

Article

A One Health Approach Molecular Analysis of *Staphylococcus aureus* Reveals Distinct Lineages in Isolates from Miranda Donkeys (*Equus asinus*) and Their Handlers

Vanessa Silva ^{1,2,3,4} , Cláudia Alfarela ¹ , Manuela Caniça ^{5,6} , Vera Manageiro ^{5,6} , Miguel Nóvoa ⁷, Belen Leiva ⁷, Maria Kress ⁷, José Luís Capelo ^{8,9} , Patrícia Poeta ^{1,4,10,11,*}  and Gilberto Igrejas ^{2,3,4} 

- ¹ Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal; vanessasilva@utad.pt (V.S.); claudiavpa@hotmail.com (C.A.)
 - ² Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal; gigrejas@utad.pt
 - ³ Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
 - ⁴ Associated Laboratory for Green Chemistry (LAQV@REQUIMTE), University NOVA of Lisboa, 2825-466 Lisbon, Portugal
 - ⁵ National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AMR/HAI), Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisbon, Portugal; manuela.canica@insa.min-saude.pt (M.C.); vera.manageiro@insa.min-saude.pt (V.M.)
 - ⁶ Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, Oporto University, 4051-401 Oporto, Portugal
 - ⁷ AEPGA, Association for the Study and Protection of Asinines, Atenor, 5225-011 Miranda do Douro, Portugal; miguelnovoaa@aepga.pt (M.N.); belenleiva.aepga@gmail.com (B.L.); mariakress.aepga@gmail.com (M.K.)
 - ⁸ BIOSCOPE Group, LAQV@REQUIMTE, Chemistry Department, Faculty of Science and Technology, NOVA University of Lisbon, 2825-466 Almada, Portugal; jlcm@fct.unl.pt
 - ⁹ Proteomass Scientific Society, 2825-466 Almada, Portugal
 - ¹⁰ CECAV—Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
 - ¹¹ Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
- * Correspondence: ppoeta@utad.pt
† These authors contributed equally to this work.



Citation: Silva, V.; Alfarela, C.; Caniça, M.; Manageiro, V.; Nóvoa, M.; Leiva, B.; Kress, M.; Capelo, J.L.; Poeta, P.; Igrejas, G. A One Health Approach Molecular Analysis of *Staphylococcus aureus* Reveals Distinct Lineages in Isolates from Miranda Donkeys (*Equus asinus*) and Their Handlers. *Antibiotics* **2022**, *11*, 374. <https://doi.org/10.3390/antibiotics11030374>

Academic Editor: Valentina Virginia Ebani

Received: 31 January 2022

Accepted: 4 March 2022

Published: 10 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Donkeys (*Equus asinus*) are in decline in Europe. Occupational exposure to farm animals has been associated with increased staphylococci carriage. We aimed to isolate *S. aureus* and coagulase-negative staphylococci (CoNS) from donkeys and handlers and characterize the antimicrobial resistance profiles and genetic lineages of *S. aureus* strains. Oral and nasal swab samples were collected from 49 Miranda donkeys and 23 handlers from 15 different farms. Staphylococci species were identified by MALDI-TOF MS. The presence of antimicrobial resistance genes and virulence factors was investigated by PCR. Molecular typing was performed in *S. aureus* isolates. From the 49 donkey samples, 4 *S. aureus* (8.2%) and 21 CoNS (42.9%) were isolated. Ten handlers (43.5%) were carriers of *S. aureus* and 4 (17.4%) carried CoNS. The CoNS isolates showed resistance to several classes of antimicrobials encoded by the *mecA*, *aph* (3′)-IIIa, *ant* (4′)-Ia, *tetM*, *tetK*, *lnuA*, *ermB*, *ermC*, *dfrA* and *dfrG* genes. *S. aureus* isolates were resistant to penicillin, aminoglycosides and tetracycline harboring the *blaZ*, *aph* (3′)-IIIa, *tetL*, *tetM* and *tetK* genes. All *S. aureus* isolates from donkeys belonged to ST49 and *spa*-type t208 while the strains isolated from the handlers were ascribed to 3 STs and 7 *spa*-types. However, human isolates were from different STs than the donkey isolates. Donkeys are mainly colonized by methicillin-resistant *S. sciuri*. *S. aureus* transmission between donkeys and their handlers appears not to have occurred since the isolates belonged to different genetic lineages.

Keywords: *Staphylococcus aureus*; CoNS; donkeys; animal-to-human transmission

1. Introduction

Staphylococcus spp. are widely disseminated worldwide and have been isolated from human infections, community humans, pets, livestock, wild animals and the natural environment [1]. Both *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) are commensal bacteria that colonize the skin and mucosa of humans and animals [2]. Nevertheless, staphylococci are opportunist pathogens that are responsible for a wide-range of infections, including skin and soft-tissue infection, septicemia and endocarditis [3]. Staphylococci, particularly *S. aureus*, have the ability to easily acquire antimicrobial resistance determinants [4]. Methicillin resistance in staphylococci is conferred by the acquisition of the *mec* genes (*mecA*, *mecB* or *mecC*), which are carried by the staphylococcal cassette chromosome *mec* (SCC*mec*) [5]. Methicillin-resistant *S. aureus* (MRSA) are usually associated with complicated infections representing a public health concern [6]. It is considered that the *mecA* gene may have originated from some species of CoNS since homologues of this gene have been found in the *S. sciuri* group species [4]. CoNS carrying the *mecA* gene may act as potential donors leading to the emergence of methicillin-resistant *S. aureus* (MRSA) clones [7].

Animals can be a source of *staphylococci* zoonotic infections, particularly the clones that possess no host specificity [2]. It has also been demonstrated that the close contact between animals and humans can facilitate the transmission of pathogens. Staphylococci transmission events have been documented mainly between pets and owners and livestock and their handlers [8,9]. Molecular typing techniques, such as multilocus sequence typing (MLST), have provided information on the evolution of human and animal strains and shown that humans were the main hosts of *S. aureus*, but due to the occurrence of transmission it had acquired the ability to infect animals [10]. The frequent human-to-animal transmission eventually led to specific strain lineages adapting to new animal hosts [10,11]. MLST analysis showed that some sequence types (STs) and clonal complexes (CCs) are predominant in animal *S. aureus*, such as CC398, CC9, CC1, CC97, CC133 and CC121, and others are prevalent in human *S. aureus* (CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45 and CC51) [10]. The emergence of *S. aureus* and CoNS in equines, including horses, donkeys and mules, has been demonstrated [12–14]. The frequency of *S. aureus* colonization in horses ranges from 4% to 39% in Europe and CC8, CC22 or CC398 are the most common lineages identified [12,15,16]. Nevertheless, although studies reporting staphylococci in horses are common, studies in healthy donkeys are still very scarce [13].

The donkey population has been decreasing since 1970 but lately this trend has reversed, and it is estimated that the donkey population is 4.3 million globally [17]. In Europe, donkeys are mainly found in Portugal, Spain, Italy and Greece. Miranda donkey is a native Portuguese donkey breed that originated from the Trás-os-Montes region [18]. Donkeys played an important role in maintaining rural communities and sustainable farming practices. However, more recently, these animals began to be used as companion animals and in therapeutic activities and ecotourism [11]. Therefore, we aimed to study the prevalence of *S. aureus* and CoNS in healthy donkeys and their handlers as well as the antimicrobial resistance and genetic lineages of the isolates in order to investigate a possible human-to-animal transmission or vice-versa.

