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Summary

Background: The human relationship with cats and dogs has been suggested to be of potential concern to public health because of the possible role of pets as reservoir of antibiotic resistant microorganisms. Here I suggest the "One Health" interdisciplinary approach to be helpful towards the understanding of the role of pets in antibiotic resistance spreading, considering also the socio-emotional context of the human-pet relationship.

Methods: I investigated the presence of multi-drug resistant (MDR) staphylococci in cats, dogs and people in the nursing homes and in the community of four Swiss Cantons (Berne, Ticino, Vaud and Zurich). The study received ethical clearance from the responsible Cantonal Ethical Committees and authorization for animal experimentation from the Cantonal and Federal Veterinary Offices. Between March 2008 and December 2009 I collected nasal swabs from 978 people and nasal and ear swabs from 256 dogs and 277 cats and checked them for the presence of staphylococci. Isolated bacteria were identified and their phenotypic antibiotic resistance profile evaluated. Questionnaires on demographic information, health status and human–pet contact were completed by each participant and for each animal investigated.

Results: Rapid and reliable identification of staphylococci by matrix assisted laser desorption ionisation – time of flight mass spectrometry (MALDI-TOF MS) was a pre-requisite to understand the distribution of *Staphylococcus* spp. in people and pets, also to differentiate among phylogenetically close related species such as *S. delphini, S. intermedius,* and *S. pseudintermedius*. The analysis of the staphylococcal population composition of healthy cats and dogs revealed that *S. pseudintermedius* was present in 27 % (70/256) of healthy dogs and 3 % (8/277) of healthy cats, whereas *S. felis* was isolated only from cats and represented 31 % of their coagulase-negative staphylococcal isolates. About 17 % (92/533) of pets carried MDR *Staphylococcus* spp. strains. Previous hospitalisation (stay in a veterinary clinic during at least one night) was identified as a risk factor for the carriage of these strains in nostril and ear of cats and dogs**.** However, although a

relevant proportion of pets and nursing home residents was found to be carrier of MDR staphylococci, the residents had no increased risk of being carriers of these strains when living in homes with pets or having contact with these animals at least once a week. Findings suggested limited strain transmission between pets and humans. I could show strong physical closeness of pets with their owners in households and the high emotional importance of this relationship, but I did not observe any evident impact of pets on carriage of MDR staphylococci in their owners.

At the end of my study I also analysed the clinical implications of methicillin-resistant *S. pseudintermedius* (MRSP) infections using as an example the isolation of this microorganism from a pyoderma lesion in a dog that underwent various antibiotic treatments before the correct diagnosis was made and an appropriate antibiotic treatment was administered**.**

Discussion and conclusions: MDR staphylococci were recovered in relevant proportions from healthy pets and people. I could document the potential for exchange of strains due to close physical contact between their hosts. My results, however, indicated negligible rates of MDR staphylococcal transmission between human and pets.

In evaluating the role of pets as reservoir of antibiotic resistant staphylococci, the network of contacts and their physical intensity, together with information on multi-drug resistance carriage in humans and pets should be considered for a correct estimation of the transmission and distribution of antibiotic resistant strains among different hosts.

Riassunto

Contesto dello studio: Recentemente è stato suggerito che il contatto delle persone con i cani e i gatti possa costituire un potenziale problema per la salute pubblica in seguito al possibile ruolo degli animali domestici come serbatoi di microorganismi resistenti agli antibiotici. In questo lavoro propongo che l'approccio interdisciplinare "One Health (una sola salute)" potrebbe essere uno strumento utile per comprendere il ruolo degli animali domestici nella diffusione della resistenza antibiotica, considerando anche il ruolo socio-emozionale della relazione uomo-animale domestico.

Metodi: Ho studiato la diffusione di stafilococchi multi resistenti agli antibiotici (MDR) in gatti, cani e persone negli istituti di lunga degenza e nella comunità di quattro cantoni svizzeri (Berna, Ticino, Vaud e Zurigo). Lo studio ha ricevuto un'autorizzazione alla sua realizzazione da parte dei comitati etici dei cantoni interessati e un'autorizzazione per la sperimentazione sugli animali da parte degli uffici veterinari cantonali e federale. Tra marzo 2008 e dicembre 2009 ho eseguito degli strisci al naso di 978 persone e al naso e orecchio di 256 cani e 277 gatti per rilevare la presenza di stafilococchi. I batteri isolati sono stati identificati ed è stato valutato il loro profilo di resistenza fenotipica agli antibiotici. Dei questionari sulla demografia, lo stato di salute e il contatto uomo-animale domestico sono stati completati da ogni partecipante e per ogni animale investigato.

