



Nasal carriage of *Staphylococcus aureus* in dogs and owners: *Spa* typing and antimicrobial resistance in two Italian veterinary hospitals

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ABSTRACT

Staphylococcus aureus is an opportunistic pathogen that colonizes humans and animals and causes a wide range of infections. Within a One Health framework, this study investigated nasal carriage, antimicrobial resistance, and genetic diversity of *S. aureus* in dogs and their owners attending two Veterinary Teaching Hospitals in Southern and Northern Italy. Strains were characterized using minimum inhibitory concentration testing and *spa* typing to assess resistance profiles and potential host- or region-associated patterns.

A total of 108 *S. aureus* isolates were recovered from 77 owners and 31 dogs. In 17 dog-owner pairs (6 from Southern Italy and 11 from Northern Italy), *S. aureus* was isolated from both hosts. Overall, resistance was most frequent to penicillin (72.2%), followed by erythromycin (38%), and ceftiofur and gentamicin (13.9% each). Isolates from Southern Italy showed higher levels of antimicrobial resistance compared to that of Northern Italy. Fifteen isolates (13.9%) were classified as methicillin resistant, and all of which were multidrug resistant.

The most common *spa* type was t1451 in both regions, while other prevalent types differed geographically. Identical *spa* types were detected in 11/17 dog-owner pairs (64.7%), suggesting possible interspecies transmission or shared environmental sources. No association was observed between *spa* type and multidrug resistance. These results highlight regional differences in *S. aureus* populations and confirm the lack of strict host specificity, underlining the importance of integrated One Health surveillance to address zoonotic transmission risks.

1. Introduction

Staphylococcus aureus is a versatile bacterium that occupies a dual role as both a commensal microorganism and an opportunistic pathogen in humans and animals. It commonly colonizes the skin, anterior nares, and mucosal surfaces without causing clinical symptoms, serving as part of the normal microbiota in a significant proportion of healthy individuals (Foster, 2020; Wertheim et al., 2005). Under predisposing conditions, such as breaches in the skin barrier, immunosuppression, or the presence of medical devices (e.g., intravenous catheters or surgical implants), *S. aureus* can shift from a benign colonizer to a potent pathogen. It is one of the leading causes of bacterial infections in humans worldwide, responsible for a spectrum of diseases ranging from superficial skin and soft tissue infections to severe, life-threatening conditions

such as pneumonia, bacteraemia, osteomyelitis, and infective endocarditis (Lowy, 1998; Tong et al., 2015).

Beyond its clinical relevance in humans, *S. aureus* also colonizes a variety of animal hosts, including companion animals such as dogs and cats, livestock, and wildlife (Weese and van Duijkeren, 2010). The detection of indistinguishable *spa* and *SCCmec* types among isolates from humans and animals suggests potential bidirectional transmission, highlighting its zoonotic significance (Haenni et al., 2017; Vincze et al., 2014). Further insights into strain relatedness and possible interspecies transmission could be obtained through the use of additional molecular typing methods, including MLST or genome-based approaches (Pan et al., 2023). In particular, methicillin-resistant *S. aureus* (MRSA) of both human-associated and livestock-associated lineages have been reported in domestic pets, underscoring the importance of the One Health

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approach in monitoring and controlling *S. aureus* dissemination across species (Boost et al., 2008; Lakhundi and Zhang, 2018). However, *spa* typing is a widely used molecular method for *S. aureus* characterization, based on sequence variation within the X region of the protein A (*spa*) gene. It is a robust method for lineage discrimination in clinical isolates in comparison with other genotyping methods like ribotyping, or multilocus sequence typing, (MLST) (Ravaoli et al., 2022).

Spa typing is valued for its simplicity, reproducibility, and standardized nomenclature, making it suitable for local outbreak detection, population structure studies, and international surveillance (Mellmann et al., 2007). Although whole-genome sequencing (WGS) provides a deeper resolution, *spa* typing remains a cost-effective and robust tool for routine molecular epidemiology of *S. aureus*. Several studies (Bramble et al., 2010; Cuny et al., 2022; Rutland et al., 2009) have demonstrated matching *spa* types in dogs and their owners, supporting the hypothesis that cross-transmission may occur in the domestic environment.

For these reasons, the integration of molecular typing tools such as *spa* typing into routine surveillance is essential to monitor clonal spread and to evaluate the risk of transmission both in households and in veterinary settings. This scenario becomes even more relevant when considering current antimicrobial resistance (AMR) trends in both human and animal *S. aureus* strains, including resistance to antibiotics like cefoxitin, penicillin, and ciprofloxacin, emphasizing the need for prudent antimicrobial use (Jin et al., 2023; Sarker et al., 2025).