2. Results

2.1. Characterization of *S. aureus* Isolates

In this study, 49 and 23 swab samples were collected from Miranda donkeys and handlers, respectively. Staphylococci were recovered from all 15 farms sampled. *S. aureus* were isolated from 10 farms while CoNS were isolated from 8 farms. From the 49 donkey samples, 4 *S. aureus* were identified while 10 *S. aureus* were isolated from handlers' samples. However, no MRSA has been identified. Three donkeys colonized by *S. aureus* were from the same farm and their ages ranged from 3 to 8 years (Supplementary Materials Table S1). However, none of these donkeys' handlers were *S. aureus* carriers. All *S. aureus* from donkeys belonging to the same farm (isolates VS3111, VS3113 and VS3114) had

resistance to penicillin and kanamycin conferred by the *blaZ* and *aph* (3′)-IIIa genes (Table 1). Furthermore, all 3 isolates were ascribed to ST49, *spa*-type t208 and *agr* type II. The *S. aureus* isolated from the remaining donkey showed resistance to aminoglycosides and tetracycline and carried the *aph* (3′)-IIIa and *tetK* genes. Yet, these isolates also belonged to ST49, t208 and *agr* II. Regarding the virulence genes, all four isolates harbored the *hla*, *hly* and *hld* genes with the exception of isolate VS3111, which also carried the *tst* gene.

Table 1. Genetic characterization and molecular typing of *S. aureus* strains recovered from donkeys and their handlers.

| Isolate | Source | Antimicrobial Resistance | | Virulence Factors | | Molecular Typing | | |
|---------|--------|--------------------------|---|-------------------|---|------------------|------------|------------|
| | | Phenotype | Genotype | IEC Type | Other Genes | ST (CC) | <i>spa</i> | <i>agr</i> |
| VS3101 | Human | PEN, CN, TOB, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | E | <i>hla</i> , <i>hld</i> , <i>eta</i> | 1290 (1) | t131 | I |
| VS3102 | Human | PEN, CN, TOB, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | E | <i>hla</i> , <i>hld</i> , <i>eta</i> | 1290 (1) | t131 | I |
| VS3103 | Human | PEN, CN, TOB, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>tst</i> | 30 (30) | t021 | III |
| VS3104 | Human | PEN, FD | <i>blaZ</i> | | <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>tst</i> | 30 (30) | t338 | III |
| VS3105 | Human | PEN, CN, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>tst</i> | 30 (30) | t012 | III |
| VS3106 | Human | PEN, CN, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>tst</i> | 30 (30) | t012 | III |
| VS3107 | Human | PEN, FD | <i>blaZ</i> | | <i>hla</i> , <i>hld</i> | 30 (30) | t1642 | III |
| VS3108 | Human | PEN, CN, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> | 30 (30) | t1642 | III |
| VS3109 | Human | Susceptible | | | <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>eta</i> | 398 (398) | t571 | I |
| VS3110 | Human | PEN, CN, TOB, KAN, TET | <i>blaZ</i> , <i>aph</i> (3′)-IIIa, <i>tetL</i> , <i>tetM</i> | | <i>hla</i> , <i>hly</i> , <i>hld</i> | 398 (398) | t011 | I |
| VS3111 | Donkey | PEN, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> , <i>tst</i> | 49 | t208 | II |
| VS3112 | Donkey | CN, KAN, TET | <i>aph</i> (3′)-IIIa, <i>tetK</i> | | <i>hla</i> , <i>hly</i> , <i>hld</i> | 49 | t208 | II |
| VS3113 | Donkey | PEN, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> | 49 | t208 | II |
| VS3114 | Donkey | PEN, KAN | <i>blaZ</i> , <i>aph</i> (3′)-IIIa | | <i>hla</i> , <i>hly</i> , <i>hld</i> | 49 | t208 | II |

Abbreviations. PEN: penicillin; CN: gentamicin; TOB: tobramycin; KAN: kamaycin; TET: tetracycline; FD: fusidic acid; ST: sequence type; CC: clonal complex.

The 10 *S. aureus* isolated from handlers had similar resistance patterns, showing resistance to penicillin, aminoglycosides, tetracycline and fusidic acid. The isolates carried the *blaZ*, *aph* (3′)-IIIa, *tetL* and *tetM* genes. *S. aureus* isolated from handlers carried the *hla* ($n = 10$), *hly* ($n = 7$), *hld* ($n = 10$), *eta* ($n = 3$) and *tst* ($n = 4$) virulence genes. Isolates VS3101 and VS3102 also carried the *scn* and *sak* genes of the immune evasion cluster (IEC) system and were ascribed to type E. Both isolates were ascribed to ST1290 (CC1), *spa*-type t131 and *agr* I. Moreover, these isolates belonged to two handlers working at the same farm. Six isolates were ascribed to ST30 (CC30) and *agr* III. However, these isolates were distributed over four different *spa*-types. Finally, two isolates belonged to ST398 (CC398) and *spa*-types t571 and t011.

2.2. Characterization of CoNS Isolates

Donkey and handler samples were also screened for the presence of methicillin-resistant CoNS (MRCoNS). Only four (17.4%) handlers carried MRCoNS, these being 3 *S. epidermidis* and one *S. sciuri* (Table 2). Out of the 49 donkeys, 21 (42.9%) were colonized by CoNS including *S. sciuri* ($n = 17$), *S. lentus* ($n = 2$), *S. xylosus* and *S. vitulinus*. Two *S. epidermidis* were multidrug-resistant since they were resistant to four classes of antimicrobials. They were resistant to penicillin, aminoglycosides, erythromycin, tetracycline and trimethoprim/sulfamethoxazole and harbored the *blaZ*, *aac* (6′)-Ie-*aph* (2′)-Ia, *aph* (3′)-IIIa, *ermC*, *dfrA* and *dfrG* genes. The only *S. sciuri* isolated from one handler showed resistance penicillin, ceftiofur and clindamycin and harbored the *mecA* and *lnuA* genes. Even though all CoNS were isolated from ORSAB medium supplemented with 2.5 mg/L of oxacillin, two *S. epidermidis*, one *S. lentus* and one *S. xylosus* isolates lacked the *mecA* gene. All the remaining isolates carried the *mecA* gene even those showing phenotypic susceptibility to all antimicrobials tested ($n = 7$). One *S. lentus* isolate also displayed a multidrug-resistant phenotype with resistances to penicillin, ciprofloxacin, macrolides and

licosamides conferred by the *mecA*, *blaZ* and *lnuA* genes. *S. sciuri* isolates showed a variety of resistances encoded by *optrA*, *aph* (3')-IIIa, *ant* (4')-Ia, *str*, *ermB*, *tetK* and *tetM*. Finally, only four CoNS carried virulence genes: two *eta*, one *tst* and one *hla*.