Risultati: Un'identificazione rapida e affidabile degli stafilococchi tramite spettrometria di massa (MALDI-TOF MS) è stata un prerequisito per la comprensione della distribuzione di *Staphylococcus* spp. nelle persone e negli animali domestici, anche per distinguere specie filogeneticamente molto vicine quali *S. delphini, S. intermedius,* e *S. pseudintermedius*. L'analisi della composizione della popolazione di stafilococchi isolati da gatti e cani sani ha rivelato che *S. pseudintermedius* era presente nel 27 % (70/256) dei cani e 3 % (8/277) dei gatti, mentre *S. felis* è stato isolato unicamente dai gatti e rappresentava il 31 % di tutti gli stafilococchi coagulasi-negativa isolati. Circa il 17 % (92/533) degli animali era portatore di almeno un ceppo di *Staphylococcus* spp. MDR. Un'ospedalizzazione precedente (soggiorno di almeno una notte in una

clinica veterinaria) è stata identificata come fattore di rischio per la presenza di questi microorganismi nel naso e nelle orecchie dei cani e dei gatti. Nonostante una proporzione relativamente alta di animali e residenti di istituti di lunga degenza fosse portatrice di stafilococchi MDR, i residenti degli istituti dove gli animali erano presenti o con un contatto con questi animali almeno una volta la settimana non avevano un rischio accresciuto di essere portatori. I risultati indicano quindi una trasmissione limitata di ceppi tra uomo e animale. Ho potuto osservare una vicinanza fisica intensa degli animali con i loro proprietari all'interno delle economie domestiche e verificare la forte importanza emotiva di questa relazione, ma non ho osservato nessun impatto evidente degli animali sulla presenza di stafilococchi MDR nei loro proprietari.

In conclusione del mio lavoro ho pure analizzato le implicazioni cliniche delle infezioni da *S. pseudintermedius* resistente alla meticillina (MRSP) prendendo spunto da un microorganismo isolato da una lesione del pioderma in un cane che aveva ricevuto diversi trattamenti antibiotici prima che una diagnosi corretta fosse eseguita e fosse somministrato un appropriato trattamento antibiotico.

Discussione e conclusioni: Ho potuto isolare degli stafilococchi MDR in proporzioni relativamente alte da animali domestici sani e da persone. Ho pure potuto documentare un potenziale per uno scambio di ceppi in seguito ad un contatto fisico stretto tra i loro ospiti. I nostri risultati indicano però che la probabilità di una trasmissione di stafilococchi MDR è trascurabile.

Nel valutare il ruolo degli animali domestici come serbatoio di stafilococchi, la rete di contatti e la loro intensità fisica, così come le informazioni sulla presenza di multiresistenze nelle persone e negli animali domestici dovrebbero essere considerati per ottenere una stima corretta della probabilità di propagazione dei ceppi resistenti agli antibiotici nei vari ospiti.

Résumé

Contexte de l'étude: Dans les dernières années il a été proposé que le contact des gens avec des chats et des chiens puisse entraîner un problème de santé publique à cause du rôle potentiel des animaux domestiques en tant que réservoir de microorganismes résistants aux antibiotiques. Dans ce travail je propose que l'approche interdisciplinaire "One Health (une seule santé)" pourrait être un moyen utile pour comprendre le rôle des animaux domestiques dans la diffusion de la résistance antibiotique, en considérant aussi le contexte socio-émotionnel de la relation homme-animal domestique.

Méthodes: J'ai étudié la présence de staphylocoques multirésistants aux antibiotiques (MDR) chez les chats, les chiens et les personnes dans des établissements médico-sociaux (EMS) et dans la communauté de quatre Cantons Suisses (Berne, Tessin, Vaud et Zürich). L'étude a reçu l'autorisation à son déroulement de la part des Comités d'étique cantonaux concernés et une autorisation pour l'expérimentation sur les animaux de la part des Offices vétérinaires cantonaux et fédéral. Entre mars 2008 et décembre 2009 j'ai effectué des frottis au nez de 978 personnes et au nez et à l'oreille de 256 chiens et 277 chats pour rechercher la présence de staphylocoques. Les bactéries isolées ont été identifiées et leur profil de résistance phénotypique aux antibiotiques a été évalué. Des questionnaires sur la démographie, l'état de santé et le contact homme-animal domestique ont été remplis par chaque participant et pour chaque animal investigué.

Résultats: Une identification rapide et fiable des staphylocoques à l'aide de la spectrométrie de masse en désorption laser assisté par matrice (MALDI-TOF MS) a été une condition préalable pour la compréhension de la distribution de *Staphylococcus* spp. chez les personnes et les animaux domestiques; elle a aussi aidé à identifier fiablement des espèces phylogénetiquement très proches comme *S. delphini, S. intermedius* et *S. pseudintermedius*. L'analyse de la composition de la population de staphylocoques de chats et chiens sains a relevé que *S. pseudintermedius* était présent chez 27 % (70/256) des chiens et 3 % (8/277) des chats, tandis que *S. felis* a été isolé uniquement

depuis les chats et il représentait 31 % de tous les isolats de staphylocoques coagulase-négatives. Environ 17 % (92/533) des animaux était porteur au moins d'une souche de *Staphylococcus* spp. MDR. Une hospitalisation préalable (séjours dans une clinique vétérinaire au moins pendant une nuit) a été identifiée comme facteur de risque pour le portage de ces souches dans le nez et l'oreille des chats et chiens. Tout de même, malgré la proportion assez haute d'animaux et de résidents d'EMS qui étaient porteurs de staphylocoques MDR, les résidents n'avaient pas un risque accru d'être porteurs de ces souches lorsqu'ils vivaient dans des EMS où les animaux étaient présents ou lorsqu'ils avaient un contact avec ces animaux au moins une fois par semaine. Les résultats indiquent donc une transmission limitée de souches entre homme et animal. J'ai pu observer une proximité physique intense des animaux avec leurs maîtres à l'intérieur des ménages familiers et une forte importance émotionnelle de cette relation, mais je n'ai remarqué aucun impact évident des animaux sur le portage de staphylocoques MDR chez leurs maîtres.

