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Prevalence and Characteristics of Livestock-Associated Methicillin-Susceptible *Staphylococcus aureus* from Pigs and Workers in Côte d'Ivoire

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A B S T R A C T

The emergence of methicillin-susceptible *Staphylococcus aureus* (MSSA), particularly the MSSA CC398 *spa*-type t571 clone, associated with livestock, represents a public health concern due to its zoonotic potential and ability to cause severe infections in humans. This study, conducted in Côte d'Ivoire between January 2022 and October 2023, is the first investigation aimed at assessing the prevalence, genetic characteristics, and antimicrobial resistance profiles of MSSA strains isolated from pigs and exposed workers. A total of 1,176 pigs and 50 workers were sampled across 15 farms in two regions (autonomous district of Abidjan and Sassandra-Marahoué district). Nasal swabs (from humans and pigs) were collected and subjected to microbiological anlysis for *S. aureus* research. The isolated strains were phenotypically identified and submitted to antibiotic susceptibility testing using the Kirby-Bauer method. They were also submitted to molecular characterization targeting the *nuc*, *spa* and *lukS-lukF* genes, as well as tetracycline resistance genes (*tet(K)*, *tet(L)*, *tet(M)*, *tet(O)*). The results show that nasal carriage prevalence of MSSA was 12.2% in pigs and 62.7% in workers, highlighting a significant occupational risk. Among pig categories, carriage was significantly higher in weaned piglets (19.2%). Molecular analysis revealed a strong predominance of the CC398 clonal complex, particularly spa-type t571, in both pig (97.2%) and human (100%) isolates, suggesting probable zoonotic transmission. High rates of tetracycline resistance were observed in the isolates (68% from those of pigs, 83.8% from those of humans), primarily linked to the presence of *tet(K)*and *tet(L)* genes. The absence of the *lukS-lukF-PV* gene (encoding the PVL toxin) in all isolates likely reflects their animal origin. Antibiotic susceptibility testing revealed resistance phenotypes to other antibiotics such as penicillin, ampicillin, and ciprofloxacin, demonstrating the multidrug resistance of these clones. Additionally, the detection of the MSSA CC398 spa-type t1250 clone and the identification of a novel spa-type (t6608) in the pig population underscored the genetic diversity of circulating strains. This study reports, for the first time in Côte d'Ivoire, the emergence of the MSSA CC398 spa-type t571 clone in pig farms. Antibiotics may have favored the selection and dissemination of this clone. Further molecular analyses are needed to more precisely assess the genetic relatedness between pig and human isolates.

Introduction

The resurgence of *Staphylococcus aureus* infections, particularly infections due to methicillin-resistant *Staphylococcus aureus* (MRSA), poses substantial public health challenges worldwide, primarily due to their role in zoonotic transmission from animals to humans (Voss *et al*., 2005). Studies have shown that in addition to MRSA, some strains of methicillin-susceptible *S. aureus* (MSSA) associated with livestock, such as clonal complex 398 (CC 398), can also colonize humans (van Cleef *et al*., 2011).

The prevalence of these livestock-associated *S. aureus* strains has been documented in multiple industrialized countries, including their occupational risk factors among farm workers exposed to direct animal contact (Khanna *et al*., 2008; van Duijkeren *et al*., 2007). In these nations, livestock-associated *Staphylococcus aureus* strains have been prevalent among pigs and pig handlers, presenting a potential risk for cross-species transmission and human infection (EFSA, 2009).

The livestock-associated CC398, particularly *spa* types such as t011 and t034, is commonly identified in swine and swine-associated human populations (Cuny *et al*., 2015). Typically, tetracycline resistance is a notable feature in CC398 *S. aureus* strains from animals, primarily driven by the *tet*(M) gene (Kadlec *et al*., 2012). Interestingly, while methicillin-resistant *S. aureus* (MRSA) variants of CC398 often lack the Panton-Valentine leukocidin (PVL) gene, some methicillin-susceptible strains (MSSA), particularly those of *spa*-type t571, have acquired PVL, heightening risks of virulence and severe infections in humans (Argudín *et al*., 2011). However, studies investigating methicillin-susceptible *Staphylococcus aureus* strains of porcine origin are limited in Africa in general, and specifically, no data is available concerning Côte d'Ivoire (Samutela *et al*., 2021).

Understanding the genetic profiles, prevalence, antimicrobial susceptibility profiles, and zoonotic potential of MSSA strains circulating in this environment (pig farming) is essential to assess zoonotic transmission risks and monitor antibiotic resistance trends. This study aims to characterize MSSA strains in pigs and pig workers in Côte d'Ivoire, focusing on genetic markers and antimicrobial resistance profiles to better understand their epidemiological impact and public health implications.

Materials and Methods

Ethical Approval and Participant Consent

All participants were previously informed of the objectives and modalities of the study, and then signed a consent form of their own free will. Furthermore, the study received approval from the National Committee for Ethics in Life and Health Sciences (CNESVS) of Côte d'Ivoire under reference 137-22/MSHP/CNESVS-km.

Description and Selection of Pig Farms

A cross-sectional study was conducted from January 2022 to October 2023, covering both peri-urban and rural areas throughout the rainy and dry seasons. The investigation took place at pig farms located in three municipalities in Côte d'Ivoire: Bingerville, Bouaflé, and Daloa.

While Bouaflé and Daloa are part of the Sassandra-Marahoué District, Bingerville belongs to the autonomous District of Abidjan, the economic capital of Côte d'Ivoire. The Autonomous District of Abidjan, known for hosting the majority of the country's pig farms, accounts for 70 % of the national pig herd and is predominantly characterized by intensive farming practices (MIRAH, 2018).

In contrast, the Sassandra-Marahoué district mainly consists of small-scale operations (MIRAH, 2018). The pig production systems in this region vary considerably, ranging from traditional to semi-intensive and intensive methods. Bingerville, Daloa, and Bouaflé were selected because they are located in the two districts (the Autonomous District of Abidjan and the Sassandra-Marahoué District), which together account for over 70 % of the national pig production (MIRAH, 2018). These localities reflect a diversity of farming contexts, from peri-urban systems (Bingerville) to rural areas with high pig farming activity (Daloa and Bouaflé).

Their selection enables a representative study of antibiotic use and the circulation of methicillin-resistant *S. aureus* in different pig farming environments. A total of 15 pig farms were included in the study, with an equal distribution across the communes of Bingerville, Bouaflé, and Daloa (five farms per commune). For convenience, the Autonomous District of Abidjan and the Sassandra-Marahoué District will be referred to as A and B, respectively.

Target Population

The study population included, on the one hand pig workers, on the other hand several categories of pigs (sows, piglets, and fattening pigs) raised in enclosures.

