three resistance genes observed in the five primary schools in Bouaké could be explained by ignorance of resistance problems in the community, self-medication by parents or students and the long-term use of different classes of antimicrobials in the agricultural sector.

Conclusion

In Côte d'Ivoire, the multi-resistance of *S. aureus* to antibiotics remains a real public health problem. Hygiene conditions in schools are still precarious and the use of antibiotics is often abused and poorly controlled. The results of the phenotypic data from our work can be used in the management and treatment of *S. aureus* skin infections. The genetic determinants *mecA*, *tetK* and *tetM* investigated in this study indicated a significant distribution of these genes in schoolchildren. The MRSA cross-infections observed are alarming. They may increase morbidity and mortality in apparently healthy schoolchildren. The virulence and toxicity of *S. aureus* strains isolated from schools should be studied for better management of skin lesions.

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Ethic statement

The ethic committee “Direction Médicale Scientifique du CHU de Bouaké” of Côte d’Ivoire (agreement number 011MSP/CHU-B/DG/DMS/ONAR/18) and the responsible of schools to Bouaké “DREN” (agreement number 1226/2018/DREN-BKE1) approved the study. Consents of a parent or legal guardian for children included were obtained.

Conflict of Interest

No conflict of interest exists for this study.

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