In Italy, antimicrobial resistance and MRSA circulation have been widely investigated in human and veterinary settings (Nocera et al., 2023; Milanesi et al., 2025); however, data on *S. aureus* carriage and molecular relatedness between dogs and their owners remain limited and geographically fragmented. Most studies have focused on single-host populations rather than paired human–animal carriage within a One Health framework. Furthermore, Italy is characterized by regional differences in antimicrobial use and resistance rates, with generally higher AMR levels reported in Southern compared with Northern regions (AIFA, 2025; Mageri et al., 2023; Nocera et al., 2023). Comparing two Veterinary Teaching Hospitals located in Northern and Southern Italy therefore allows exploration of potential geographic variability in resistance patterns, clonal distribution, and interspecies transmission dynamics.

The aim of the present study was to describe the antibiotic resistance profiles, and the *spa* types of *S. aureus* isolates from dogs and their owners at two Italian Veterinary Teaching Hospitals, located in distinct geographic areas, to explore clonal dynamics and transmission risk for supporting One Health oriented infection control strategies.

2. Materials and methods

2.1. Ethical statement

This study received approval for sampling dogs and humans from the Veterinary Service Center of the University of Naples Federico II (certificate n. PG/2023/0120379 of the 5 October 2023) and from the Bioethics Committee of the University of Turin (certificate n. 0251347 of the 1 February 2024). All procedures were carried out in accordance with Article 2 of the Italian Legislative Decree 26/2014, implementing Directive 2010/63/EU. Prior to sample collection, all owners received written information about the study and provided signed informed consent authorizing their participation and the sampling of their dogs.

2.2. Strain collection

A cross-sectional sampling was conducted in two Veterinary Teaching Hospitals, one located in Southern Italy (VTH1, University of Naples Federico II) and one in Northern Italy (VTH2, University of Turin). The sampling strategy was specifically designed to isolate *S. aureus* from one-to-one dog–owner pairs, with one owner enrolled for each dog. Recruitment took place during the dogs' admission for surgical

procedures.

Paired nasal swabs (eSwab®, Copan Diagnostics, Brescia, Italy) from each owner–dog pair were collected immediately before surgery using a standardized protocol. For owners, self-sampling was performed under direct supervision of trained personnel following detailed instructions to ensure adequate rotation of the swab in both anterior nares for a standardized duration. In dogs, nasal swabs were collected under general anaesthesia, ensuring consistent access to the nasal vestibule while minimizing stress. Samples were transported to the laboratory at ambient temperature and processed within 2 h of collection for the isolation and identification of *S. aureus*, following identical microbiological procedures for both hosts.

2.3. *S. aureus* isolation and identification

All samples were cultured on Columbia CNA Blood Agar (Oxoid, Thermo Fischer Scientific, Milan, Italy), Mannitol salt agar (MSA, Oxoid) and Chromatic *Staph aureus* plates (Liofilchem, Teramo, Italy) and incubated aerobically at 37 °C for 24–48 h. Presumptive *Staphylococcus* colonies were evaluated based on morphology, mannitol fermentation, haemolysis, and Gram staining. Finally, distinct colonies were sub-cultured on Columbia blood agar (Oxoid) to obtain pure cultures. Microbial identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany), a rapid and reliable method for routine bacterial species identification based on the generation of characteristic protein mass spectral fingerprints matched against a validated reference database (Seng et al., 2009; Clark et al., 2013). Species-level identification was assigned according to the manufacturer's recommended score thresholds, whereby scores ≥ 2.0 were interpreted as highly confident species-level identification.

After identification, the isolated *S. aureus* strains were preserved at -80 °C using Microbank tubes (Pro-Lab Diagnostics, Round Rock, TX, USA) for further studies. *S. aureus* TUCC00000086 (<http://www.tucc.unito.it>) was used as an internal reference strain for molecular analyses, whereas *S. aureus* ATCC 33591 was included as control strain for antimicrobial susceptibility testing.

2.4. Antimicrobial susceptibility testing and detection of methicillin resistance determinant

Antimicrobial susceptibility testing (AST) was performed by determination of minimum inhibitory concentrations (MICs) using the broth microdilution (BMD) method with the Sensititre™ Gram Positive GPALL1F system (Thermo Fisher Scientific, Milan, Italy). The GPALL1F kit contains dehydrated antimicrobial agents arranged in microdilution panels, which were rehydrated with standardized bacterial inocula. Briefly, isolates were subcultured on non-selective agar (Tryptic Soy agar, TSA, Oxoid) and a bacterial suspension was prepared in sterile saline and adjusted to a turbidity equivalent to 0.5 McFarland using a densitometer. The standardized suspension was further diluted in cation-adjusted Mueller–Hinton broth (Thermo Fisher Scientific) to obtain the final inoculum recommended by the manufacturer (approximately 5×10^5 CFU/mL per well). Microdilution plates were inoculated and incubated at 35 ± 1 °C for 16–20 h under aerobic conditions.