Table 2. Diversity, antimicrobial resistance and virulence of CoNS isolated from donkeys and handlers.

| Isolate | Source/Species | Antimicrobial Resistance | | Virulence Factors |
|---------|------------------------------|----------------------------------|--|-------------------|
| | | Phenotype | Genotype | |
| VS3115 | Human/ <i>S. epidermidis</i> | PEN, CN, TOB, KAN, TET, SXT | <i>blaZ</i> , <i>aac</i> (6')-Ie- <i>aph</i> (2'')-Ia, <i>aph</i> (3')-IIIa, <i>dfrA</i> | |
| VS3116 | Human/ <i>S. epidermidis</i> | PEN, CN, KAN, ERY, SXT | <i>mecA</i> , <i>ermC</i> , <i>aac</i> (6')-Ie- <i>aph</i> (2'')-Ia, <i>aph</i> (3')-IIIa, <i>dfrA</i> , <i>dfrG</i> | <i>tst</i> |
| VS3117 | Human/ <i>S. epidermidis</i> | PEN, FD | - | <i>eta</i> |
| VS3118 | Human/ <i>S. sciuri</i> | PEN, FOX, CD, FD | <i>mecA</i> , <i>lnuA</i> | |
| VS3119 | Donkey/ <i>S. lentus</i> | FD | - | |
| VS3120 | Donkey/ <i>S. lentus</i> | PEN, CIP, ERY, CD, TET, FD | <i>mecA</i> , <i>blaZ</i> , <i>lnuA</i> | |
| VS3121 | Donkey/ <i>S. xylosum</i> | TET | - | |
| VS3122 | Donkey/ <i>S. vitulinus</i> | PEN, TET | <i>mecA</i> | |
| VS3123 | Donkey/ <i>S. sciuri</i> | PEN, CD, FD | <i>mecA</i> | |
| VS3124 | Donkey/ <i>S. sciuri</i> | PEN, FD | <i>mecA</i> | |
| VS3125 | Donkey/ <i>S. sciuri</i> | PEN, FOX, TOB, KAN | <i>mecA</i> , <i>aph</i> (3')-IIIa | |
| VS3126 | Donkey/ <i>S. sciuri</i> | PEN, FOX, LNZ, ERY, CN, TOB, KAN | <i>mecA</i> , <i>optrA</i> , <i>ermB</i> , <i>aph</i> (3')-IIIa | <i>eta</i> |
| VS3127 | Donkey/ <i>S. sciuri</i> | PEN, FOX, CD, FD | <i>mecA</i> | |
| VS3128 | Donkey/ <i>S. sciuri</i> | PEN, CN, FD | <i>mecA</i> , <i>aph</i> (3')-IIIa | |
| VS3129 | Donkey/ <i>S. sciuri</i> | PEN, CN, TOB, KAN, FD | <i>mecA</i> , <i>aph</i> (3')-IIIa, <i>ant</i> (4')-Ia, <i>str</i> | |
| VS3130 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | |
| VS3131 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | |
| VS3132 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | |
| VS3133 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | |
| VS3134 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | |
| VS3135 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | <i>hla</i> |
| VS3136 | Donkey/ <i>S. sciuri</i> | PEN, TET | <i>mecA</i> , <i>tetK</i> | |
| VS3137 | Donkey/ <i>S. sciuri</i> | PEN | <i>mecA</i> | |
| VS3138 | Donkey/ <i>S. sciuri</i> | PEN, KAN, TOB, TET | <i>mecA</i> , <i>aph</i> (3')-IIIa, <i>ant</i> (4')-Ia, <i>str</i> , <i>tetM</i> | |
| VS3139 | Donkey/ <i>S. sciuri</i> | PEN, CD, FD | <i>mecA</i> | |

Abbreviations. PEN: penicillin; FOX: cefoxitin; LNZ: linezolid; CIP: ciprofloxacin; CN: gentamycin; TOB: tobramycin; KAN: kanamycin; ERY: erythromycin; CD: clindamycin; TET: tetracycline; FD, fusidic acid; SXT: trimethoprim-sulfamethoxazole.

3. Discussion

In this study we collected oral and nasal swab samples from 49 Miranda donkeys and 23 handlers to investigate the frequency of staphylococci in donkeys and a possible transmission between animals and humans due to the close proximity. It has been shown that the close contact between pets and humans offers favorable conditions for transmission by direct contact [19]. In our study, *S. aureus* were isolated from 4 (8.2%) donkeys and 10 (43.5%) handlers among the 49 and 23 donkeys and handlers' samples, respectively, and were further characterized by molecular typing in order to investigate a possible transmission. Studies reporting the infection or colonization of donkeys by *S. aureus* are very scarce. Nevertheless, one study conducted with nasal samples of healthy donkeys and another with conjunctival swabs reported a frequency of *S. aureus* of 50% and 47.8%, respectively [13,20]. Another study investigated the occurrence of *S. aureus* in healthy donkeys and donkeys with respiratory tract disease and reported an incidence of 13.2% [21]. As for handlers, the proportion of samples that were positive for *S. aureus* is in line with the normal frequency of human colonization, which is approximately 30% [22]. *S. aureus* from donkeys were ascribed to the same clonal lineage while *S. aureus* isolates from handlers belonged to several different lineages. All *S. aureus* isolated from donkeys belonged to ST49, *spa*-type t208 and *agr* II, which was a clonal lineage distinct from those found in

humans. Furthermore, no *S. aureus* was isolated from handlers of those donkeys. In fact, isolates from humans were from different STs than the ones from donkeys. Three donkeys were from the same farm and all isolates presented the same resistance phenotype and genotype, which suggests a possible animal-to-animal transmission. One *S. aureus* was isolated from a donkey living in a different farm. Gharsa et al. conducted a study on *S. aureus* from donkeys destined to food consumption in Tunisia and reported a wider diversity of clones with ST133 as the main lineage (present in 44% of the isolates) which is frequently found in ruminants [13]. Little et al. studied the occurrence of *S. aureus* in diseased equines, including six donkeys, and isolated two *S. aureus* from donkeys which were ascribed to ST8-t064 and ST398-t011 [12]. *S. aureus* ST49-t208 has been widely reported as a cause of infection in European red squirrels [23–25] and it has also been found in wild rodents [26] and in pigs [27]. In Portugal, this particular clonal lineage has been reported in surface waters and as the dominant clone in wild night raptors [28,29]. Therefore, it seems that *S. aureus* ST49-t208 lacks host specificity, but it is associated with animals. Although studies reporting *S. aureus* in donkeys are rare, studies conducted with horses showed that the most common lineages in Europe are ST1, ST254, ST22 and ST398 [30]. Regarding the human *S. aureus* strains isolated from donkey handlers, two isolates were ascribed to ST1290, t131 and *agr* I and carried the same antimicrobial and virulence genes. Both strains were isolated from handlers from the same farm, which may suggest a possible human-to-human transmission. Furthermore, both isolates carried the IEC system genes (ascribed to type E), which indicates a human origin since the adaptation of human strains to animals required genome alterations including the loss of elements that contain the IEC [31]. *S. aureus* ST1290-t131 was first reported to be associated with community-acquired MRSA and it has also been reported among vancomycin-intermediate *S. aureus* from bloodstream infection and wild rodents from Portugal [32–34]. Most *S. aureus* isolates from handlers were ascribed to ST30 and *agr* III which, in turn, belonged to four different *spa*-types (t021, t338, t012 and t1642). *S. aureus* ST30 is primarily associated with human colonization and infection but it has spread to pets and farm animals [35]. *S. aureus* CC30 is associated with the carriage of the virulence genes PVL and *tst* [36,37]. In our study, four of the six ST30 *S. aureus* carried the *tst* gene but all were negative for the gene encoding PVL. Finally, two handlers' *S. aureus* isolates belonged to ST398 (CC398) and *spa*-types t571 and t011. *S. aureus* ST398 is a lineage initially described as colonizing livestock pigs and later other farm animals, such as poultry, horses, cows and veal calves, and it has also been described as colonizing humans [38–41]. Most ST398 found among humans are associated with methicillin-susceptible *S. aureus*, whereas in animals ST398 is more often found linked to MRSA strains [42,43]. Furthermore, most ST398 *S. aureus* isolated from humans belong to *spa*-type t571 while t011 is more predominant in animals [43]. In fact, in our study, the ST398-t011 isolate was resistant to tetracycline carrying the *tetL* and *tetM* genes and according to other studies tetracycline-resistance may be a phenotypic marker of animal-associated ST398 [44]. By contrast, the ST398-t571 isolate not only lacked tetracycline resistance but it was also susceptible to all antimicrobials tested. Some of the donkey handlers tested in this study live off agriculture and it is very likely that they have more farm animals; the detection of a tetracycline-resistant ST398-t011 *S. aureus* may thus have actually resulted from an animal-to-human transmission.