Sampling and Inclusion Criteria

Pigs

In this investigation, both the physiological stage and the approximate age of the pigs were taken into account. The required sample size was calculated based on an expected prevalence of 50 % (Lozano *et al*., 2016; Samutela *et al*., 2021), a 5 % margin of error, and a 95 % confidence interval, using the formula recommended by the World Health Organization (WHO, 1991). This calculation established a minimum sample size of 385 animals. A stratified random sampling method was then applied to distribute the samples among different groups: Suckling piglets, weaned piglets, sows, and fattening pigs. In total, 1176 pigs were sampled from 15 farms: 642 in Bingerville, 240 in Bouaflé, and 294 in Daloa. Detailed information regarding the samples is provided in Table 1.

Human participants

The study involved healthy adults aged 18 and over. Individuals were excluded if they had taken antibiotics in the two weeks prior to sampling, had been employed at their current workplace for less than one year, worked in sectors not exclusively related to pig farming, or held positions without direct animal contact, such as administrative roles. Recruitment was carried out on the basis of informed consent, with every eligible participant required to sign a consent form before participating. In total, 50 volunteers agreed to take part in the study. Forty-five people were recruited in the commune of Bingerville, two in Bouaflé, and three in Daloa.

Sample collection and transport

Nasal swabs were obtained from both pigs and pig workers following the protocols outlined below.

Pigs

At each farm, nasal swabs were collected from three distinct pig groups: piglets (Suckling piglets, Weaned piglets), fattening pigs, and sows. For every group, between 2 and 10 animals were randomly chosen for sampling. Using sterile swabs (IZ, Becton-Dickinson), nasal secretions were gathered by gently inserting the swab into one nostril and performing five complete rotations to ensure adequate collection; the same procedure was then repeated in the other nostril with the same swab. The samples were transferred into tubes containing a liquid transport medium (Stuart type, Oxoid), with each tube meticulously labeled to indicate the animal's category, approximate age, and the farm's identity and geographic location. Once sealed, the tubes were stored at 4 °C in a cooler equipped with cold packs and subsequently transported to the National Public Health Institute (INSP) laboratory within 24 hours. In the laboratory, each sample was inoculated into 2 mL of an enrichment broth specially prepared with 10 g/L tryptone, 75 g/L NaCl, 10 g/L mannitol, and 2.5 g/L yeast extract, following the guidelines established by Bergey *et al*. (1986). The enrichment broths were then incubated at 35 °C for a period of 24 to 48 hours to facilitate comprehensive analysis.

Pig workers

Nasal swabbing was conducted on eligible pig workers using a similar approach. Sterile swabs (IZ, Becton-Dickinson) were employed to collect samples by gently inserting the swab about 2 to 3 cm into one nostril, rotating it five complete times to collect nasal secretions; the procedure was then repeated in the opposite nostril using the same swab. The collected samples were deposited into tubes containing a Stuart-type liquid transport medium (Oxoid). Prior to collection, each tube was clearly labeled with detailed socio-demographic information of the participant, as well as the farm's identity and geographic location. After sealing, the samples were maintained at 4 °C in a cooler with cold packs and delivered to the INSP laboratory within 24 hours. In the laboratory, each sample was introduced into 2 mL of an enrichment broth composed of 10 g/L tryptone, 75 g/L NaCl, 10 g/L mannitol, and 2.5 g/L yeast extract, in accordance with the recommendations of Bergey *et al*. (1986). The inoculated broths were then incubated at 35 °C for 24 to 48 hours to allow for detailed analysis.

Isolation and phenotypic identification of *S. aureus*

After a 24-hour incubation at 35 °C, 10 µL of the enrichment culture is streaked onto selective Mannitol

Salt Agar (MSA) plates (Bio-Rad, Marnes-la-Coquette, France). The plates are then incubated at 35 °C for 24 to 48 hours and examined for the presence of *S. aureus*. From each sample, three colonies displaying the typical morphology: round, convex, yellow-pigmented with a surrounding yellow halo are selected as presumptive *S. aureus* candidates.

These colonies are subsequently confirmed by assessing their Gram stain appearance and by performing the catalase test, tube coagulase test, and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad). Suspect *S. aureus* colonies (Gram+, catalase +, coagulase +) are then re-isolated on Chapman agar. Prior to this step, they are enriched in Tryptic Soy Broth (TSB; Thermo Fisher Scientific, Oxoid™, United Kingdom) supplemented with 75 g/L NaCl and incubated at 35 °C for 24 hours to secure pure colonies.

The confirmed *S. aureus* strains are stored in cryotubes (Deltalab, Spain) at –80 °C for subsequent molecular analyses and antibiotic susceptibility testing. All isolates are verified to lack the *mec*A gene and demonstrate susceptibility to both cefoxitin and oxacillin, although detailed data are not provided in this report.

Molecular Analysis

The molecular characterization of the presumed *Staphylococcus aureus* strains was carried out in two stages. First, total DNA was extracted from the bacterial strains. Then, two genotypic approaches were employed: polymerase chain reaction (PCR) and sequencing, to confirm the identity of the isolates and analyze their genetic profiles.

Extraction of total DNA

Staphylococcus aureus isolates stored at -80 °C were subcultured on trypticase soy agar plates for 18–24 hours at 35 °C. After incubation, two to three colonies were picked and suspended in vials containing 3 mL of sterile nuclease-free physiological saline (API^{R} NaCl 0.85%, Biomérieux SA, Marcy-l'Etoile, France).

After vortex homogenization, 200 µL of the bacterial suspension was used for DNA extraction using the QIAamp DNA Mini Kit according to the manufacturer's guidelines (Qiagen, Germany, GmbH). The extracted DNA samples were stored in 1.5 mL conical tubes that were previously labeled and frozen at -20 °C for later use.

PCR

The following genes were investigated in *Staphylococcus aureus* strains using the conventional PCR method: the *nuc* gene (species-specific), the tetracycline resistance genes (*tet*(K), *tet*(L), *tet*(M) and *tet*(O), the Panton-Valentine toxin gene (*lukS-lukF*), as well as the gene encoding protein A (*spa*). A set of primers listed in Table 2 was used in accordance with the protocols described below to detect each of these genes.

Detection of the *nuc* gene

The *nuc* gene was amplified to confirm *S. aureus* identification, as previously described (Maes *et al*., 2002; Brakstad *et al*., 1992).

Detection of tetracycline resistance genes (*tet*(K), *tet*(L), *tet*(M) and *tet*(O))

Tetracycline resistance genes were detected using specific primers, as described in prior studies (Platteeuw *et al*., 1995; Guay *et al*., 1993; Nesin *et al*., 1990; Le Blanc *et al*., 1988).