Each plate includes several antibiotics active against Gram-positive bacteria; however, only those relevant for *S. aureus* and used for antimicrobial resistance classification (Magiorakos et al., 2012) are reported here.

In the case of MRSA, methicillin resistance -reflecting non-susceptibility to multiple β -lactam agents- was considered sufficient for inclusion in the MDR category, irrespective of the number of additional resistant classes, as outlined by Magiorakos et al. (2012). XDR strains were defined as non-susceptible to all but one or two antimicrobial classes, and PDR strains as non-susceptible to all agents in all classes tested.

The antibiotics included in this study were ceftiofur (CXI), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), daptomycin (DAP), erythromycin (ERY), gentamicin (GEN), levofloxacin (LEVO), linezolid (LZD), moxifloxacin (MXF), nitrofurantoin (NIT), penicillin (PEN), quinupristin/dalfopristin (SYN), rifampin (RIF), tetracycline (TET), tigecycline (TGC), trimethoprim/sulfamethoxazole (SXT), and vancomycin (VAN), all tested in serial twofold dilutions. MIC values were interpreted using EUCAST 2025 breakpoints whenever these were compatible with the dilution ranges provided in the GPALL1F panel. For selected antimicrobial agents (CHL, CLI, RIF and TET), whose EUCAST breakpoints fell outside the concentration range tested on the panel, categorical interpretation was performed according to CLSI M100 (Ed. 34, Clinical and Laboratory Standards Institute (CLSI), 2024) criteria. According to European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2025), *S. aureus* isolates with ceftiofur MIC values >4 mg/L are classified as methicillin-resistant, mostly due to the presence of the *mecA* or *mecC* gene. Detection of methicillin resistance determinants was performed by PCR targeting both *mecA* and *mecC* genes, as previously described (Stegger et al., 2012). The assay allows specific discrimination between the two homologous genes. PCR amplification was carried out with the TopTaq DNA Polymerase kit (Qiagen, Hilden, Germany) using genomic DNA extracted as described in Section 2.5. Appropriate positive and negative controls were included in each run. Amplicons were analyzed by agarose gel electrophoresis.

2.5. *Spa* typing

On all *S. aureus* isolates, genomic DNA was extracted using InstaGene Matrix (Bio-Rad Laboratories, USA) following the manufacturer's protocol. Extracts were stored at 4 °C for short-term use or -20 °C for longer-term storage. The polymorphic X region of the protein A gene (*spa*) was amplified using the primers *spa* 1095F (5'-AGACGATCCTTCGGTGAGC-3') and *spa* 1517R (5'-GCTTTTGCAATGTCATTACTG-3') (Stegger et al., 2012). Consensus sequences were submitted to the Ridom StaphType database (Ridom GmbH, <http://spaserver.ridom.de/>) to determine *spa* repeat patterns and assign *spa* types. Each extraction and PCR batch included a known *spa* type reference strain (*S. aureus* TUCC0000086 *spa* type t094 MSSA) to ensure extraction, amplification, and sequencing quality.

2.6. Statistical analysis

The association between resistance to each individual antimicrobial agent and four explanatory variables (geographic origin, presence of a dog-owner pair, host source of the isolate, and the interaction between pair status and host source) was assessed using logistic regression models of the form:

$$\text{logit}(Atb.) = \beta_0 + \beta_1 VTH + \beta_2 Pair + \beta_3 Host + \beta_4 (Pair \times Host)$$

For each model, coefficients (β_0 - β_4) were extracted. Odds ratios (ORs) were obtained by exponentiating the coefficients, 95% confidence intervals were derived from the normal approximation of the log(OR), and *p* values were computed from the coefficients and their standard errors. The same modelling framework was applied to evaluate factors associated with multidrug resistance.

The association between *spa* types and geographic origin, as well as between resistance profiles and *spa* types, was assessed using Fisher's exact test due to the small expected counts in some contingency table cells.

All analyses were conducted in R (version 4.4.3), using the lme4 package (version 1.1.36) for model fitting (Bates et al., 2015).

3. Results

3.1. Sample population

During the study period, 564 nasal swabs were collected from 282 owner-dog couples, of which 120 pairs were sampled at VTH1 and 162 pairs at VTH2. Of the total, *S. aureus* was isolated from 77 owners and 31 dogs with a prevalence of 27% and 11%, respectively. Regarding the source, 33 positive samples (13.75%) were detected at VTH1 and 75 (23.15%) at VTH2. The distribution of strains isolated in dogs and owners at the two hospitals is shown in Table 1.

Notably, in 17 dog-owner pairs (6 from VTH1 and 11 from VTH2) *S. aureus* was recovered from both human and canine samples. In 14 pairs, it was isolated only from the canine samples, whereas in 60 couples it was isolated exclusively from owner samples.