A la fin de mon travail j'ai aussi analysé les implications cliniques des infections par *S. pseudintermedius* résistant à la méticilline (MRSP) en utilisant comme exemple une isolation de ce microorganisme depuis une lésion du pyoderme chez un chien qui avait reçu plusieurs traitements antibiotiques avant qu'une diagnostique correcte soit faite et un traitement antibiotique efficace ait été donné.

Discussion et conclusions: Des staphylocoques MDR ont été isolés en proportion remarquable depuis des animaux et des personnes saines. Nous avons documenté le potentiel qui existe quant à l'échange de souches dû au contact physique étroit entre les différents hôtes. Cependant nos résultats indiquent une proportion négligeable d'échange de staphylocoques résistants aux antibiotiques entre animaux et humains.

Dans l'évaluation du rôle des animaux domestiques en tant que réservoir de staphylocoques résistant aux antibiotiques, le réseau de contacts et leur intensité physique, ainsi que l'information quant au portage de multirésistance aux antibiotiques chez les humains et les animaux domestiques

devraient être considérés à fin d'avoir une estimation correcte de la probabilité de propagation de souches résistantes aux antibiotiques parmi les différents hôtes.

Zusammenfassung

Hintergrund: In den letzten Jahren hat man den Kontakt zwischen Menschen und Katzen sowie zwischen Menschen und Hunden zunehmend als potentiell besorgniserregend für die öffentliche Gesundheit eingestuft, vor allem wegen der möglichen Rolle von Haustieren als Reservoir antibiotikaresistenter Mikroorganismen. In meiner Arbeit schlage ich, den "One Health (eine einzige Gesundheit)" interdisziplinären Ansatz, der auch die sozialen und emotionalen Aspekte der Mensch-Tier Beziehung berücksichtigt, als ein mögliches Werkzeug vor, um die Rolle der Tiere bei der Verbreitung der Antibiotikaresistenz zu verstehen.

Methoden: Ich studierte die Prävalenz multiresistenter (MDR) *Staphylococcus*-Stämme bei gesunden Menschen, Katzen und Hunden in der Gemeinschaft und in ausgewählten Alters- und Pflegeheimen von vier Schweizer Kantonen (Bern, Tessin, Waadt und Zürich). Das Projekt wurde jeweils von den zuständigen Ethischen Komitees sowie durch die Veterinärämter der Kantone und das Bundesamt für Veterinärwesen (BVET) bewilligt. Von März 2008 bis Dezember 2009 sammelte ich Nasentupfer von 978 Personen und Nasen- und ein Ohrtupfer von 256 Hunden und 277 Katzen und prüfte sie auf das Vorhandensein von Staphylokokken. Die isolierten Bakterien wurden molekularbiologisch bestimmt und ihre phänotypische Resistenz gegen Antibiotikas ausgewertet. Von jedem Teilnehmer und für jedes Tier wurden demographische Daten, Gesundheitszustand und Angaben über Mensch-Tier Kontakt gesammelt und ausgewertet.

Ergebnis: „Matrix assisted Laser Desorption Ionisation - time of flight“ Massenspektrometrie (MALDI-TOF-MS) wurde zur genauen und schnellen Bestimmung von phylogenetisch nahverwandten Arten wie *S. delphini*, *S. intermedius* und *S. pseudintermedius* angewandt. *S. pseudintermedius* wurde aus 27 % (70/256) der Hunde und 3 % (8/277) der Katzen isoliert; *S. felis* dagegen wurde nur von Katzen isoliert und stellte insgesamt 31 % der Koagulase-negativen isolierten Staphylokokken dar. Über 17 % (92/533) der Haustiere beherbergten MDR *Staphylococcus*-Stämme. Ein Aufenthalt in einer Tierklinik während mindestens einer Nacht des

vorhergehenden Jahres vor Studienanfang stellte einen Risikofaktor für die Anwesenheit von MDR-Staphylokokken in der Nase und im Ohr von Katzen und Hunden dar. Meine Studie lässt einerseits eine potentielle Übertragung von MDR-Staphylokokken zwischen Haustieren und Menschen vermuten. Andererseits habe ich aber keine klare Rolle der Haustiere für die Übertragung von MDR-Staphylokokken auf ihre Besitzer zeigen können, obwohl ich während der ganzen Studie einen ausgeprägten, körperlichen Kontakt zwischen Haustieren und ihren Besitzern und die daraus entstehende emotionale Bedeutung dieser Beziehung beobachtet hatte.

Am Schluss meiner Arbeit bespreche ich auch die klinische Bedeutung von Methicillin-resistenten *S. pseudintermedius* (MRSP)-Infektionen: als Beispiel dient die unwirksame Behandlung mit verschiedenen Antibiotika eines aus einer Pyodermie isolierten MRSP, bevor die richtige Diagnose gestellt und die entsprechende antibiotische Behandlung verschrieben worden war.

Diskussion und Schlussfolgerungen: Multiresistente Staphylokokken wurden in relativ hohen Mengen in gesunden Haustieren und Menschen nachgewiesen. Daraus könnte man sich eine potentielle Übertragung dieser Mikroorganismen durch den körperlichen Kontakt zwischen den Wirten vorstellen. Die Ergebnisse meiner Studie zeigen jedoch, dass die Wahrscheinlichkeit einer Übertragung von MDR-Staphylokokken zwischen Menschen und Haustieren vernachlässigbar klein ist.