Detection of the Panton–Valentine leucocidin gene *lukF-lukS*

The *lukS-lukF* gene was amplified as previously described (Holmes *et al*., 2005).

Detection of *spa* gene

The *spa* gene was amplified using specific primers, as described by Mathema *et al*. (2008).

Sequencing and *Spa* typing

DNA sequencing was performed using the same primers employed to detect the *Staphylococcus aureus* protein. PCR amplicons were sequenced via the Sanger method at the Beijing Genomics Institute (BGI) laboratory in Hong Kong, China. The obtained sequences were then submitted to the National Reference Center (CNR) for Staphylococci in Lyon, France, for *spa* type identification. The analysis was conducted using the international Ridom SpaServer database (https://www.spaserver.ridom.de), following accredited bioinformatics procedures in compliance with the NF EN ISO 15189 standard. *spa* types were systematically

determined using the curated SpaServer database, which assigns repeat sequences to established sequence types (STs) or clonal complexes (CCs). In cases where direct matches were unavailable, STs and CCs were phylogenetically inferred based on documented associations between specific *spa* types and genetic lineages in the literature.

Antimicrobial Susceptibility testing

Antimicrobial susceptibility testing was performed via the disk diffusion method, adhering to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020). The antibiotics evaluated included: chloramphenicol (30 µg), erythromycin (15 µg), ampicillin (10 µg), gentamicin (15 µg), sulfamethoxazole-trimethoprim (23.75/1.25 µg), penicillin (6 µg) cefotaxime (5 µg), tetracycline (30 µg), ciprofloxacin (5 µg), tobramycin (10 µg), and kanamycin (30 µg). All discs were obtained from BIO-RAD (Marnes-la-Coquette, France). Frozen *Staphylococcus aureus* strains isolated, preserved at -80°C were revitalized on trypticase soy agar (TSA; Thermo Fisher Scientific, Oxoid™, United Kingdom). Using sterile forceps, a cryobead carrying bacterial culture was aseptically transferred to the agar surface, streaked for isolated colonies, and incubated at 35 ± 2°C for 18–24 hours. A bacterial suspension was subsequently prepared in saline, adjusted to 0.5 McFarland standard turbidity (≈1.5 × 10^8 CFU/mL), and utilized as the inoculum. For testing, the inoculum was evenly spread on Mueller-Hinton agar plates with a sterile swab. Antibiotic discs were applied, and plates were incubated at 35°C for 24 hours. Inhibition zone diameters were measured and interpreted per CLSI criteria as susceptible (S), intermediate (I), or resistant (R) (CLSI, 2020). *Staphylococcus aureus* ATCC 25923 served as the quality control strain.

Statistical analysis

The collected data were entered and organized using Microsoft Excel 2007, then analyzed with RCommander software (version R 3.3.3.0). Statistical analyses were conducted using methods appropriate to the nature of the variables and the objectives of the study. For each categorical independent variable, univariate logistic regression was performed to estimate Odds Ratios (ORs) and their 95% Confidence Intervals (95% CI), in order to quantify the association between each group of interest and the likelihood of MSSA detection. Proportions were compared using significance tests suited to the observed sample sizes. The Chi-square (Chi^2) test was applied when all cell counts in the contingency table were five or more. When one or more expected cell counts were below this threshold, Fisher's exact test was used, in accordance with statistical guidelines for small sample analysis.

Results and Discussion

Prevalence of methicillin-susceptible *Staphylococcus aureus* (MSSA) carriage on livestock farms

The MSSA strains were identified using phenotypic methods and then confirmed by conventional PCR targeting the *nuc* gene (Figure 1). Comparison of the two methods revealed 7.7% (12/156) false positives in pigs compared to 5.1% (2/39) in humans (Table 3). The prevalence rates of MSSA strains in pigs and humans across both regions (Autonomous District of Abidjan and Sassandra-Marahoué District) were 12.2% (144/1176) and 62.7% (37/50), respectively (Table 4). Regarding pig categories, the highest prevalence (19.2%) was observed in weaned piglets. Additionally, in pigs, MSSA carriage decreased significantly with physiological age ($p < 0.001$), from 19.2% (weaned piglets) to 2.8% (sows). MSSA carriage was significantly higher in people working on pig farms than in pigs (OR = 11.95, 95% CI: 6.09–23.47, $p < 0.0001$) (Table 3). In pigs, the prevalence of MSSA strains was significantly lower in region B than in region A (OR = 0.37, 95% CI: 0.25–0.55, $p < 0.0001$). It should be noted that no MSSA cases were detected among workers in region B, in stark contrast to the 82% carrier rate in region A. However, this difference was not statistically significant ($p = 1$, Fisher's exact test) (Table 4).

Genotype distribution of MSSA isolates

The results of *spa* typing of MSSA are summarized in Table 5. Pigs isolates had three distinct *spa* types, including a new *spa* type (t6608, no associated ST or CC reported). Based on their *spa* type, nearly (98.6 %) MSSA were assigned to ST398 or CC398. CC398 *spa*-type t571 was predominant (97.2 %) among MSSA compared to the CC398 *spa*-type t1250 (1.4 %). In humans, the only *spa* type found was t571, which is also common in animals, with a prevalence of 100 % (CC398 *spa*-type t571). The *luk*-PV genes encoding Panton–

Valentine leukocidin (PVL) were absent in all porcine and human isolates (Tables 6 and 7). Analysis revealed that *tet(K)*, *tet(L)*, and *tet(M)* genes constituted the tetracycline resistance genes in MSSA strains (Figure 2). The resistance rates and tetracycline resistance genes for all MSSA strains are presented in the Table 8. Overall, 68% (98/144) of porcine strains were resistant to tetracycline, compared to 83.8% (31/37) in human strains. Among the porcine strains, *tet(L)* was slightly more frequently identified (27.6%) than *tet(K)* (24.5%), while the detection rate of *tet(M)* (17.3%) was significantly lower compared to the other two genes. Regarding the co-occurrence of resistance genes, 11 strains (11.2%) carried both *tet(K)* and *tet(L)*, 6 strains (6.1%) carried *tet(K)* and *tet(M)*, and 8 strains (8.2%) carried *tet(L)* and *tet(M)*. Additionally, 5 strains (5.1%) carried all three resistance genes: *tet(K)*, *tet(L)* and *tet(M)*. In humans, similar patterns were observed, with *tet(L)* (29.2%), *tet(K)* (22.6%) and *tet(M)* (6.4%). Regarding the co-occurrence of resistance genes, 7 strains (22.6%) carried both *tet(K)* and *tet(L)*, 2 strains (6.4%) carried *tet(K)* and *tet(M)*, and 2 (6.4%) carried *tet(L)* and *tet(M)*. Additionally, 2 strains (6.4%) harbored all three genes: *tet(K)*, *tet(L)* and *tet(M)*. The *tet(O)* gene was absent in all porcine and human strains.