3.2. Antibiotic resistance

Regarding antibiotic resistance (Fig. 1), 72.2% of isolates were resistant to PEN, followed by 38% resistance to ERY, 13.9% to CXI and GEN. Resistance to other molecules was less represented. Interestingly, no strain was resistant to LZD, NIT, TGC and VAN. Comparing isolates from VTH1 and VTH2 we observed that CHL, DAP, SYN and RIF resistance was found only in isolates from VTH1. Moreover, the proportion of isolates resistant to CXI ($p = 0.0108$), CLI ($p = 0.006$), ERY ($p = 0.005$), GEN ($p = 0.03$), TET ($p = 0.0278$) and SXT ($p = 0.051$) was significantly higher in VTH1 when compared to VTH2 (Fig. 2).

As for differences between hosts, we observed a significantly higher proportion of resistance in dogs, when compared to humans, for the following antibiotics (Fig. 3): CLI ($p = 0.028$), DAP ($p = 0.0476$), SYN ($p = 0.0473$) and TET ($p = 0.029$). In the statistical model, we also included the host category (strain isolated from a single dog, a single owner, or from a dog-owner pair), but no significant differences were observed among these three groups.

Nineteen (17.6%) out of 108 strains were susceptible to all antibiotics; 24/108 strains (22.2%) were MDR, with 13 strains isolated from VTH1 (39.4%) and 11 from VTH2 (14.7%). Therefore, a statistically significant difference was observed between hospitals with a higher proportion of MDR strains in VTH1 ($p = 0.005$) with no significant differences observed in host species. None of the isolates were XDR or PDR.

Overall, 15 of 108 *S. aureus* isolates (13.9%) were classified as MRSA by MICs and confirmed by molecular investigation of the *mec* gene. Consistent with the classification criteria described above, all MRSA isolates were categorized as MDR. Of these, 14/15 also met the conventional definition of MDR, based on non-susceptibility to ≥ 3 antimicrobial classes.

MRSA strains were isolated in humans and dogs at both hospitals: 9 strains (humans $n = 7$, dogs $n = 2$) at VTH1 and 6 strains (humans $n = 4$, dogs $n = 2$) at VTH2. MRSA strains were mainly observed at VTH1 ($p = 0.0108$) with no differences observed regarding the host species.

A variety of antimicrobial susceptibility profiles were observed ($n = 20$). The most frequent was the single resistance to PEN ($n = 42/108$), followed by resistance to PEN and ERY ($n = 10/108$) and resistance to ERY alone ($n = 9/108$); other profiles were less represented (Table 2).

Table 1

Distribution of *S. aureus* strains isolated from dogs and owners at two hospitals (VTH1 and VTH2) located in Southern and Northern Italy, respectively.

Hospital	<i>S. aureus</i> from dogs	<i>S. aureus</i> from humans	Total
	N. (%)	N. (%)	N. (%)
VTH1	9 (8.3%)	24 (22.2%)	33 (30.5%)
VTH2	22 (20.4%)	53 (49.1%)	75 (69.5%)
Total	31 (28.7%)	77 (71.3%)	108 (100%)

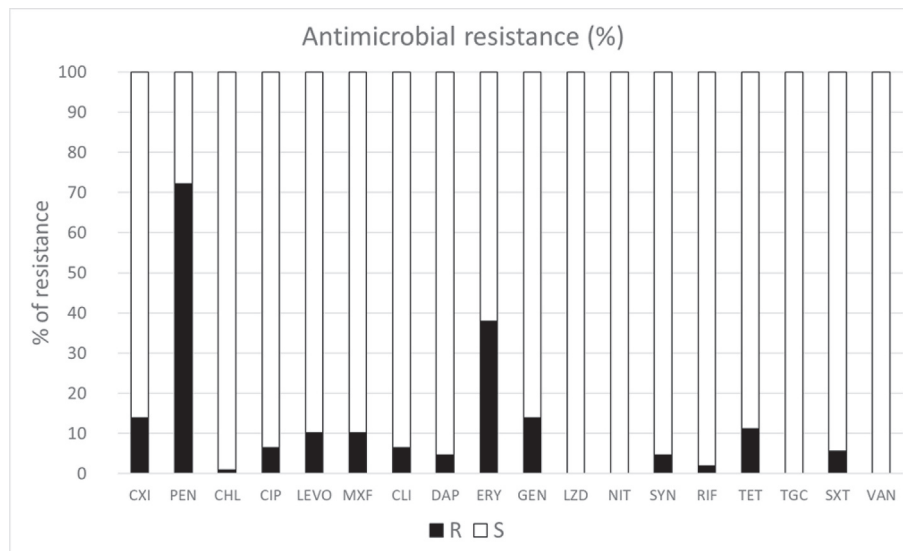


Fig. 1. Proportion of antimicrobial resistance to tested antimicrobials among *S. aureus* strains isolated in Teaching Veterinary Hospitals (VTH1 and VTH2). CXI: ceftiofur, PEN: penicillin, CHL: chloramphenicol, CIP: ciprofloxacin, LEVO: levofloxacin, MXF: moxifloxacin, CLI: clindamycin, DAP: daptomycin, ERY: erythromycin, GEN: gentamicin, LZD: linezolid, NIT: nitrofurantoin, SYN: quinupristin/dalfopristin, RIF: rifampin, TET: tetracycline, TGC: tigecycline, SXT: trimethoprim/sulfamethoxazole, VAN: vancomycin.