Künftig sollten auch das Netzwerk von Kontakten und ihre körperliche Intensität in die Bewertung der Rolle der Haustiere als Reservoir antibiotikaresistenter Staphylokokken einbezogen werden. Zusammen mit Informationen über die Anwesenheit von MDR-Staphylokokken bei Menschen und Haustieren sollten sie zu einer korrekten Schätzung der Verbreitung antibiotikaresistenter Stämme in verschiedenen Wirten führen.

Abbreviations

AOR	Adjusted odds ratio
CA-MRSA	Community acquired - methicillin resistant *Staphylococcus aureus*
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CNS	Coagulase-negative *Staphylococcus* spp.
CPS	Coagulase-positive *Staphylococcus* spp.
DALYs	Disability Adjusted Life Years
HA-MRSA	Hospital acquired - methicillin resistant *Staphylococcus aureus*
hsp60 gene	Heat shock protein 60 gene
m/z	Mass to charge ratio
MALDI-TOF MS	Matrix assisted laser desorption ionisation – time of flight mass spectrometry
MDR	Multi-drug resistance
mecA gene	Gene encoding for methicillin resistance
MIC	Minimal inhibitory concentration
MLST	Multilocus sequence typing
MRSA	Methicilli-resistant *Staphylococcus aureus*
MRSP	Methicillin resistant *Staphylococcus pseudintermedius*
OR	Odds ratio
PBP2a	Penicillin binding protein 2a
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PVL	Panton-Valentine Leukocidin toxin
QoL	Quality of life
rpoB gene	RNA polymerase beta-subunit gene
RR	Relative risk
SARAMIS	Spectral archive and microbial identification system
SCC*mec*	Staphylococcal cassette chromosome
SIG	*Staphylococcus intermedius* group
WHO	World Health Organisation

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1. Introduction

1.1. The "One Health" approach

Reports on the occurrence of probable exchange of antibiotic resistant microorganisms between human and pets have raised concerns about the role of pets as reservoir of multi-drug resistant (MDR) bacteria. Pets are increasingly used to assist therapy in nursing homes and the interaction between cats, dogs and their owners in the household has become very close. Thus, public health would benefit by investigating this issue with a "One Health" approach. "One Health" is a conceptual thinking that promote interdisciplinary cooperation between human and animal health (1).

My thesis aims to look at the carriage of MDR staphylococci from a perspective that embraces both human and veterinary medicine. The presence of antibiotic resistant staphylococci is, in fact, of concern for both disciplines and both can therefore benefit from common investigations in this field. Studies on the interactions between people and animals need to take into account the socio-cultural components and the context in which the study is carried out (2). This is why, in my work, I have examined also these aspects which are of primary importance in the relationship between pets and humans.

1.2. Socio-cultural and psychological context

In my work I mainly concentrated on the microbiological and epidemiological aspects of the human-pet relationship. However, although psychological analysis was definitely not the focus of my thesis, I believe it is important to investigate the role of pets as potential reservoir of MDR for humans by evaluating it also in a broader context, which considers also the psychological aspects of their interaction.

1.2.1. Challenges of the modern society

In the last decades social factors, such as the high divorce rate, the number of people living alone, the increased number of widowed elderly, the stress associated with urbanisation and geographic mobility, have fostered the appearance of several psychological disorders such as depression and loneliness (3). The World Health Organisation (WHO) defines mental health as "a state of well-being in which each individual realizes his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully and is able to make a contribution to her or his community" (4). Depression is an important handicap for mental health: according to the WHO, it affects about 121 million people worldwide. Future projections show that by 2020 depression will concern all class of ages and will reach the 2nd place of the ranking of Disability Adjusted Life Years (DALYs), after the cardio-vascular diseases (5). Loneliness is not as well defined as depression, but both mental states are interleaved forms of sadness or unhappiness, with loneliness being a subtype of depression characterised by a deficiency in the interpersonal relationships (3).

Improvements in the medical field, with development and availability of new technologies, have changed the demographic picture of the society, raising life expectancy and thus the proportion of older people. In Switzerland in 2008 the rate of 65 years old or older people was 21.2 % and this rate is expected to reach 33.3 % by 2050 (6). As a consequence of changes in the family structure, older people in need of care or no longer self-sufficient often do not live at home with their relatives. Since increased age was shown to be related to the occurrence and degree of multimorbidity, elderly people often spend a substantial part of their time in nursing homes where health care is provided (7).

1.2.2. Evolution of the human-pet relationship

The relationship between humans and pets has its origins in the ancient times when wolfs have been progressively domesticated and wild cats captured by early humans (8, 9). This interaction

successively evolved and reached an equilibrium that can be described as a social mutualism (10). Today this symbiotic relationship provides an atmosphere in which the two partners have an emotional interaction without any threatening at the physiological level (11). In the last decades the relationship between pets and their owners has become closer: cats and dogs are members of the family network, even representing sometimes the only daily life companion for people living marginally to society. Pets can help people in developing healthful components, providing companionship and pleasurable activity, facilitating exercise, play and happiness, being something to care for and a source of consistency, allowing feeling of security, being a comfort to touch and pleasurable to watch (12). It has been shown that people interacting with animals may benefit from improved physical health and psychological and social well-being (13). Actively looking after pets might also be an incentive to keep a moderate level of physical activity, thus reducing the risk of being overweight (14). At present pets have an important role of companionship in our society as they have never had previously in human history.