Antimicrobial resistance profiles

Regarding antibiotic resistance phenotypes in animals, 97.2% of MSSA CC398 *spa*-type t571 strains (140/144), 1.4 % of MSSA CC398 *spa*-type t1250 strains (2/144), and 1.4% of non-CC398 MSSA *spa*-type t6608 strains (2/144) were resistant to multiple antibiotic classes.

All porcine-origin MSSA strains exhibited higher phenotypic resistance levels to tetracycline (90.2%) and penicillin (56.2%) (Table 6). The same trends were observed in humans (Table 7).

Prevalence of methicillin-susceptible *Staphylococcus aureus* (MSSA) carriage on livestock farms

The findings on MSSA carriage in pigs and humans highlight several key epidemiological and zoonotic aspects of *Staphylococcus aureus* in livestock farming systems. The prevalence of MSSA in pigs (12.2%) was notably lower than the prevalence in humans (62.7%), which aligns with previous studies indicating that *S. aureus* colonization is more common in humans than in animals (Momoh *et al*., 2018; Fall *et al*., 2012). However, the significantly higher MSSA carriage in pig farm workers (OR = 11.95, $p < 0.0001$) suggests occupational exposure as a major risk factor, corroborating findings from other African studies where frequent animal contact increased colonization rates (Samutela *et al*., 2021). The higher prevalence of MSSA in weaned piglets (19.2%) compared to sows (2.8%) ($p< 0.001$) may reflect age-dependent immune maturation or differences in management practices, such as antibiotic use in younger pigs. Similar trends have been reported in European studies, where piglets showed higher *S. aureus* carriage due to immature immunity and higher stocking densities (De Neeling *et al*., 2007). The regional disparity in MSSA prevalence (lower in Region B vs. Region A) could stem from variations in farm hygiene, antibiotic usage, or livestock density, though the lack of statistical significance in human carriers (p =1) suggests confounding factors like small sample sizes or undetected environmental reservoirs. The 7.7% false-positive rate in pigs (phenotypic vs. PCR confirmation) underscores the importance of molecular methods for accurate *S. aureus* identification, as phenotypic tests may misclassify coagulase-negative staphylococci (Igbinosa *et al*., 2016). This aligns with global recommendations to use *nuc* gene PCR for definitive detection (Samutela *et al*., 2021). The reviewed article by Samutela *et al*. (2021) reported MSSA prevalence rates of 0–55% in African pigs, with human carriage up to 30.8% among farm workers.

The current study's pig prevalence (12.2%) falls within this range but is lower than the 43.2–55% rates reported in Nigeria and South Africa (Dweba *et al*., 2019; Okunlola et Ayandele, 2015). This discrepancy may reflect differences in sampling (nasal vs. oral/rectal swabs) or regional antimicrobial use. Notably, Samutela *et al*. (2021) emphasized the predominance of human-associated clonal complexes (CC5, CC88) in African livestock, suggesting anthropogenic transmission. This hypothesis is further supported by the high MSSA carriage rate observed among pig workers in our study (62.7%).

The absence of MSSA in Region B workers contrasts sharply with Samutela *et al*. (2021) findings of widespread carriage, possibly due to stricter biosecurity or lower pig-human contact in that region. Further, the declining MSSA prevalence with pig age mirrors trends observed in European LA-MRSA studies (Köck *et al*., 2009), reinforcing the role of host factors in colonization dynamics.

Genotype distribution of MSSA isolates

The genotypic analysis of MSSA isolates revealed a predominance of CC398, particularly the *spa*-type t571, among both porcine and human samples. This finding aligns with previous studies demonstrating the adaptability, zoonotic potential, and livestock association of the CC398 lineage, especially in pigs (Samutela *et al*., 2021; Cuny *et al*., 2015; Zarazaga *et al*., 2010). The high prevalence of CC398 *spa*-type t571 (97.2%) in pigs and its exclusive presence in human isolates suggest potential zoonotic transmission or shared environmental reservoirs, consistent with the concept of livestock-associated (LA) *S. aureus*. The absence of PVL genes (*luk-PV*) in all isolates further supports the livestock-associated nature of these strains, as LA-MSSA CC398 strains typically lack this virulence factor, which is more common in community-associated *S. aureus* lineages (Katakweba *et al*., 2016; Lina *et al*., 1999). This contrasts with reports from other studies where PVL-positive strains are more prevalent in both community and hospital settings. However, the ability of MSSA CC398 *spa*-type t571 to acquire new virulence factors, such as the *luk-PV* gene encoding Panton-Valentine Leukocidin (PVL), represents an additional concern. Fatal cases of necrotizing pneumonia due to PVL-positive MSSA CC398 t571 strains have been reported, although the PVL-positive phenotype remains rare in this lineage (Rasigade *et al*., 2010). Such evolution underscores the pandemic potential of this clone and its capacity to adapt rapidly to different ecological niches, including humans and potentially wildlife reservoirs (Gómez *et al*., 2016).

The detection of a novel *spa*-type (t6608) in pigs highlights the underlying genetic diversity within MSSA populations, even within a predominantly clonal lineage like CC398. Although its clinical significance remains unclear due to the lack of associated ST or CC data, this finding underscores the need for ongoing surveillance to monitor emerging genotypes in animal and human populations. Notably, previous African reports mostly identified other *spa*-types (e.g., t034, t108, t567) in pig-related *S. aureus* (Samutela *et al*., 2021), and Schaumburg *et al*. (2015) primarily reported human-associated sequence types such as ST1 and ST6 without detecting *spa*-type t571. This difference may be explained by variations in farming practices, regional epidemiological patterns, or the importation of breeding animals from countries where carriage of CC398 *spa*-type t571 is widespread in the pig population, particularly in Europe.