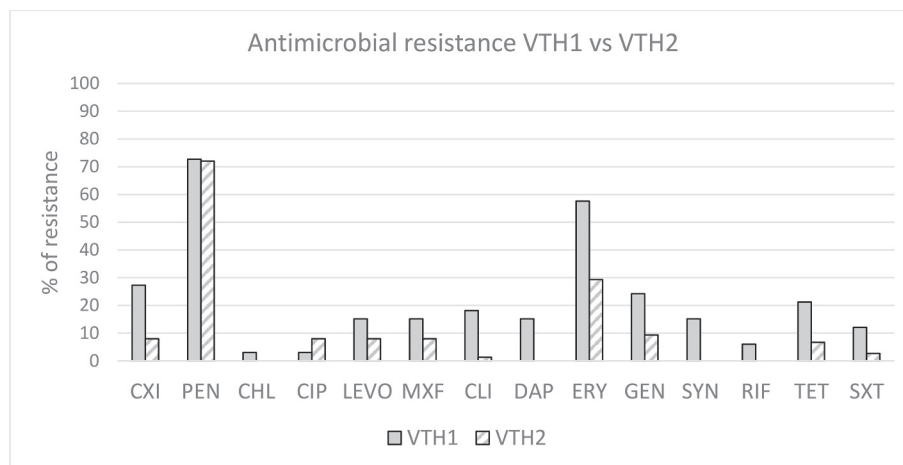


Fig. 2. Proportion of antimicrobial resistance to tested antimicrobials among *S. aureus* strains by geographic area: Southern Italy (VTH1) and Northern Italy (VTH2). CXI: ceftiofur, PEN: penicillin, CHL: chloramphenicol, CIP: ciprofloxacin, LEVO: levofloxacin, MXF: moxifloxacin, CLI: clindamycin, DAP: daptomycin, ERY: erythromycin, GEN: gentamicin, SYN: quinupristin/dalfopristin, RIF: rifampin, TET: tetracycline, SXT: trimethoprim/sulfamethoxazole.

3.3. *Spa* typing of *S. aureus* isolates

All *S. aureus* strains underwent *spa* typing. Of these, 5 strains, despite being tested repeatedly, did not provide correct repeat sequences, while 10 strains showed repeat sequences not yet present in the *spa* typer database. A high genetic diversity was detected among the *S. aureus* isolates, with the identification of numerous *spa* types ($n = 44$). Among these, t1451 was the only type observed at a relatively higher frequency ($n = 22$), and it was detected in isolates from both the Northern and the Southern Italian hospitals. Twelve *spa* types were found less frequently, and the others were found only once. Considering *spa* types identified more than once, we observed geographical differences in their distribution ($p = 0.001$). While, considering only the three most represented ones (t1451, t005, t084) the observed difference was no longer significant.

Twelve dog-owner pairs showed the same *spa* type (Fig. 4), whereas additional types were found exclusively in human isolates ($n = 23$) or exclusively in canine isolates ($n = 10$). No statistically significant

differences were found for *spa* type distribution between hosts.

Among *spa* types found more than once and with more than one antimicrobial susceptibility profile (ASP), we observed significant association between *spa* type and ASP ($p < 0.001$). Overall, 15.3% of isolates belonging to *spa* types observed more than once were MDR (Table 3). Although all t718 isolates were MDR, no association was observed between *spa* types and multidrug resistance (Fisher's exact $p = 0.096$).

4. Discussion

In this study, a comparative analysis of nasal carriage, antimicrobial resistance, and genetic diversity of *S. aureus* was conducted in dogs and their owners attending two Italian Veterinary Teaching Hospitals (VTH1 and VTH2), using an integrated approach combining *spa* typing and MIC profiling. This strategy enabled a robust characterization of both clonal structure and phenotypic resistance patterns, providing insights into *S. aureus* epidemiology at the human-animal interface.