MRCoNS were detected in almost half of the donkeys (42.9%) and in four (17.4%) handlers. Although studies showing the frequency of *S. aureus* in donkeys are scarce, studies of CoNS are even rarer. Moreover, the few studies conducted with donkeys focused on non-*S. aureus* staphylococci and determined only one species, mainly *S. pseudintermedius* [45,46]. Foti et al. described the bacterial flora present in the normal conjunctiva of donkeys and detected four species of CoNS species including *S. xylosum* ($n = 22$), *S. chromogenes* ($n = 4$), *S. cohnii* ($n = 2$) and *S. lentus* ($n = 2$) [20]. In the same study, CoNS were more frequent than *S. aureus*, which is in accordance with our results [20]. In a study by Gutema et al., CoNS were isolated from 9.2% of healthy donkeys and 10.6% of donkeys with respiratory problems [21]. However, in that study, the CoNS species were not identified. *S. sciuri* was

the most prevalent species among donkeys. *S. sciuri* and *S. lentus* were also among the most frequently detected CoNS species in horses [47–51]. However, other studies report other species of CoNS as the most common in horses such as *S. capitis* and *S. equorum* [14,52]. *S. lentus* is commonly detected among farm animals [53]. Staphylococci from the *S. sciuri* group (*S. sciuri*, *S. lentus*, *S. xylosus*) are commensal bacteria of the skin and mucous membranes of different animal species and are known to have a broad host range [48]. Nevertheless, they are occasionally responsible for opportunistic infections both in animals and humans [54–56]. All *S. sciuri* isolates carried the *mecA* gene, including the strain isolated from one handler, as previously reported among equine staphylococcal isolates, which was not a surprise since it had been hypothesized that *S. sciuri* may be the evolutionary precursor of the *mecA* gene [4,52,57]. One handler was colonized by *S. sciuri* and one of their donkeys (VS3127) was also a carrier of *S. sciuri*. Transmission of *S. sciuri* strains between horses and their handlers have been previously documented [58]. Three handlers were colonized by *S. epidermidis*, which are one of the most common members of the healthy cutaneous microbiome both in humans and animals [59,60]. However, two *S. epidermidis* isolates were multidrug-resistant. Furthermore, although *S. epidermidis* possesses fewer virulence factors than *S. aureus*, two of the isolates carried virulence genes [61]. *eta* is an exfoliative toxin and was detected in two CoNS isolates—one *S. epidermidis* and one multidrug-resistant *S. sciuri*—and it has been previously reported in different staphylococcal species [62,63]. The *S. sciuri* isolate carrying the *eta* gene was also the only isolate showing resistance to linezolid conferred by the *optrA* gene. This gene was first found in enterococci in China, and later in *S. sciuri* isolated from pigs [64–66]. By contrast to *cfr*, which also confers resistance to linezolid and other antimicrobial classes, *optrA* only confers resistance to oxazolidinones [64].

4. Materials and Methods

4.1. Sample Collection and Bacterial Isolates

Oral and nasal swab samples (one swab per animal/handler) were collected from 49 Miranda donkeys (*Equus asinus*) and 23 handlers from October 2019 to January 2020 in collaboration with the Association for the Study and Protection of Donkey Cattle (Associação para o Estudo e Proteção do Gado Asinino—AEPGA). Donkeys were housed in 15 different farms. The number of samples collected in each farm are shown in Table S1. The 49 donkeys consisted of 12 males and 37 females and ranging in age from 4 months to 21 years, with a median age of 8 years (Table S1). The handlers consisted of 13 men and 10 women with a median age of 54 years. All samples were correctly identified and sent to the laboratory within a maximum of 2 days after being collected. The swabs were inserted into tubes containing brain heart infusion (BHI) broth with 6.5% of NaCl and incubated at 37 °C for 24 h. Then, 100 µL of inoculum was seeded onto Baird–Parker agar and oxacillin resistance screening agar base (ORSAB) plates for the isolation of *S. aureus* and methicillin-resistant staphylococci. Baird–Parker plates were incubated at 37 °C for 24 h whereas ORSAB plates were incubated for 24 to 48 h. One colony was recovered from each plate. The isolate species were identified by matrix-assisted laser desorption/ionisation–time of flight mass spectrometry (MALDI-TOF MS).

4.2. Phenotypic Antimicrobial Resistance and Susceptibility

The resistance phenotype of each of the isolates was established by the Kirby–Bauer disk diffusion method. The susceptibility of the isolates was tested against 14 antimicrobial agents according to the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018) [67] with the exception of kanamycin, which followed the American Guidelines of the Clinical & Laboratory Standards Institute (CLSI 2017) [68] at the following concentrations per disk: penicillin 1 U, cefoxitin 30 µg, tetracycline 30 µg, linezolid 10 µg, timetroprim/sulfamethoxazole 1.25/23.75 µg, ciprofloxacin 5 µg, erythromycin 15 µg, clindamycin 5 µg, gentamicin 10 µg, tobramycin 10 µg, chloramphenicol, 30 µg,

fusidic acid 10 µg, kanamycin 30 µg and mupirocin 200 µg. *S. aureus* strain ATCC 25,923 was used as quality control in the susceptibility assays.

4.3. Detection of Antimicrobial and Virulence Genes

According to the phenotypic resistance of each isolate, the following antimicrobial resistance genes were studied by PCR: β-lactams (*blaZ* and *mecA*), macrolides and lincosamides (*ermA*, *ermB*, *ermC*, *ermT*, *msr* (A/B), *mphC*, *lnuA*, *lnuB*, *vgaA* and *vgaB*), tetracycline (*tetM*, *tetK*, *tetL* and *tetO*), aminoglycosides (*aac* (6′)-Ie-aph (2′′)-Ia, *ant* (4′)-Ia, *aph* (3′)-IIIa and *str*), trimethoprim/sulfamethoxazole (*dfrA*, *dfrG*, *dfrK* and *dfrD*) and fusidic acid (*fusA*, *fusB*, *fusC* and *fusD*) [69].