Tetracycline resistance genes and antimicrobial resistance profiles

The high prevalence of tetracycline resistance genes in MSSA isolates from both pigs (68%) and humans (83.8%) reflects the widespread use of tetracyclines in livestock farming, which exerts selective pressure favoring resistant strains (Samutela *et al*., 2021; Schwarz *et al*., 2001). The predominance of *tet*(L) and *tet*(K) over *tet*(M) in both porcine and human isolates is consistent with previous findings that these genes are more commonly associated with livestock-associated *S. aureus* (Jones *et al*., 2006; Trzcinski *et al*., 2000). The co-occurrence of multiple tetracycline resistance genes (e.g., *tet(K)*, *tet(L)*, *tet(M)*) in some strains suggests the accumulation of resistance determinants, likely due to horizontal gene transfer and selective pressure from antimicrobial use (Roberts, 2005). The absence of *tet(O)* in all isolates aligns with its known rarity in *S. aureus*, as it is typically found in other Gram-positive bacteria (Chopra and Roberts, 2001). The high phenotypic resistance rates to both tetracycline and penicillin observed in porcine and human isolates reflect the historical and continued use of these antibiotics and underscore the challenges posed by antimicrobial resistance in livestock-associated MSSA CC398. The multidrug resistance observed in 97.2% of MSSA CC398 *spa*-type t571 strains is particularly concerning, limiting treatment options and raising the risk of persistence and dissemination of resistant strains across animal and human populations (Samutela *et al*., 2021; Aarestrup *et al*., 2008). Comparisons with other studies reveal regional variations in resistance patterns. For instance, Jones *et al*. (2006) reported higher tet(K) prevalence in MRSA isolates from North America, while our study found tet(L) to be slightly more common in MSSA isolates. This discrepancy may reflect differences in antibiotic usage or the genetic backgrounds of the studied strains. Notably, the high tetracycline resistance rate in human isolates (83.8%) exceeds that reported in some European studies, suggesting localized antibiotic pressure or clonal expansion. The similarity of resistance patterns between animal and human isolates supports the hypothesis of zoonotic transmission and highlights the importance of One Health strategies to address antimicrobial resistance (Price *et al*., 2012).

Unlike the traditional livestock-associated MRSA CC398 strains, the MSSA CC398 *spa*-t571 isolates are typically tetracycline-susceptible, PVL-negative, and *scn*-positive (indicative of human adaptation via phage φ3

acquisition) (Uhlemann *et al*., 2012). These traits distinguish them from the classical LA-MRSA CC398 strains, which often carry the *tet(M)* gene and show broad tetracycline resistance. Importantly, CC398 t571 isolates generally lack *tet(M)* but may carry other tetracycline resistance genes like *tet(K)* and *tet(L)*, acquired under selective antimicrobial pressure, as seen in certain human and animal isolates (Uhlemann *et al*., 2012).

Table.1 Distribution of samples collected per farm

		Pig categories by age (weeks)				
Farm	**Localisation**	**Suckling piglets (0-3)**	**Weaned piglets (4-8)**	**Fattening pigs (10-24)**	**Sows (24 and over)**	**Total**
F1	Bingerville	48	96	79	25	248
F2	Bingerville	45	64	23	19	151
F3	Bingerville	24	37	40	15	116
F4	Bingerville	21	31	32	12	96
F5	Bingerville	2	2	21	6	31
F6	Bouaflé	12	24	12	2	50
F7	Bouaflé	6	10	10	2	28
F8	Bouaflé	8	19	14	2	43
F9	Bouaflé	16	34	30	4	84
F10	Bouaflé	5	16	12	2	35
F11	Daloa	15	37	43	5	100
F12	Daloa	4	6	5	4	19
F13	Daloa	8	18	20	3	49
F14	Daloa	9	19	26	3	57
F15	Daloa	14	29	22	4	69
	Total	237	442	389	108	1176

Table.2 Primers used for the identification of *nuc*, *tet*(K), *tet*(L), *tet*(M), *tet*(O), *lukS-lukF* and *spa* genes

Gene	**Primer (5'-3')**	**Amplicon size (bp)**	**References**
nuc	F-GCGATTGATGGTGATACGGTT	279	**Maes *et al.*, (2002)**
	R-AGCCAAGCCTTGACGAACTAAAGC		**Brakstad *et al.*, (1992)**
***tet*(K)**	F-TATTTTGGCTTTGTATTCTTTCAT	1159	**Guay *et al.*, (1993)**
	R-GCTATACCTGTTCCCTCTGATAA		
***tet*(L)**	F-ATAAATTGTTTCGGGTCGGTAAT	1077	**Platteeuw *et al.*, (1995)**
	R-AACCAGCCAACTAATGACAATGAT		
***tet*(M)**	F-AGTTTTAGCTCATGTTGATG	1862	**Nesin *et al.*, (1990)**
	R-TCCGACTATTTAGACGACGG		
***tet*(O)**	F-AGCGTCAAAGGGGAATCACTATCC	1723	**Le Blanc *et al.*, (1988)**
	R-CGGCGGGGTTGGCAAATA		
lukS-lukF	F-ATCATTAGGTAAAATGTCTGCACATGATCCA	433	**Holmes *et al.*, (2005)**
	R-GCATCAASTGTATTGGATAGCCAAAAGC		
Spa	F-GCCAAAGCGCTAACCTTTTA	700	**Mathema *et al.*, (2008)**
	R-TCCAGCTAATAACGCTGCAC		

Table.3 Overall prevalence of MSSA isolated from pigs and humans

Origine	Sample size	No. of MSSA + samples (phenotypic methods)	No. of (%) MSSA + samples (PCR)	[1]OR	[1]CI95%	*p-value*	Test used
Pigs							
Weaned piglets	442	92	85 (19.2)	1.0	[*]Reference		
Suckling piglets	237	25	22 (9.3)	0.43	[0.26–0.71]	0.0009	[2]Chi²
Fattening pigs	389	35	34 (8.7)	0.40	[0.26–0.61]	< 0.0001	Chi²
Sows	108	4	3 (2.8)	0.12	[0.04–0.39]	0.0004	[3]Fisher
Total	1176	156	144 (12.2)				
Humans							
Pig workers	50	39	37 (62.7)	11.95	[6.09–23.47]	< 0.0001	Chi²

[*]Reference: Weaned piglets selected as the main comparison group (highest prevalence among pigs) ;[1]OR (Odds Ratio) and CI95**%** (confidence inerval) Calculated using logistic regression (univariate model) ;[2]Chi² for expected counts ≥5; [3]Fisher's exact test for expected counts <5

Table.4 Prevalence of MSSA by region

Origin	Sample size (A)	Sample size (B)	No. of (%) MSSA+ samples (A)	No. of (%) MSSA+ samples (B)	[1]OR (B vs A)	[1]CI95%	*p*-value	Test used
Sows	77	31	1 (1.3)	2 (6.4)	5.24	[0.46-60.03]	0.183	[3]Fisher
Fattening pigs	195	194	26 (13.3)	8 (4.1)	0.28	[0.12-0.63]	0.0023	[2]Chi²
Pig workers	45	5	37 (82.2)	0 (0)	0.0	[0.0- inf]	1.0	Fisher
Suckling piglets	140	97	17 (12.1)	5 (5.1)	0.39	[0.14-1.1]	0.0766	Chi²
Weaned piglets	230	212	63 (27.4)	22 (10.4)	0.31	[0.18-0.52]	< 0.0001	Chi²
Total pigs	642	534	107 (16.6)	37 (6.9)	0.37	[0.25, 0.55]	< 0.0001	Chi²