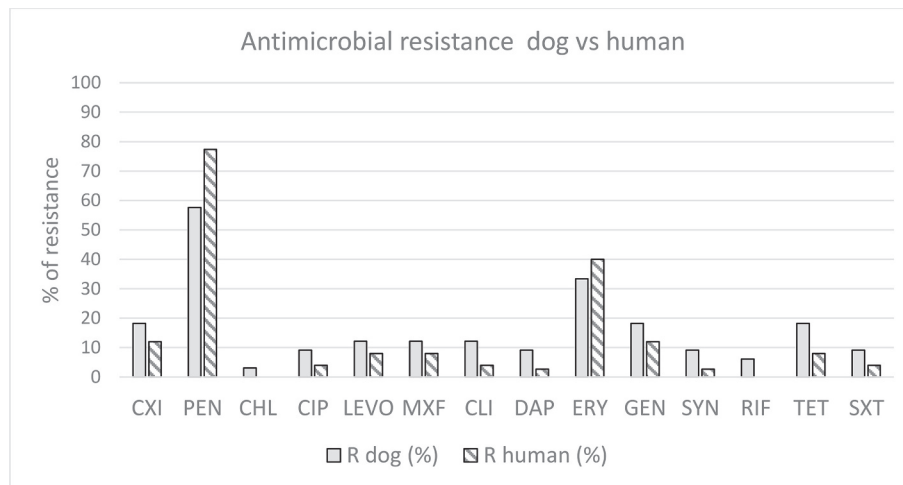


Fig. 3. Proportion of antimicrobial resistance to tested antimicrobials among canine and human *S. aureus* strains. CXI: ceftiofur, PEN: penicillin, CHL: chloramphenicol, CIP: ciprofloxacin, LEVO: levofloxacin, MXF: moxifloxacin, CLI: clindamycin, DAP: daptomycin, ERY: erythromycin, GEN: gentamicin, SYN: quinupristin/dalfopristin, RIF: rifampin, TET: tetracycline, SXT: trimethoprim/sulfamethoxazole.

Table 2
Antimicrobial resistance profiles of 108 *S. aureus* strains isolated from VTH1 and VTH2 with their relative frequency and description.

	Total (n)	VTH1 (n)	VTH2 (n)	Dog (n)	Owner (n)	Antibiotic resistance profile
A	1	1	0	1	0	CXI, PEN, CIP, LEVO, MXF, CLI, DAP, ERY, GEN, SYN, RIF, TET, SXT
B	1	1	0	1	0	CXI, PEN, CHL, CLI, DAP, ERY, GEN, SYN, RIF, TET, SXT
C	3	3	0	1	2	CXI, PEN, LEVO, MXF, CLI, DAP, ERY, GEN, SYN, TET
D	1	0	1	1	0	CXI, PEN, CIP, LEVO, MXF, CLI, ERY, GEN, TET
E	1	0	1	0	1	CXI, PEN, CIP, LEVO, MXF, ERY, TET
F	1	1	0	0	1	CXI, PEN, LEVO, MXF, CLI, ERY, GEN
G	3	0	3	1	2	CXI, PEN, CIP, LEVO, MXF, ERY
H	1	0	1	0	1	PEN, CIP, LEVO, MXF
I	4	2	2	1	3	PEN, ERY, GEN
J	3	2	1	1	2	PEN, ERY, SXT
K	3	2	1	1	2	CXI, PEN, ERY, TET
L	2	0	2	1	1	PEN, GEN, TET
M	10	4	6	0	10	PEN, ERY
N	1	0	1	0	1	ERY, SXT
O	1	0	1	0	1	PEN, GEN
P	1	1	0	0	1	PEN, CXI
Q	42	7	35	10	32	PEN
R	9	3	6	3	6	ERY
S	1	0	1	0	1	GEN
T	19	6	13	11	8	no resistance

CXI: ceftiofur, PEN: penicillin, CHL: chloramphenicol, CIP: ciprofloxacin, LEVO: levofloxacin, MXF: moxifloxacin, CLI: clindamycin, DAP: daptomycin, ERY: erythromycin, GEN: gentamicin, LZD: linezolid, NIT: nitrofurantoin, SYN: quinupristin/dalfopristin, RIF: rifampin, TET: tetracycline, TGC: tigecycline, SXT: trimethoprim/sulfamethoxazole, VAN: vancomycin.

The prevalence of *S. aureus* detected in this study in canine (11%) and human (27%) nasal samples was consistent with previously reported data. In general, nasal carriage of *S. aureus* has been estimated to range from approximately 8–11% in dogs and 24–38% in humans (Abdullahi et al., 2022; Boost et al., 2008; Halablab et al., 2010; Olsen et al., 2013), depending on the clinical context, population characteristics and geographic area. Indeed, variability in colonization patterns likely reflects host-related factors, environmental conditions, and

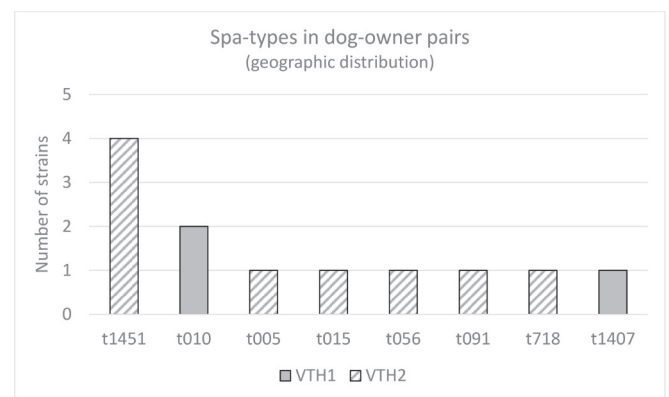


Fig. 4. Geographic distribution of the *spa* types identified in dog–owner pairs in Italian Teaching Veterinary Hospitals (VTH1 and VTH2).