1.2.3. Pet-assisted therapy

Pets are increasingly used in the therapy of chronically diseased or elderly patients. These animals are extensively trained and have a clear therapeutic goal (15, 16). Studies conducted on hospitalised patients and elderly people residing in institutional settings reported a general health benefit from pet-assisted therapy, including reduced feeling of anxiety, loneliness and isolation (17, 18). Benefits of such approach apply also to children care, showing that, in acute care paediatric setting, children who underwent pet-assisted therapy experienced a significant reduction in pain level compared to children not having this kind of treatment (19).

Despite the psychological and social benefits shown to arise from pet-assisted therapy, there is a debate on the zoonotic potential of human-pet contact, which is at the basis of this therapeutic approach (20, 21). Indeed, pets might act as source of diseases (20). To date, no regulations on pet-

therapy animals and their handling in healthcare settings were available. Thus, Enoch et al., while documenting the carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet-therapy dog, suggested guidelines in an attempt to prevent potential spread of nosocomial infections, such as MRSA, from dogs employed in healthcare-associated settings (22).

1.3. Microbiological context

1.3.1. Contact with pets and risk of pathogen transmission

Zoonoses refer to "any disease or infection that is naturally transmissible from vertebrate animals to humans" (23). They may originate from bacteria, fungi, virus or parasites that can be transmitted by physical contact, faecally–orally, or through vectors (24). Scratches and bites from cats and dogs can lead to infections (25, 26). Guay (20) reviewed a panel of zoonoses that might be expected in long-tern care setting in association with pet-assisted therapy, providing examples of medically important infectious diseases acquired from cats and dogs, such as dermatophytosis, bartonellosis and toxoplasmosis.

Pets have been suggested to be reservoirs for antimicrobial-resistant bacteria (27-29). Companion animals represent indeed potential sources of spread of antimicrobial resistance, owing to the extensive use of antimicrobial agents in veterinary practices dealing with small animals (30, 31) and close contact with humans. The transmission of antibiotic resistant microorganisms between pets and humans in different settings (e.g. veterinary clinics and household) was recently documented (32-36).

1.3.2. Antibiotic use and antibiotic resistance

In 2002 the worldwide use of antibiotics was estimated to be around 100,000-200,000 tonnes per year (37). Large differences in antibiotic consumption were observed across countries (38). In

Switzerland the outpatient antibiotic consumption in human medicine was shown to be determined by socioeconomic factors (e.g. pro capita income, antibiotic price, density of medical practices, demographic, cultural and educational parameters) (39).

Since the discovery and the use of the first antimicrobial drugs in the 1930s and 1940s, bacteria showed the capacity to resist to antibiotics by developing different strategies (40-42). Three mechanisms of resistance toward antimicrobial agents are known: (i) enzymatic inactivation of the antibiotic; (ii) decreased intracellular accumulation of the antibiotic; and (iii) alteration or protection of the cellular target (43). The presence of resistance genes located in genetic elements (e.g. plasmids, transposons, chromosomal cassettes) greatly enhances their mobility and ability to spread from one bacterial strain to the other. Horizontal gene transfer, by exchange and acquisition of new genetic material by transduction, transformation or conjugation, is the primary mechanism by which microorganisms acquire antibiotic resistance (44).

Antibiotic resistance is commonly associated to fitness costs. Microorganisms must pay an evolutionary cost for their resistances that results in a loss of fitness (45) and can be measured at biological level (e.g. reduced growth, physiological weakness) (46). We are concerned with this evolutionary issue when thinking at the strategies for control of spread of antibiotic resistance. It has been suggested that a reduction in antibiotic use would benefit the susceptible bacteria strains that are fit under low antibiotic pressure and would thus be able to outcompete resistant strains over time (47, 48). However, reversibility is function of the time required to reduce the abundance of resistant bacteria and this is inversely related to the fitness cost of resistance (49, 50). Therefore, if the fitness cost associated to a given antibiotic resistance is low, the reversibility process might be so slow that, in most cases, it is unlikely to be of practical importance (46).

1.3.3. Antibiotic resistance in Staphylococcus spp.

Bacteria of the genus *Staphylococcus* are grouped in coagulase-positive (CPS) and coagulase-negative (CNS) species. They are common members of the normal cutaneous and mucosal bacterial community of humans and animals, but they may also cause significant and widespread bacterial infections in their hosts (51-62). Staphylococci have developed resistance to a wide range of antibiotics and the reduced treatment options in case of infections caused by MDR staphylococci is a critical issue and a challenge for clinicians and veterinarians (63-66).