[1]OR (Odds Ratio) and CI95**%** (confidence inerval) Calculated using logistic regression (univariate model) ;[2]Chi² for expected counts ≥5; [3]Fisher's exact test for expected counts <5; A: Autonomous District of Abidjan; B: Sassandra-Marahoué District

Table.5 Summary of *spa* types and motifs from MSSA isolates found in this study

spa types	Associated MLST	Motif	Origin of MSSA strains		
			Pigs	Humans	Overall
t571	ST398	08-16-02-25-02-25-34-25	140/144 (97.2 %)	37/37 (100 %)	177/181 (97.8 %)
t1250	ST398	08-16-02-25-02-25	2/144 (1.4 %)	0	2/144 (1.4 %)
t6608*	None	08-23-25-02-25-34-25	2/144 (1.4 %)	0	2/144 (1.4 %)

*New *spa* type

Table.6 Genotypic and phenotypic characteristics of methicillin-susceptible *Staphylococcus aureus* (MSSA) strains isolated from pigs

Region	Pig category	*spa*-types	CC/ST[a]	No. of *spa*-types MSSA isolates	Antimicrobial resistance phenotype[b] (n)	No. of Tet^R genes[c] MSSA (n)
A	Weaned piglets	t571	398	14	PEN (14), AMP (10), CIP (8), TET (14)	*tet*K(10)
A	Weaned piglets	t571	398	6	ERY (3), CTX (4), CHL (5), TET (6)	*tet*K(5)
A	Weaned piglets	t571	398	10	AMP (6), CIP (7), CTX (8), TET (10)	*tet*L(6)
A	Weaned piglets	t571	398	6	PEN (5), CHL (4), TET (6)	*tet*L(5)
A	Weaned piglets	t1250	398	2	PEN (1), AMP (1), CIP (1), CHL (1), TET (2)	*tet*L(2)
A	Weaned piglets	t6608*	None	1	PEN (1), AMP (1), CIP (1), GMI (1), ERY (1), CTX (1), TET (1)	*tet*L(1)
A	Weaned piglets	t571	398	4	AMP (1), CIP (4), SXT (2), KMN (1), TET (3)	*tet*M(4)
A	Weaned piglets	t571	398	8	PEN (8), AMP (5), CIP (5), SXT (3), KMN (5), TET (7)	*tet*M(2)
A	Weaned piglets	t1250	398	1	PEN (1), AMP (1), CIP (1), CHL (1), GMI (1), TET (1)	*tet*M(1)
A	Weaned piglets	t6608	None	1	PEN (1), AMP (1), CIP (1), GMI (1), ERY (1), TMN (1), TET (1)	*tet*M(1)
A	Weaned piglets	t571	398	5	AMP (3), CIP (4), SXT (2), TET (5)	*tet*K+*tet*L(5)
A	Weaned piglets	t571	398	1	PEN (1), AMP (1), CIP (1), SXT (1), ERY (1), TET (1)	*tet*K+*tet*M(1)
A	Weaned piglets	t571	398	2	PEN (1), AMP (1), CIP (1), SXT (1), CTX (1), ERY (1), GMI (1), TET (2)	*tet*L+*tet*M(2)
A	Weaned	t571	398	2	PEN (1), AMP (1), CIP (1),	*tet*K+*tet*L+ *tet*M(1)

	piglets				SXT (1), CTX (1), ERY (1), GMI (1), KMN (1), TMN (1), TET (1)	
A	Suckling piglets	t571	398	4	PEN (3), AMP (2), CIP (4), GMI (1), KMN (1), TMN (1), TET (4)	*tet*K(2)
A	Suckling piglets	t571	398	5	PEN (5), AMP (1), CTX (2), GMI (2), KMN (1), TMN (1), TET (3)	*tet*L(3)
A	Suckling piglets	t571	398	3	PEN (2), AMP (1), CTX (1), GMI (3), KMN (1), TMN (1), SXT (1), TET (2)	*tet*M(2)
A	Suckling piglets	t571	398	1	AMP (1), CTX (1), CIP (1), GMI (1), TET (1)	tetK+ tetL(1)
A	Suckling piglets	t571	398	1	AMP (1), CHL (1), CIP (1), GMI (1), SXT (1), TET (1)	tetK+tetM(1)
A	Suckling piglets	t571	398	2	PEN (2), CHL (1), CIP (1), GMI (1), TET (2)	*tet*L+*tet*M(2)
A	Suckling piglets	t571	398	1	CHL (1), CIP (1), ERY (1), TET (1)	*tet*K+ *tet*L+*tet*M(1)
A	Fattening pigs	t571	398	4	AMP (2), CHL (1), CIP (2), TET (4)	*tet*K(4)
A	Fattening pigs	t571	398	6	PEN (6), AMP (1), TET (5)	tet*L*(5)
A	Fattening pigs	t571	398	3	PEN (3), AMP (1), GMI (1), TET (3)	*tet*M(3)
A	Fattening pigs	t571	398	4	AMP (2), GMI (1), CTX (2), CIP (3), TET (4)	*tet*K+*tet*L(4)
A	Fattening pigs	t571	398	3	AMP (2), GMI (3), CHL (1), TET (2)	*tet*K+*tet*M(3)
A	Fattening pigs	t571	398	3	AMP (2), TET (3)	*tet*L+*tet*M(3)
A	Fattening pigs	t571	398	3	PEN (2), AMP (1), TET (3)	*tet*K+*tet*L+*tet*M(3)
A	Adult sows	t571	398	1	TET (1)	*tet*K(1)
B	Weaned piglets	t571	398	7	PEN (7), AMP (2), CIP (4), CTX (2), CHL (2), TET (6)	*tet*K(3)
B	Weaned piglets	t571	398	3	AMP (1), CIP (3), CTX (1), CHL (1), TET (2)	*tet*L(2)
B	Weaned piglets	t571	398	4	GMI (2), CTX (4), CHL (1), TET (3)	*tet*M(2)
B	Weaned piglets	t571	398	4	PEN (4), CIP (3), CTX (1), TET (3)	tetK+tetM(1)
B	Weaned piglets	t571	398	4	CTX (1), CHL (2), GMI (2), TET (4)	*tet*K+ *tet*L(1)
B	Suckling piglets	t571	398	3	PEN (1), CTX (1), GMI (2), TET (3)	*tet*L(2)