Table 3
Contingency table of the frequency of *spa* types and multi-drug resistance (MDR) among *S. aureus* isolates from dogs and owners in two teaching veterinary hospitals. *Spa* types observed less than twice were excluded.

Spa types	Total		MDR n. (%)	Methicillin resistance profile	
	n.dogs	n.owners		MSSA	MRSA
t1451	7	15	5 (22.7%)	20	2
t005	2	4	0 (0.0%)	6	0
t084	2	3	0 (0.0%)	5	0
t010	2	2	0 (0.0%)	4	0
t091	2	2	0 (0.0%)	2	0
t701	1	3	0 (0.0%)	4	0
t002	0	3	1 (33.3%)	3	0
t056	1	2	0 (0.0%)	3	0
t718	1	2	3 (100%)	0	3
t015	1	1	0 (0.0%)	2	0
t571	0	2	0 (0.0%)	2	0
t1255	0	2	0 (0.0%)	2	0
t1407	1	1	0 (0.0%)	2	0
Total	20	42	9 (15.3%)	57	5

differences in healthcare or veterinary contact (Boost et al., 2008; Morris et al., 2017; Nocera and De Martino, 2024). Regarding antimicrobial resistance, the highest resistance rates were observed for penicillin, followed by erythromycin, ceftiofur, and gentamicin, whereas all isolates remained susceptible to last-line agents, including vancomycin,

linezolid, and tigecycline. This resistance profile aligns with carriage studies reporting high resistance to long-established and widely used antibiotics, while preserving susceptibility to critically important antimicrobials (Becker et al., 2014; Danelli et al., 2020). Marked differences were observed between the two hospital settings. Overall antimicrobial resistance, as well as the prevalence of multidrug-resistant (MDR) and methicillin-resistant *S. aureus* (MRSA), was significantly higher in VTH1 than in VTH2, suggesting increased selective pressure in the former. This difference was particularly evident among canine isolates, which showed higher resistance frequencies than human isolates. Resistance to daptomycin, quinupristin/dalfopristin, rifampicin, clindamycin, macrolides, and tetracyclines was significantly more prevalent in VTH1. Notably, among these antimicrobials, daptomycin, quinupristin/dalfopristin, rifampicin are classified by the EMA “Antimicrobial Advice ad hoc Expert Group” (AMEG) as category A (Avoid) or agents whose use in animals should be restricted to exceptional circumstances only (European Medicines Agency (EMA), 2020). The detection of resistance to such critically important drugs, even when limited to isolates from Southern Italy, represents a warning signal and highlights the importance of sustained antimicrobial resistance surveillance within a One Health framework, particularly for staphylococcal species circulating at the human-animal interface. These data could indicate the circulation of clinically relevant resistance traits beyond their intended therapeutic context (Weese and van Duijkeren, 2010; Haenni et al., 2017; Shariati et al., 2020).

Approximately 22% of the strains were classified as MDR, with a significantly higher prevalence in VTH1 compared with VTH2. Accordingly, MRSA strains were detected in both hospitals and host species, but were significantly more frequent in VTH1. In contrast, no significant differences in MDR or MRSA prevalence were observed between human and canine isolates, indicating that environmental and institutional factors may play a more prominent role than host species alone. All MRSA isolates were initially identified phenotypically based on cefoxitin resistance and subsequently confirmed by PCR detection of methicillin resistance determinants. In our dataset, only *mecA* was detected, while *mecC* was not identified in any isolate, consistent with the current epidemiology of MRSA in companion animals, where *mecC*-positive strains have been only sporadically reported at low frequency in Europe (Couto et al., 2016; Ruzauskas et al., 2015).

These findings are consistent with the previously regional differences in antimicrobial use and resistance in Italy, with Southern regions generally reporting higher AMR levels than Northern areas (AIFA, 2025; Maugeri et al., 2023; Nocera et al., 2023) across both human and veterinary medicine. Such regional disparities highlight the impact of antimicrobial use practices and selective pressure on resistance epidemiology. As samples were collected prior to hospitalization, the colonization detected in both dogs and owners likely reflects community or household acquisition rather than nosocomial transmission. However, colonized animals and humans entering veterinary hospital settings may contribute to the introduction and subsequent dissemination of resistant strains. This underscores the importance of infection prevention measures and integrated antimicrobial resistance surveillance within a One Health framework.