The presence of *hla*, *hly*, *hld*, *lukF/lukS-PV*, *eta*, *etb* and *tst* genes encoding for virulence factors such as hemolysins, Pantone–Valentine leucocidin, exfoliatins and toxic shock syndrome toxin were investigated by PCR [69]. In addition, the presence of the IEC was also evaluated, first by investigating the presence of *scn* gene and then, in positive isolates, the presence of the other IEC genes (*chp*, *sak*, *sea* and *sep*) to determine the IEC group [70]. Positive and negative controls used in all experiments belonged to the strain collection of the University of Trás-os-Montes and Alto Douro.

4.4. Molecular Typing

All *S. aureus* isolates were typed by multilocus sequence typing (MLST) based on seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmK*, *pta*, *tpiA* and *yqiL*) as described by Enright et al. [71]. Each isolate was assigned to a ST and then to a CC according to the MLST database (<http://www.mlst.net/>, accessed on 14 December 2021). The isolates were also typed by *spa*-typing described by Harmsen et al. [72]. PCR products were subjected to DNA sequencing and the isolates were assigned to the specific *spa* types according to the Ridom SpaServer database (<http://www.spaserver.ridom.de>, accessed on 13 December 2021). Finally, *agr* typing was also performed in all *S. aureus* strains as previously described [73].

5. Conclusions

S. aureus was detected in 8% of the donkeys screened. However, colonization with different species of MRCoNS isolates with multidrug resistance were detected. *S. aureus* transmission between donkeys and their handlers appears not to have occurred since the clonal types of *S. aureus* isolates were distinct from each other. Furthermore, most *S. aureus* clones isolated from handlers were associated with human origin except for one CC398 isolate harboring resistance to tetracycline.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antibiotics11030374/s1>, Table S1: Description of donkeys and handlers' samples, farms, sampling locations and staphylococci recovery.

Author Contributions: Conceptualization, V.S. and P.P.; methodology, V.S. and V.M.; validation, M.C. and P.P.; investigation, V.S. and C.A.; resources, M.N., B.L. and M.K.; data curation, V.S.; writing—original draft preparation, V.S.; writing—review and editing, V.S.; supervision, J.L.C., G.I. and P.P.; funding acquisition, P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the R&D Project CAREBIO2: Comparative assessment of antimicrobial resistance in environmental biofilms through proteomics—towards innovative therapeutic biomarkers, with reference NORTE-01-0145-FEDER-030101 and PTDC/SAU-INF/30101/2017, financed by the European Regional Development Fund (ERDF) through the Northern Regional Operational Program (NORTE 2020) and the Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry-LAQV, which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020) and by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Vanessa Silva is grateful to FCT (Fundação para a Ciência e a Tecnologia) for financial support through the PhD grant SFRH/BD/137947/2018.

Institutional Review Board Statement: The study was conducted according to the Helsinki Declaration (ICH-GCP principles), in compliance with Schedule Y/ICMR Guidelines, the Oviedo Convention, and was approved by the Ethics Committee of University of Trás-os-Montes e Alto Douro (EC-UTAD, 8 November 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Igrejas, G.; Correia, S.; Silva, V.; Hébraud, M.; Caniça, M.; Torres, C.; Gomes, C.; Nogueira, F.; Poeta, P. Planning a One Health Case Study to Evaluate Methicillin Resistant Staphylococcus aureus and Its Economic Burden in Portugal. *Front. Microbiol.* **2018**, *9*, 2964. [[CrossRef](#)] [[PubMed](#)]
2. Papadopoulos, P.; Papadopoulos, T.; Angelidis, A.S.; Boukouvala, E.; Zdragas, A.; Papa, A.; Hadjichristodoulou, C.; Sergelidis, D. Prevalence of Staphylococcus aureus and of methicillin-resistant *S. aureus* (MRSA) along the production chain of dairy products in north-western Greece. *Food Microbiol.* **2018**, *69*, 43–50. [[CrossRef](#)] [[PubMed](#)]
3. Coates, R.; Moran, J.; Horsburgh, M.J. Staphylococci: Colonizers and pathogens of human skin. *Future Microbiol.* **2014**, *9*, 75–91. [[CrossRef](#)] [[PubMed](#)]
4. Silva, V.; Capelo, J.L.; Igrejas, G.; Poeta, P. Molecular Epidemiology of Staphylococcus aureus Lineages in Wild Animals in Europe: A Review. *Antibiotics* **2020**, *9*, 122. [[CrossRef](#)]
5. Becker, K.; Ballhausen, B.; Köck, R.; Kriegeskorte, A. Methicillin resistance in Staphylococcus isolates: The “mec alphabet” with specific consideration of mecC, a mec homolog associated with zoonotic *S. aureus* lineages. *Int. J. Med. Microbiol.* **2014**, *304*, 794–804. [[CrossRef](#)]
6. Hamza, D.A.; Abd-Elsalam, R.M.; Nader, S.M.; Elhariri, M.; Elhelw, R.; El-Mahallawy, H.S. Pathways of methicillin-resistant Staphylococcus aureus in animal model: New insights regarding public health. *Infect. Drug Resist.* **2020**, *13*, 1593. [[CrossRef](#)]
7. Hanssen, A.-M.; Ericson Sollid, J.U. SCC mec in staphylococci: Genes on the move. *FEMS Immunol. Med. Microbiol.* **2006**, *46*, 8–20. [[CrossRef](#)]
8. Grøntvedt, C.A.; Elstrøm, P.; Stegger, M.; Skov, R.L.; Andersen, P.S.; Larssen, K.W.; Urdahl, A.M.; Angen, Ø.; Larsen, J.; Åmdal, S.; et al. Methicillin-Resistant Staphylococcus aureus CC398 in Humans and Pigs in Norway: A “One Health” Perspective on Introduction and Transmission. *Clin. Infect. Dis.* **2016**, *63*, 1431–1438. [[CrossRef](#)]
9. Harrison, E.M.; Weinert, L.A.; Holden, M.T.G.; Welch, J.J.; Wilson, K.; Morgan, F.J.E.; Harris, S.R.; Loeffler, A.; Boag, A.K.; Peacock, S.J. A shared population of epidemic methicillin-resistant Staphylococcus aureus 15 circulates in humans and companion animals. *mBio* **2014**, *5*, e00985-e13. [[CrossRef](#)]
10. Haag, A.F.; Fitzgerald, J.R.; Penadés, J.R. Staphylococcus aureus in Animals. In *Gram-Positive Pathogens*; Wiley: Hoboken, NJ, USA, 2019; pp. 731–746.
11. Weinert, L.A.; Welch, J.J.; Suchard, M.A.; Lemey, P.; Rambaut, A.; Fitzgerald, J.R. Molecular dating of human-to-bovid host jumps by Staphylococcus aureus reveals an association with the spread of domestication. *Biol. Lett.* **2012**, *8*, 829–832. [[CrossRef](#)]
12. Little, S.V.; Hillhouse, A.E.; Lawhon, S.D.; Bryan, L.K. Analysis of Virulence and Antimicrobial Resistance Gene Carriage in Staphylococcus aureus Infections in Equids Using Whole-Genome Sequencing. *mSphere* **2022**, *6*, e00196-e20. [[CrossRef](#)] [[PubMed](#)]
13. Gharsa, H.; Ben Sallem, R.; Ben Slama, K.; Gómez-Sanz, E.; Lozano, C.; Jouini, A.; Klibi, N.; Zarazaga, M.; Boudabous, A.; Torres, C. High diversity of genetic lineages and virulence genes in nasal Staphylococcus aureus isolates from donkeys destined to food consumption in Tunisia with predominance of the ruminant associated CC133 lineage. *BMC Vet. Res.* **2012**, *8*, 203. [[CrossRef](#)] [[PubMed](#)]
14. Bino, E.; Lauková, A.; Ščerbová, J.; Kubašová, I.; Kandričáková, A.; Strompfová, V.; Miltko, R.; Belzecki, G. Fecal coagulase-negative staphylococci from horses, their species variability, and biofilm formation. *Folia Microbiol.* **2019**, *64*, 719–726. [[CrossRef](#)] [[PubMed](#)]
15. Tirosh-Levy, S.; Steinman, A.; Carmeli, Y.; Klement, E.; Navon-Venezia, S. Prevalence and risk factors for colonization with methicillin resistant Staphylococcus aureus and other Staphylococci species in hospitalized and farm horses in Israel. *Prev. Vet. Med.* **2015**, *122*, 135–144. [[CrossRef](#)]
16. Kaspar, U.; von Lützu, K.; Schlattmann, A.; Rösler, U.; Köck, R.; Becker, K. Zoonotic multidrug-resistant microorganisms among non-hospitalized horses from Germany. *One Health* **2019**, *7*, 100091. [[CrossRef](#)]
17. Ramalho Sousa, S.; Anastácio, S.; Nóvoa, M.; Paz-Silva, A.; Madeira de Carvalho, L.M. Gastrointestinal Parasitism in Miranda Donkeys: Epidemiology and Selective Control of Strongyles Infection in the Northeast of Portugal. *Animals* **2021**, *11*, 155. [[CrossRef](#)]
18. Couto, M.; Santos, A.S.; Laborda, J.; Nóvoa, M.; Ferreira, L.M.; Madeira de Carvalho, L.M. Grazing behaviour of Miranda donkeys in a natural mountain pasture and parasitic level changes. *Livest. Sci.* **2016**, *186*, 16–21. [[CrossRef](#)]