B	Suckling piglets	t571	398	2	PEN (2), AMP (1), CIP (1), CTX (1), CHL (1), GMI (1), TMN (1), SXT (2), TET (2)	*tet*M(1)
B	Fattening pigs	t571	398	8	PEN (8), AMP (3), TET (7)	*tet*M(1)
B	Adult sows	t571	398	2	PEN (2), TET (1)	*tetL*(1)
			Total	144	PEN (81), AMP (56), CIP (59), TET (130), ERY (9), CTX (32), CHL (23), SXT (14), KMN (10), TMN (6), GMI (26)	98

[a]CC and ST were assigned based on known associations with *spa* types (**Oliveira and de Lencastre, 2002**).
[b]CTX: cefotaxime; CHL: chloramphenicol; GMI: gentamicin; TMN: tobramycin; KMN: kanamycin; PEN: penicillin; AMP: ampicillin; TET: tetracycline; CIP: ciprofloxacin; ERY: erythromycin; SXT: co-trimoxazole
[c]Tetracycline resistance genes: *tet*(K), *tet*(L), *tet*(M) and *tet*(O) (absence of the *tet*(O) gene in all strains studied)
*New *spa* type
A: Autonomous District of Abidjan; B: Sassandra-Marahoué District

Table.7 Genotypic and phenotypic characteristics of methicillin-susceptible *Staphylococcus aureus* (MSSA) strains isolated from humans

Region	*spa* type	CC/ST[a]	No. of *spa*-types MSSA isolates	Antimicrobial resistance phenotype[b]	No. of Tet[R] genes[c] MSSA (n)
A	t571	398	3	PEN (3), AMP (2), CIP (3), ERY (1), CTX (1), TET (3)	*tet*K(3)
A	t571	398	3	PEN (2), AMP (1), CIP (3), TET (2)	*tet*K(2)
A	t571	398	3	PEN (2), SXT (1), TET (3)	*tet*K(2)
A	t571	398	5	CIP (3), CTX (4), CHL (3), TET (5)	*tet*L(4)
A	t571	398	6	PEN (6), AMP (4), GMI (1), TET (5)	*tet*L(5)
A	t571	398	3	PEN (2), CIP (3), SXT (1), KMN (1), TET (2)	*tet*M(2)
A	t571	398	7	PEN (5), AMP (2), CIP (4), SXT (2), CTX (5), TET (7)	*tet*K_ *tet*L(7)
A	t571	398	3	PEN (2), AMP (1), SXT (2), GMI (2), TET (3)	*tet*K_ *tet*M(2)
A	t571	398	2	AMP (1), SXT (2), GMI (1), TET (2)	*tet*L_ *tet*M(2)
A	t571	398	2	PEN (2), AMP (1), TET (2)	*tet*K_ *tet*L_ *tet*M(2)
		Total	37	PEN (24), AMP (12), CIP (16), ERY (1), CTX (10), TET (34), SXT (8), CHL (3), GMI (4), KMN (1)	31

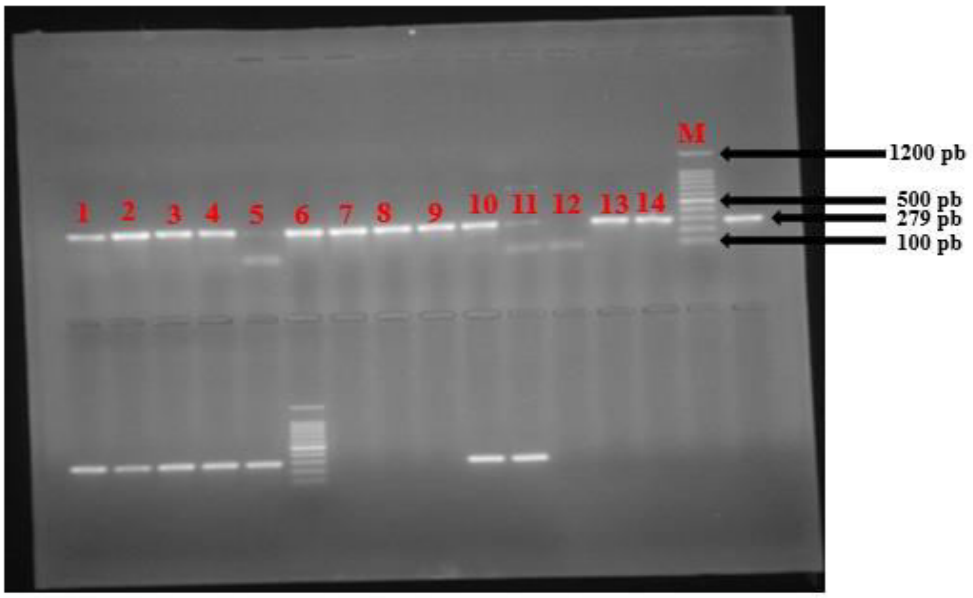
[a]CC and ST were assigned based on known associations with *spa* types (**Oliveira and de Lencastre, 2002**).
[b]CTX: cefotaxime; CHL: chloramphenicol; GMI: gentamicin; TMN: tobramycin; KMN: kanamycin; PEN: penicillin; AMP: ampicillin; TET: tetracycline; CIP: ciprofloxacin; ERY: erythromycin; SXT: co-trimoxazole
[c]Tetracycline resistance genes: *tet*(K), *tet*(L), *tet*(M) and *tet*(O) (absence of the *tet*(O) gene in all strains studied)
*New *spa* type
A: Autonomous District of Abidjan; B: Sassandra-Marahoué District

Table.8 Regional distribution of tetracycline resistance determinants in MSSA strains isolated from pigs and humans