Molecular epidemiological analysis revealed distinct population structures in the two hospitals. Isolates from VTH1 were mainly represented by *spa* type t010, whereas those from VTH2 were predominantly associated with *spa* type t1451. The detection of identical *spa* types in 12 dog-owner pairs supports the possibility of interspecies transmission or shared environmental exposure, while unrelated *spa* types in other pairs suggest independent colonization events. These findings are consistent with previous studies reporting geographic variability in *S. aureus* lineages and highlighting the role of humans and companion animals as potential reservoirs that may facilitate bidirectional transmission under conditions of close contact (Haenni et al., 2017; Loeffler et al., 2011; Vincze et al., 2014; Weese and van Duijkeren, 2010). Nevertheless, the present study design does not allow definitive attribution of isolates to

hospital-associated (HA), community-associated (CA), or livestock-associated (LA) MRSA lineages. Dogs may acquire *S. aureus* from multiple sources, including close human contacts, other animals, or environmental reservoirs. Therefore, while shared *spa* types suggest possible interspecies transmission or common exposure, the directionality and precise source of acquisition cannot be conclusively determined.

The detection of *spa* type t1451 is of particular interest, as it is associated with the CC398 lineage, traditionally linked to livestock (Hetem et al., 2013; Santos et al., 2020; Silva et al., 2024), but increasingly detected in companion animals, environmental sources, and invasive human infections (Abdullahi et al., 2021; Mama et al., 2021). This finding underscores the expanding ecological and epidemiological relevance of CC398 lineages within a One Health framework.

Finally, among *spa* types detected more than once and associated with more than one antimicrobial susceptibility profile (ASP), a statistically significant association between *spa* type and antimicrobial susceptibility profile was observed ($p < 0.001$), indicating that despite some intra-clonal phenotypic variability, clonal background remains an important determinant of resistance patterns, in agreement with previous studies on *S. aureus*. Importantly, because sampling was performed prior to hospitalization, the isolates identified in this study reflect pre-existing colonization rather than acquisition within the veterinary hospital setting. Nevertheless, colonized animals and owners entering clinical environments may act as reservoirs and facilitate the introduction of resistant strains into healthcare settings, reinforcing the need for appropriate infection control measures.

4.1. Study limitations

Some methodological aspects should be considered when interpreting the present findings.

Isolation was performed by direct plating without a selective enrichment step. While this approach ensured recovery of well-isolated colonies suitable for downstream characterization, it may have resulted in a conservative estimate of colonization prevalence, particularly in samples with low bacterial load. The use of validated liquid-based transport systems (eSwab®) likely supported bacterial recovery.

Dogs were enrolled at admission for surgical procedures; prior antimicrobial exposure, repeated veterinary visits, or underlying clinical conditions may therefore have influenced colonization and resistance patterns. As detailed clinical metadata were not systematically available, this represents a potential source of bias when interpreting antimicrobial resistance frequencies.

Finally, although *spa* typing provided valuable information on clonal distribution, higher-resolution approaches such as multilocus sequence typing (MLST) or whole-genome sequencing (WGS) would allow more precise assessment of strain relatedness and transmission dynamics.

Despite these limitations, the standardized sampling procedures and parallel analysis of dog-owner pairs provided a valuable population-level overview of *S. aureus* colonization and antimicrobial resistance patterns at the human-animal interface.

5. Conclusions

The combined application of *spa* typing and MIC determination provided a comprehensive assessment of genetic diversity and antimicrobial resistance in *S. aureus* at the human-animal interface. The results highlight significant regional differences in resistance profiles, with a higher prevalence of MDR and MRSA isolates in Southern Italy. The detection of shared *spa* types between dogs and their owners supports the potential for interspecies transmission or common environmental reservoirs.

Moreover, animals colonized in the community may enter veterinary clinical environments as carriers, potentially contributing to dissemination within these settings. These findings highlight the importance of integrated antimicrobial stewardship and surveillance strategies in

veterinary healthcare facilities, in line with a One Health approach.

CRedit authorship contribution statement

Patrizia Nebbia: Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Patrizia Robino:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Giulia Iamone:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Rosana Schena:** Investigation, Formal analysis. **Sinem Arslan:** Investigation, Formal analysis. **Stefano Cavalli:** Investigation, Formal analysis. **Annunziata Romano:** Investigation, Formal analysis. **Alessandro Bellato:** Writing – review & editing, Software, Investigation, Formal analysis. **Iaria Prandi:** Investigation, Formal analysis. **Davide Mancusi:** Investigation, Formal analysis. **Matteo Olimpo:** Investigation, Formal analysis. **Gerardo Fatone:** Writing – review & editing, Investigation, Conceptualization. **Francesca Paola Nocera:** Writing – review & editing, Supervision, Conceptualization. **Luisa De Martino:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no financial conflicts of interest or personal relationships that could influence the work reported in this article.

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Data availability

The data that support the findings of this study are available from the corresponding author, upon request.

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