19. Damborg, P.; Broens, E.M.; Chomel, B.B.; Guenther, S.; Pasmans, F.; Wagenaar, J.A.; Weese, J.S.; Wieler, L.H.; Windahl, U.; Vanrompay, D.; et al. Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspectives for Targeted Research and Policy Actions. *J. Comp. Pathol.* **2016**, *155*, S27–S40. [[CrossRef](#)]
20. Foti, M.; Fisichella, V.; Giacopello, C. Detection of methicillin-resistant Staphylococcus aureus (MRSA) in the microbial flora from the conjunctiva of healthy donkeys from Sicily (Italy). *Vet. Ophthalmol.* **2013**, *16*, 89–92. [[CrossRef](#)]
21. Gutema, D.F.; Duguma, B.E.; Dinka, A.G. Isolation and identification of aerobic bacterial flora from the upper respiratory tract of donkeys in central Ethiopia. *Int. J. Appl. Res. Vet. Med.* **2009**, *7*, 181–189.
22. Abimana, J.B.; Kato, C.D.; Bazira, J. Methicillin-Resistant Staphylococcus aureus Nasal Colonization among Healthcare Workers at Kampala International University Teaching Hospital, Southwestern Uganda. *Can. J. Infect. Dis. Med. Microbiol.* **2019**, *2019*, 4157869. [[CrossRef](#)] [[PubMed](#)]
23. Hofer, U. Squirrel-killing Staphylococcus aureus. *Nat. Rev. Microbiol.* **2021**, *19*, 481. [[CrossRef](#)] [[PubMed](#)]
24. Simpson, V.; Davison, N.; Hudson, L.; Enright, M.; Whatmore, A.M. Staphylococcus aureus ST49 infection in red squirrels. *Vet. Rec.* **2010**, *167*, 69. [[CrossRef](#)] [[PubMed](#)]
25. Fountain, K.; Blackett, T.; Butler, H.; Carchedi, C.; Schilling, A.-K.; Meredith, A.; Gibbon, M.J.; Lloyd, D.H.; Loeffler, A.; Feil, E.J. Fatal exudative dermatitis in island populations of red squirrels (*Sciurus vulgaris*): Spillover of a virulent Staphylococcus aureus clone (ST49) from reservoir hosts. *Microb. Genom.* **2021**, *7*, 565. [[CrossRef](#)]
26. Mrochen, D.M.; Schulz, D.; Fischer, S.; Jeske, K.; El Gohary, H.; Reil, D.; Imholt, C.; Trübe, P.; Suchomel, J.; Tricaud, E.; et al. Wild rodents and shrews are natural hosts of Staphylococcus aureus. *Int. J. Med. Microbiol.* **2018**, *308*, 590–597. [[CrossRef](#)]
27. Overesch, G.; Büttner, S.; Rossano, A.; Perreten, V. The increase of methicillin-resistant Staphylococcus aureus (MRSA) and the presence of an unusual sequence type ST49 in slaughter pigs in Switzerland. *BMC Vet. Res.* **2011**, *7*, 30. [[CrossRef](#)]
28. Silva, V.; Ferreira, E.; Manageiro, V.; Reis, L.; Tejedor-Junco, M.T.; Sampaio, A.; Capelo, J.L.; Caniça, M.; Igrejas, G.; Poeta, P. Distribution and Clonal Diversity of Staphylococcus aureus and Other Staphylococci in Surface Waters: Detection of ST425-t742 and ST130-t843 mecC-Positive MRSA Strains. *Antibiotics* **2021**, *10*, 1416. [[CrossRef](#)]
29. Silva, V.; Lopes, A.F.; Soeiro, V.; Caniça, M.; Manageiro, V.; Pereira, J.E.; Maltez, L.; Capelo, J.L.; Igrejas, G.; Poeta, P. Nocturnal Birds of Prey as Carriers of Staphylococcus aureus and Other Staphylococci: Diversity, Antimicrobial Resistance and Clonal Lineages. *Antibiotics* **2022**, *11*, 240. [[CrossRef](#)]
30. Aires-de-Sousa, M. Methicillin-resistant Staphylococcus aureus among animals: Current overview. *Clin. Microbiol. Infect.* **2017**, *23*, 373–380. [[CrossRef](#)]
31. Cuny, C.; Abdelbary, M.; Layer, F.; Werner, G.; Witte, W. Prevalence of the immune evasion gene cluster in Staphylococcus aureus CC398. *Vet. Microbiol.* **2015**, *177*, 219–223. [[CrossRef](#)]
32. Rasigade, J.-P.; Laurent, F.; Lina, G.; Meugnier, H.; Bes, M.; Vandenesch, F.; Etienne, J.; Tristan, A. Global Distribution and Evolution of Pantone–Valentine Leukocidin-Positive Methicillin-Susceptible Staphylococcus aureus, 1981–2007. *J. Infect. Dis.* **2010**, *201*, 1589–1597. [[CrossRef](#)] [[PubMed](#)]
33. Silva, V.; Gabriel, S.I.; Borrego, S.B.; Tejedor-Junco, M.T.; Manageiro, V.; Ferreira, E.; Reis, L.; Caniça, M.; Capelo, J.L.; Igrejas, G.; et al. Antimicrobial Resistance and Genetic Lineages of Staphylococcus aureus from Wild Rodents: First Report of mecC-Positive Methicillin-Resistant *S. aureus* (MRSA) in Portugal. *Animals* **2021**, *11*, 1537. [[CrossRef](#)] [[PubMed](#)]
34. Bakthavatchalam, Y.D.; Veerarahavan, B.; Peter, J.V.; Rajinikanth, J.; Inbanathan, F.Y.; Devanga Ragupathi, N.K.; Rajamani Sekar, S.K. Novel observations in 11 heroresistant vancomycin-intermediate methicillin-resistant Staphylococcus aureus strains from South India. *Genome Announc.* **2016**, *4*, e01425-e16. [[CrossRef](#)] [[PubMed](#)]
35. Seinige, D.; von Altrock, A.; Kehrenberg, C. Genetic diversity and antibiotic susceptibility of Staphylococcus aureus isolates from wild boars. *Comp. Immunol. Microbiol. Infect. Dis.* **2017**, *54*, 7–12. [[CrossRef](#)]
36. Papadimitriou-Olivgeris, M.; Drougka, E.; Fligou, F.; Dodou, V.; Kolonitsiou, F.; Filos, K.S.; Anastassiou, E.D.; Petinaki, E.; Marangos, M.; Spiliopoulou, I. Spread of Tst-Positive Staphylococcus aureus Strains Belonging to ST30 Clone among Patients and Healthcare Workers in Two Intensive Care Units. *Toxins* **2017**, *9*, 270. [[CrossRef](#)]
37. Achermann, Y.; Seidl, K.; Kuster, S.P.; Leimer, N.; Durisch, N.; Ajdler-Schäffler, E.; Karrer, S.; Senn, G.; Holzmann-Bürgel, A.; Wolfensberger, A. Epidemiology of methicillin-susceptible Staphylococcus aureus in a neonatology ward. *Infect. Control Hosp. Epidemiol.* **2015**, *36*, 1305–1312. [[CrossRef](#)]
38. Diene, S.M.; Corvaglia, A.R.; François, P.; van der Mee-Marquet, N. Prophages and adaptation of Staphylococcus aureus ST398 to the human clinic. *BMC Genom.* **2017**, *18*, 133. [[CrossRef](#)]
39. Dweba, C.C.; Zishiri, O.T.; El Zowalaty, M.E. Isolation and Molecular Identification of Virulence, Antimicrobial and Heavy Metal Resistance Genes in Livestock-Associated Methicillin-Resistant Staphylococcus aureus. *Pathogens* **2019**, *8*, 79. [[CrossRef](#)]
40. Geenen, P.L.; Graat, E.A.M.; Haenen, A.; Hengeveld, P.D.; van Hoek, A.H.A.M.; Huijsdens, X.W.; Kappert, C.C.; Lammers, G.A.C.; van Duijkeren, E.; van de Giessen, A.W. Prevalence of livestock-associated MRSA on Dutch broiler farms and in people living and/or working on these farms. *Epidemiol. Infect.* **2013**, *141*, 1099–1108. [[CrossRef](#)]
41. Anker, J.C.H.; Koch, A.; Ethelberg, S.; Mølbak, K.; Larsen, J.; Jepsen, M.R. Distance to pig farms as risk factor for community-onset livestock-associated MRSA CC398 infection in persons without known contact to pig farms—A nationwide study. *Zoonoses Public Health* **2018**, *65*, 352–360. [[CrossRef](#)]