			No. (%) of MSSA isolates								
Origin	**Region**	**MSSA strains**	**Total Tet^R**	***tet*(K)**	***tet*(L)**	***tet*(M)**	***tet*(O)**	***tet*(K)+ *tet*(L)**	***tet*(K)+ *tet*(M)**	***tet*(L)+ *tet*(M)**	***tet*(K)+ *tet*(L)+ *tet*(M)**
Weaned piglets	**A**	63	47 (74.6)	15 (32.0)	14 (30.0)	8 (17.0)	0	5 (10.6)	1 (2.0)	3 (6.4)	1 (2.0)
Suckling piglets	**A**	17	12 (70.6)	2 (16.7)	3 (25.0)	2 (16.7)	0	1 (8.3)	1(8.3)	2 (16.7)	1 (8.3)
Fattening pigs	**A**	26	24 (92.3)	3 (12.5)	5 (21.0)	3 (12.5)	0	4 (16.5)	3 (12.5)	3 (12.5)	3 (12.5)
Adult sows	**A**	1	1(100.0)	1 (100.0)	0	0	0	0	0	0	0
Subtotal	**A**	107	84 (78.5)	21 (25.0)	22 (26.0)	13 (15.5)	0	10 (12.0)	5 (6.0)	8 (9.5)	5 (6.0)
Weaned piglets	**B**	22	9 (40.9)	3 (33.4)	2 (22.2)	2 (22.2)	0	1 (11.1)	1(11.1)	0	0
Suckling piglets	**B**	5	3 (60.0)	0	2 (66.7)	1 (33.3)	0	0	0	0	0
Fattening pigs	**B**	8	1(12.5)	0	0	1 (100.0)	0	0	0	0	0
Adult sows	**B**	2	1 (50,0)	0	1 (100.0)	0	0	0	0	0	0
Subtotal	**B**	37	14 (37.8)	3 (21.5)	5 (35.7)	4 (28.6)	0	1 (7.1)	1 (7.1)	0	0
Total	**A+B**	144	98 (68.0)	24 (24.5)	27 (27.6)	17 (17.3)	0	11 (11.2)	6 (6.1)	8 (8.2)	5 (5.1)
Pig workers	**A**	37	31(83.8)	7 (22.6)	9 (29.2)	2 (6.4)	0	7 (22.6)	2 (6.4)	2 (6.4)	2 (6.4)
Pig workers	**B**	0	0	0	0	0	0	0	0	0	0
Total	**A+B**	37	31(83.8)	7 (22.6)	9 (29.2)	2 (6.4)	0	7 (22.6)	2 (6.4)	2 (6.4)	2 (6.4)

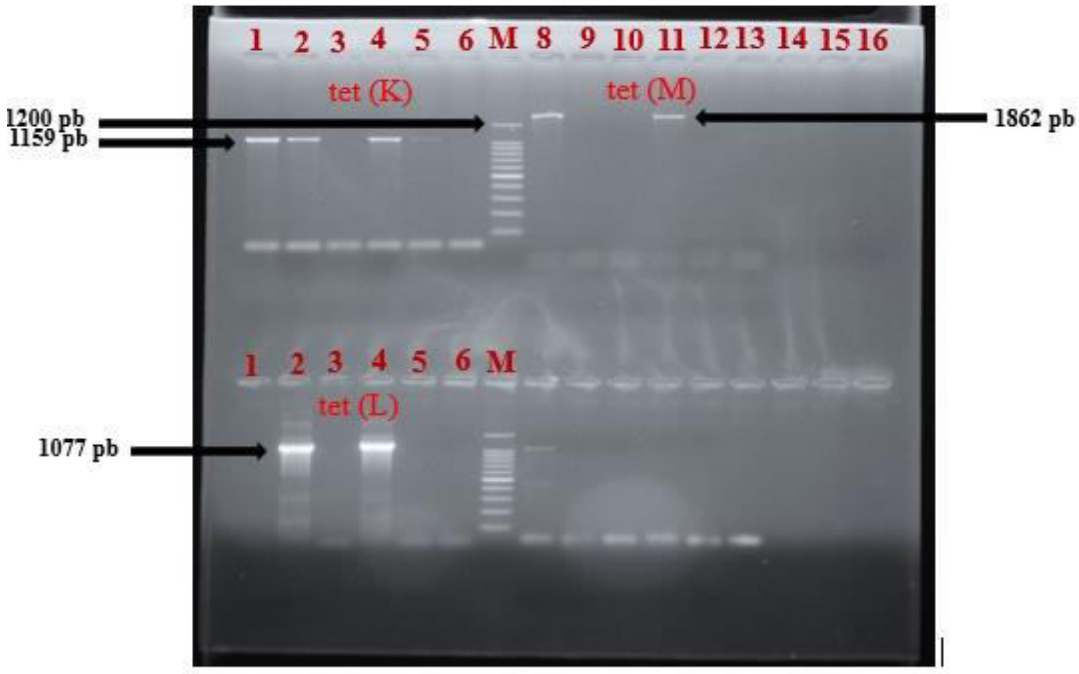
A: Autonomous District of Abidjan; B: Sassandra-Marahoué District

Figure.1 Agarose gel electrophoresis for *nuc* gene



Lane 1 to lane 11: isolates; lane 12: Negative control; lane 13: Positive control; lane 14: isolate; **M**: Molecular weight Marker

Figure.2 Agarose gel electrophoresis for *tet*(K); *tet*(M) and *tet*(L) genes



***tet*(K):** Lane 1: Positive control, Lane 2 to Lane 5: Isolates; Lane6: Control negative; **M**: Molecular weight Marker; ***tet*(M):** Lane 8: Positive control; Lane 9: Control negative; Lane 10 to Lane 13: Isolates; ***tet*(L):** Lane 1: Control negative; Lane 2: Positive control; Lane 3 to Lane 6: Isolates; **M**: Molecular weight Marker

However, in some pig-derived isolates, MSSA CC398 *spa*-type t571 has been found to be tetracycline-resistant, suggesting either a re-acquisition of resistance genes or ongoing selective pressure within animal populations (Hasman *et al*., 2010). Additionally, the absence of PVL genes in all isolates distinguishes these livestock-associated MSSA strains from community-associated MRSA, where PVL is a key virulence factor (Lina *et al*., 1999).

This study provides the first molecular and epidemiological data on porcine-associated MSSA carriage in Côte d'Ivoire. The predominant detection of the CC398 *spa*-type t571 clone, which is highly resistant to tetracyclines, in both pigs and workers suggests potential transmission between animals and humans within farm settings. The absence of major virulence genes such as *luk-PV* currently limits the clinical severity of potential infections. However, the emergence of multidrug-resistant clones in the context of widespread antibiotic use could poses a significant public health risk. It is therefore crucial to implement continuous surveillance, integrated within a One Health approach, to prevent the amplification of resistant zoonotic *S. aureus* clones and their spread into human communities.

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Author Contributions

TRAORÉ Mamadou: Developed the initial concept, performed sampling and typing and authored the manuscript.; TANO Konan Dominique: Supervised the study; GOHOUN Wèyème Carole Zita: Supervised the technical work; GNAGNE Akpa Paterne: Collaborated in the analysis of *spa* sequences; KOUAME Kohi Alfred: Contributed to the revision of manuscript; BOUATENIN Koffi Maïzan Jean-Paul: Contributed to the revision of manuscript; YAVO William: Participated in the planning and approved of the final manuscript; KOUSSEMON Marina: Participated in the planning, assessed the results, and approved the final manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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