Among the antibiotic resistance mechanisms found in staphylococci, methicillin resistance is of concern because of the synthesis of the low-affinity penicillin-binding protein PBP2' (PBP2a), encoded by the *mecA* gene, which confers resistance to all beta-lactams (67). The PBP2a has a decreased affinity for beta-lactams due to the modification of its active site, preventing beta-lactams and their derivates from inhibiting the final stages of peptidoglycan biosynthesis (68). The *mecA* gene is located in a mobile genetic element called staphylococcal cassette chromosome (SCC*mec*). The SCC*mec* can harbour antibiotic resistance genes others than the *mecA,* but also virulence determinants. There is evidence of horizontal gene transfer of SCC*mec* between different staphylococcal species (69). SCC*mec* have been classified and characterised according to their putative cassette chromosome recombinase gene (*ccr*) and their overall genetic composition (70). Six different types of SCC*mec*, (SCC*mec* I-VI), have been described; their discrimination, coupled with other typing methods, e.g. pulsed field gel electrophoresis (PFGE), allowed identifying genetically related staphylococcal isolates and thus confirmed also their epidemiological relationship (71, 72).

1.4. Clinical and epidemiological context

Species of *Staphylococcus* are opportunistic pathogens that can be found both as commensal microorganisms and as causative infectious agents of skin and soft tissues. As a general rule, CPS

species (*Staphylococcus aureus, S. intermedius, S. schleiferi* subsp. *coagulans, S. hyicus, S. lutrae, S. delphini* and *S. pseudintermedius*) are more virulent pathogens than CNS species (e.g. *S. epidermidis, S. hominis, S. felis, S. pettenkoferi*,…), the latter having more subtle clinical manifestations, with a subacute or even chronic clinical course lacking immediate signs of infection (73, 74). MDR staphylococci, both CPS and CNS, have become widespread in hospitals around the world, and currently methicillin-resistant strains represent the most common causes of bacterial nosocomial infections (75). Prevalence of community-associated MRSA (CA-MRSA) is increasing worldwide (76) with up to 63% of CA-MRSA that were isolated from cases of community skin and soft-tissue infections due to *S. aureus* (77); and methicillin resistance is of concern also in veterinary setting (78, 79).

1.4.1. Staphylococci in humans

In humans, CPS bacteria are of major interest because they include *S. aureus*, which is present in the anterior nares of about 25-30 % healthy people (80). This microbe has developed resistance to a wide range of antibiotics: in fact, methicillin-resistant *S. aureus* (MRSA) represents a considerable challenge of treatment for human clinicians (80, 81). MRSA strains can be classified into two groups: hospital acquired MRSA (HA-MRSA), and community acquired MRSA (CA-MRSA). The majority of CA-MRSA strains are characterized by the presence of Panton-Valentine leukocidin (PVL) toxin, (82, 83). HA-MRSA and CA-MRSA are further differentiated by their antimicrobial resistance patterns. MDR MRSA isolates are usually considered HA-MRSA, whereas CA-MRSA are resistant only to beta-lactams and macrolides (84). In Swiss nursing homes the prevalence of MRSA carriage in old patients can reach 5 % (85, 86); in 1995 up to 20 % of all *S. aureus* isolated in Swiss hospitals were MRSA (87). Meanwhile, outbreaks of CA-MRSA infections are becoming an important public health problem worldwide as a consequence of the unique combinations of

virulence factors and resistance traits of these strains that have been associated with high morbidity and mortality in the community (88).

S. aureus is the most investigated CPS, being documented and recognized as pathogenic agent, but in the literature cases of infection caused by CPS other than this species were occasionally reported. *S. schleiferi* subsp. *coagulans* (89, 90) and recently also *S. pseudintermedius* infections were identified in humans (54, 57, 58). We can find also reports of infections due to *S. intermedius* (91-93), but these are probably misidentified *S. pseudintermedius*. *S. pseudintermedius* was described for the first time in 2005 and it is indistinguishable from *S. intermedius* by phenotypic analyses; in addition, the discrimination power of *16S* in these closely related staphylococcal species is questionable (94-96). To my knowledge, there are no reports on human infections caused by the CPS species *S. hyicus, S. lutrae* or *S. delphini*.

CNS have also emerged as a considerable cause of nosocomial infections, with about 80-90 % of human isolated strains producing an inducible beta-lactamase (97). Patients with CNS infections are usually immunocompromised, with indwelling or implanted foreign bodies (e.g. catheters) (98). Even if clinical manifestations of CNS are subtle when compared to CPS (73, 74), the important role of these pathogens and their increasing incidence has been recognized (98-101). For example CNS have been documented as infectious agents in neutropenic patients (102), accounting for about one quarter of all bloodstream infections (103). Moreover, several studies showed clonal intra- and inter-hospital spread of *S. epidermidis* strains, suggesting that similar infection control measures may be necessary for MDR CNS isolates as for MRSA (98, 104-107). Accurate identification of CNS is necessary to provide a better understanding of the pathogenic potential of the various species (108, 109). Since the SCC*mec* in CNS are identical to those found in MRSA strains, and evidence of horizontal gene transfer of SCC*mec* between CNS and *S. aureus* has been reported, methicillin-resistant CNS might indeed act as reservoirs for methicillin resistance in *S. aureus* (69).

Risk factors associated with carriage and infections by MRSA were extensively investigated in last years. Colonisation by MRSA was associated with a 4-fold increase in the risk of infections caused by this microorganism (OR = 4.08, 95 % confidence interval (CI): 2.10-7.44) (110). Factors reported to increase the risk of colonisation or infection by MRSA include age above 60 years, hospitalisation in the previous year, antibiotic use in the previous 3 months, presence of wounds and recent skin infection, urinary catheter, diabetes mellitus, and peripheral vascular disease (111-114). Risk factors for the emergence and spread of CNS clones in hospitals include duration of hospital stay (especially in intensive care units), duration of antibiotic treatment, antibiotic pressure in the environment, and hygienic standards (115). Additionally, intravascular catheters, low gestational age, and long hospital stays have been described as important risk factors for the development of putative CNS infections in neonatal intensive care (116).