42. Price, L.B.; Stegger, M.; Hasman, H.; Aziz, M.; Larsen, J.; Andersen, P.S.; Pearson, T.; Waters, A.E.; Foster, J.T.; Schupp, J.; et al. Staphylococcus aureus CC398: Host adaptation and emergence of methicillin resistance in livestock. *mBio* **2012**, *3*, e00305-e11. [[CrossRef](#)] [[PubMed](#)]
43. Uhlemann, A.-C.; Porcella, S.F.; Trivedi, S.; Sullivan, S.B.; Hafer, C.; Kennedy, A.D.; Barbian, K.D.; McCarthy, A.J.; Street, C.; Hirschberg, D.L. Identification of a highly transmissible animal-independent Staphylococcus aureus ST398 clone with distinct genomic and cell adhesion properties. *mBio* **2012**, *3*, e00027-e12. [[CrossRef](#)] [[PubMed](#)]
44. Elhani, D.; Gharsa, H.; Kalai, D.; Lozano, C.; Gómez, P.; Boutheina, J.; Aouni, M.; Barguellig, F.; Torres, C.; Slama, K. Ben Clonal lineages detected amongst tetracycline-resistant methicillin-resistant Staphylococcus aureus isolates of a Tunisian hospital, with detection of lineage ST398. *J. Med. Microbiol.* **2015**, *64*, 623–629. [[CrossRef](#)] [[PubMed](#)]
45. Ruscher, C.; Lübke-Becker, A.; Wleklinski, C.-G.; Šoba, A.; Wieler, L.H.; Walther, B. Prevalence of Methicillin-resistant Staphylococcus pseudintermedius isolated from clinical samples of companion animals and equidae. *Vet. Microbiol.* **2009**, *136*, 197–201. [[CrossRef](#)]
46. Ruscher, C.; Lübke-Becker, A.; Semmler, T.; Wleklinski, C.-G.; Paasch, A.; Šoba, A.; Stamm, I.; Kopp, P.; Wieler, L.H.; Walther, B. Widespread rapid emergence of a distinct methicillin- and multidrug-resistant Staphylococcus pseudintermedius (MRSP) genetic lineage in Europe. *Vet. Microbiol.* **2010**, *144*, 340–346. [[CrossRef](#)] [[PubMed](#)]
47. Bonvegna, M.; Grego, E.; Sona, B.; Stella, M.C.; Nebbia, P.; Mannelli, A.; Tomassone, L. Occurrence of Methicillin-Resistant Coagulase-Negative Staphylococci (MRCoNS) and Methicillin-Resistant Staphylococcus aureus (MRSA) from Pigs and Farm Environment in Northwestern Italy. *Antibiotics* **2021**, *10*, 676. [[CrossRef](#)]
48. Othman, A.A.; Hiblu, M.A.; Abbassi, M.S.; Abouzeed, Y.M.; Ahmed, M.O. Nasal colonization and antibiotic resistance patterns of Staphylococcus species isolated from healthy horses in Tripoli, Libya. *J. Equine Sci.* **2021**, *32*, 61–65. [[CrossRef](#)]
49. Couto, N.; Belas, A.; Tilley, P.; Couto, I.; Gama, L.T.; Kadlec, K.; Schwarz, S.; Pomba, C. Biocide and antimicrobial susceptibility of methicillin-resistant staphylococcal isolates from horses. *Vet. Microbiol.* **2013**, *166*, 299–303. [[CrossRef](#)]
50. Mallardo, K.; Nizza, S.; Fiorito, F.; Pagnini, U.; de Martino, L. A comparative evaluation of methicillin-resistant staphylococci isolated from harness racing-horses, breeding mares and riding-horses in Italy. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 169–173. [[CrossRef](#)]
51. Mehmet, D.; Bülent, B.A.Ş.; Yarsan, E. *Antimicrobial Resistance of Streptococcus spp. and Staphylococcus spp. Isolated from Respiratory Tract of Race Horses in Turkey*; Ankara Üniversitesi Veteriner Fakültesi Dergisi: Ankara, Turkey, 2022.
52. Karakulska, J.; Fijałkowski, K.; Nawrotek, P.; Pobuciewicz, A.; Poszumski, F.; Czernomysy-Furowicz, D. Identification and methicillin resistance of coagulase-negative staphylococci isolated from nasal cavity of healthy horses. *J. Microbiol.* **2012**, *50*, 444–451. [[CrossRef](#)]
53. Zhang, Y.; Agidi, S.; LeJeune, J.T. Diversity of staphylococcal cassette chromosome in coagulase-negative staphylococci from animal sources. *J. Appl. Microbiol.* **2009**, *107*, 1375–1383. [[CrossRef](#)] [[PubMed](#)]
54. Meservey, A.; Sullivan, A.; Wu, C.; Lantos, P.M. Staphylococcus sciuri peritonitis in a patient on peritoneal dialysis. *Zoonoses Public Health* **2020**, *67*, 93–95. [[CrossRef](#)] [[PubMed](#)]
55. Beims, H.; Overmann, A.; Fulde, M.; Steinert, M.; Bergmann, S. Isolation of Staphylococcus sciuri from horse skin infection. *Open Vet. J.* **2016**, *6*, 242–246. [[CrossRef](#)]
56. Al-Azawi, I.H.; Al-Hamadani, A.H.; Hasson, S.O. Association between biofilm formation and susceptibility to antibiotics in staphylococcus lentus isolated from urinary catheterized patients. *Nano Biomed. Eng.* **2018**, *10*, 97–103. [[CrossRef](#)]
57. Mama, O.M.; Gómez, P.; Ruiz-Ripa, L.; Gómez-Sanz, E.; Zarazaga, M.; Torres, C. Antimicrobial Resistance, Virulence, and Genetic Lineages of Staphylococci from Horses Destined for Human Consumption: High Detection of *S. aureus* Isolates of Lineage ST1640 and Those Carrying the lukPQ Gene. *Animals* **2019**, *9*, 900. [[CrossRef](#)]
58. De Martino, L.; Lucido, M.; Mallardo, K.; Facello, B.; Mallardo, M.; Iovane, G.; Pagnini, U.; Tufano, M.A.; Catalanotti, P. Methicillin-Resistant Staphylococci Isolated from Healthy Horses and Horse Personnel in Italy. *J. Vet. Diagn. Investig.* **2010**, *22*, 77–82. [[CrossRef](#)]
59. Pastar, I.; O'Neill, K.; Padula, L.; Head, C.R.; Burgess, J.L.; Chen, V.; Garcia, D.; Stojadinovic, O.; Hower, S.; Plano, G.V.; et al. Staphylococcus epidermidis Boosts Innate Immune Response by Activation of Gamma Delta T Cells and Induction of Perforin-2 in Human Skin. *Front. Immunol.* **2020**, *11*, 2253. [[CrossRef](#)]
60. Méric, G.; Mageiros, L.; Pensar, J.; Laabei, M.; Yahara, K.; Pascoe, B.; Kittiwat, N.; Tadee, P.; Post, V.; Lambie, S.; et al. Disease-associated genotypes of the commensal skin bacterium Staphylococcus epidermidis. *Nat. Commun.* **2018**, *9*, 5034. [[CrossRef](#)]
61. Otto, M. Virulence factors of the coagulase-negative staphylococci. *Front. Biosci.* **2004**, *9*, 841–863. [[CrossRef](#)]
62. Chajęcka-Wierzchowska, W.; Gajewska, J.; Wiśniewski, P.; Zadernowska, A. Enterotoxigenic Potential of Coagulase-Negative Staphylococci from Ready-to-Eat Food. *Pathogens* **2020**, *9*, 734. [[CrossRef](#)]
63. Zell, C.; Resch, M.; Rosenstein, R.; Albrecht, T.; Hertel, C.; Götz, F. Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. *Int. J. Food Microbiol.* **2008**, *127*, 246–251. [[CrossRef](#)] [[PubMed](#)]
64. Wang, Y.; Lv, Y.; Cai, J.; Schwarz, S.; Cui, L.; Hu, Z.; Zhang, R.; Li, J.; Zhao, Q.; He, T. A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in Enterococcus faecalis and Enterococcus faecium of human and animal origin. *J. Antimicrob. Chemother.* **2015**, *70*, 2182–2190. [[CrossRef](#)] [[PubMed](#)]

65. Li, D.; Wang, Y.; Schwarz, S.; Cai, J.; Fan, R.; Li, J.; Feßler, A.T.; Zhang, R.; Wu, C.; Shen, J. Co-location of the oxazolidinone resistance genes *optrA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. *J. Antimicrob. Chemother.* **2016**, *71*, 1474–1478. [[CrossRef](#)] [[PubMed](#)]
66. Fan, R.; Li, D.; Wang, Y.; He, T.; Feßler, A.T.; Schwarz, S.; Wu, C. Presence of the *optrA* gene in methicillin-resistant *Staphylococcus sciuri* of porcine origin. *Antimicrob. Agents Chemother.* **2016**, *60*, 7200–7205. [[CrossRef](#)] [[PubMed](#)]
67. EUCAST European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters Version 8.0*; EUCAST: Växjö, Sweden, 2018.
68. CLSI Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2017.
69. Silva, V.; Vieira-Pinto, M.; Saraiva, C.; Manageiro, V.; Reis, L.; Ferreira, E.; Caniça, M.; Capelo, J.L.; Igrejas, G.; Poeta, P. Prevalence and Characteristics of Multidrug-Resistant Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) CC398 Isolated from Quails (*Coturnix Coturnix Japonica*) Slaughtered for Human Consumption. *Animals* **2021**, *11*, 2038. [[CrossRef](#)]
70. Van Wamel, W.J.B.; Rooijackers, S.H.M.; Ruyken, M.; van Kessel, K.P.M.; van Strijp, J.A.G. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. *J. Bacteriol.* **2006**, *188*, 1310–1315. [[CrossRef](#)]
71. Enright, M.C.; Day, N.P.; Davies, C.E.; Peacock, S.J.; Spratt, B.G. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol.* **2000**, *38*, 1008–1015. [[CrossRef](#)]
72. Harmsen, D.; Claus, H.; Witte, W.; Rothgänger, J.; Claus, H.; Turnwald, D.; Vogel, U. Typing of Methicillin-Resistant *Staphylococcus aureus* in a University Hospital Setting by Using Novel Software for *spa* Repeat Determination and Database Management. *J. Clin. Microbiol.* **2003**, *41*, 5442–5448. [[CrossRef](#)]
73. Shopsin, B.; Gomez, M.; Montgomery, S.O.; Smith, D.H.; Waddington, M.; Dodge, D.E.; Bost, D.A.; Riehman, M.; Naidich, S.; Kreiswirth, B.N. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J. Clin. Microbiol.* **1999**, *37*, 3556–3563. [[CrossRef](#)]