1.4.2. Staphylococci in cats and dogs

The most frequently isolated CPS species from dog clinical samples is *S. pseudintermedius* (117). This microorganism can show resistance to methicillin (118-121) and can also harbour the gene of a presumptive PVL analogue (l*ukS-I*) (122, 123). This species, together with *S. intermedius* and *S. delphini*, belongs to the so called "*Staphylococcus intermedius* Group" (SIG) (124). The three species are indistinguishable by biochemical and morphological characters; only recently the epidemiological relevance of *S. pseudintermedius* became evident, when new molecular identification methods (e.g. partial sequence of *hsp60* and *sodA* genes, multiplex-PCR) were developed (34, 94, 125). We lack therefore indications on the epidemiology of this species before 2005. From a phylogenetic point of view, however, *S. pseudintermedius* is not a new emerging species among dogs, but rather a misidentified biotype of *S. intermedius* (126). Additionally, SIG strains share many phenotypic characteristics with *S. aureus,* further complicating their identification (127, 128). In the last 5 years methicillin-resistant *S. pseudintermedius* (MRSP)

carriage or infections were increasingly reported in veterinary settings both in cats and dogs (59, 60, 119, 129, 130). Other CPS species found in these pets include *S. schleiferi* subsp. *coagulans* (131-133) and *S. aureus* (22, 134, 135).

CNS are also part of the normal bacterial community of skin and mucosae of pets, but developed resistance mechanisms to various antibiotics as well (136, 137). Nevertheless, their pathogenic potential and the capacity to transfer resistance genes to the CPS species are still under-investigated. Previous studies mainly focused on CPS with very little investigation on CNS (138). However, *S. felis*, a CNS species, was recognized as a possible feline urinary tract pathogen (53).

Only recently risk factors associated with the carriage of MRSA in cats and dogs were investigated in veterinary practices and referral hospitals (139, 140). Antimicrobial drugs (β-lactams or fluoroquinolones), number of antimicrobial treatments, number of days admitted to veterinary clinics, intravenous catheterisation and surgical implants were found to be associated with the carriage of MRSA in cats and dogs, thus suggesting similarities with the risk factors described for humans (139, 140). Unifactorial contingency tables were used to explore possible risk factors associated to the carriage of MRSP, showing prior hospitalisation and/or antibiotic therapy to be potential associated factors (141).

1.4.3. Potential exchange of staphylococci between humans and pets

Specific categories of people working with animals (e.g. pig farmers) are at risk of colonisation or infection by animal related methicillin-resistant staphylococcal strains (142-145). Cases of carriage or infection by *S. pseudinteremedius* in humans after contact with pets have been reported (57, 146). In the last decade the carriage of *S. aureus* in pets, with strain types typically found in humans, was also documented (22, 32, 146). Hanselman et al. described a high prevalence of *S. aureus* in dogs and *S. pseudinteremedius* in pet owners thus suggesting that transmission of CPS may occur between humans and companion animals residing within the same household (146).

1.5. References

1. Zinsstag J, Schelling E, Bonfoh B, Fooks AR, Kasymbekov J, Waltner-Toews D, et al. Towards a 'One Health' research and application tool box. Vet Ital. 2009;45:121-33.

2. Zinsstag J, Schelling E, Wyss K, Mahamat MB. Potential of cooperation between human and animal health to strengthen health systems. Lancet. 2005;366:2142-5.

3. Shaver PR, Brennan KA. Measures of depression and loneliness. In: Robinson JP, Shaver PR, Wrightsman LS, editors. Measures of personality and social psychological attitudes. San Diego, California, USA: Academic Press, Elsevier; 1991. p. 195-277.

4. WHO. World Health Organisation. http://www.who.int/features/qa/62/en/index.html.

5. WHO. World Health Organisation. http://www.who.int/mental_health/management/depression/definition/en/index.html.

6. BFS. Bundesamt für Statistik. http://www.bfs.admin.ch/bfs/portal/fr/index/themen/01/03/blank/key/10/02.html.

7. Ribbe MW, Ljunggren G, Steel K, Topinkova E, Hawes C, Ikegami N, et al. Nursing homes in 10 nations: a comparison between countries and settings - Continuing and rehabilitative care for elderly people: A comparison of countries and settings. CBSNews. 2007; http://findarticles.com/p/articles/mi_m2459/is_n6_v26/ai_20206888/.

8. Steven L. Origins and domestication. In: Steven L, editor. Handbook of applied dog behavior and training - Vol 1 - Adaptation and learning. Iowa: Iowa State Press - Blackwell Publishing Company; 2000. p. 3-30.

9. Serpell JA. Domestication and history of the cat. In: Turner DC, Bateson P, editors. The domestic cat: the biology of its behaviour, 2nd edition. Cambridge, UK: Cambridge University Press; 2000. p. 179-92.

10. Steven L. Human-dog companionship: cultural and psychological significance. In: Steven L, editor. Handbook of applied dog behavior and training - Vol 1 - Adaptation and learning. Iowa: Iowa State Press - Blackwell Publishing Company; 2000. p. 361-92.

11. Odendaal JS. Animal-assisted therapy - magic or medicine? J Psychosom Res. 2000;49:275-80.

12. Katcher AH, Friedmann E. Potential health value of pet ownership. Compendium of continuing education practice veterinarian. 1980;2:117-21.

13. Serpell JA. Beneficial effects of pet ownership on some aspects of human health and behaviour. J R Soc Med. 1991;84:717-20.

14. Cutt H, Giles-Corti B, Knuiman M, Timperio A, Bull F. Understanding dog owners' increased levels of physical activity: results from RESIDE. Am J Public Health. 2008;98:66-9.

15. TDI. Therapy Dogs International - Testing requirements. Brochure of the Therapy Dogs International. http://www.tdi-dog.org/images/New%20Testing%20Brochure%20(Final).pdf.

16. Turner DC. Das (Heim-) Tier als Therapeutikum. Der informierte Arzt - Gazette Médicale. 1999;20:368-9.

17. Colombo G, Buono MD, Smania K, Raviola R, De Leo D. Pet therapy and institutionalized elderly: A study on 144 cognitively unimpaired subjects. Arch Gerontol Geriat. 2006;42:207-16.

18. Coakley AB, Mahoney EK. Creating a therapeutic and healing environment with a pet therapy program. Complement Ther Clin Pract. 2009;15:141-6.

19. Braun C, Stangler T, Narveson J, Pettingell S. Animal-assisted therapy as a pain relief intervention for children. Complement Ther Clin Pract. 2009;15:105-9.

20. Guay DR. Pet-assisted therapy in the nursing home setting: potential for zoonosis. Am J Infect Control. 2001;29:178-86.

21. Brodie SJ, Biley FC, Shewring M. An exploration of the potential risks associated with using pet therapy in healthcare settings. J Clin Nurs. 2002;11:444-56.

22. Enoch DA, Karas JA, Slater JD, Emery MM, Kearns AM, Farrington M. MRSA carriage in a pet therapy dog. J Hosp Infect. 2005;60:186-8.

23. WHO. World Health Organisation. http://www.who.int/topics/zoonoses/en/.

24. Tan JS. Human zoonotic infections transmitted by dogs and cats. Arch Intern Med. 1997;157:1933-43.

25. Goldstein EJC. Bite wounds and infection. Clin Infect Dis. 1992;14:633-40.

26. Dire DJ. Cat bite wounds: risk factors for infection. Ann Emerg Med. 1991;20:973-9.

27. Damborg P, Top J, Hendrickx AP, Dawson S, Willems RJ, Guardabassi L. Dogs are a reservoir of ampicillin-resistant *Enterococcus faecium* lineages associated with human infections. Appl Environ Microbiol. 2009;75:2360-5.

28. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

29. Lloyd DH. Reservoirs of antimicrobial resistance in pet animals. Clin Infect Dis. 2007;45:S148-52.

30. Prescott JF. Antimicrobial use in food and companion animals. Anim Health Res Rev. 2008;9:127-33.

31. Regula G, Torriani K, Gassner B, Stucki F, Muntener CR. Prescription patterns of antimicrobials in veterinary practices in Switzerland. J Antimicrob Chemother. 2009;63:805-11.

32. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, et al. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol. 2006;115:148-55.

33. van Duijkeren E, Wolfhagen MJ, Heck ME, Wannet WJ. Transmission of a Panton-Valentine leucocidin-positive, methicillin-resistant *Staphylococcus aureus* strain between humans and a dog. J Clin Microbiol. 2005;43:6209-11.

34. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol. 2007;45:1118-25.

35. van Duijkeren E, Wolfhagen M, Box ATA, Heck M, Wannet WJB, Fluit AC. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis. 2004;10:2235-7.

36. Guardabassi L, Loeber ME, Jacobson A. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Vet Microbiol. 2004;98:23-7.

37. Wise R. Antimicrobial resistance: priorities for action. J Antimicrob Chemother. 2002;49:585-6.

38. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet. 2005;365:579-87.

39. Filippini M, Masiero G, Moschetti K. Socioeconomic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland. Health Policy. 2006;78:77-92.

40. Cohen FL, Tartasky D. Microbial resistance to drug therapy: a review. Am J Infect Control. 1997;25:51-64.

41. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10:S122-9.

42. Walsh C. Molecular mechanisms that confer antibacterial drug resistance. Nature. 2000;406:775-81.

43. Jayaraman R. Antibiotic resistance: an overview of mechanisms and a paradigm shift. Current Science. 2009;96:1475-84.

44. Buckley M. Antibiotic resistance: an ecological perspective on an old problem. Colloquium on antibiotic resistance; Annecy, France: A report from the american academy of microbiology; 2009.

45. MacLean RC, Hall AR, Perron GG, Buckling A. The population genetics of antibiotic resistance: integrating molecular mechanisms and treatment contexts. Nat Rev Genet. 2010;11:405-14.

46. Andersson DI, Hughes D. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol. 2010;8:260-71.

47. Levin BR. The population genetics of antibiotic resistance. Clin Infect Dis. 1997;24:S9-S16.

48. Andersson DI, Levin BR. The biological cost of antibiotic resistance. Curr Opin Microbiol. 1999;2:489-93.

49. Spratt BG. Antibiotic resistance: counting the cost. Curr Biol. 1996;6:1219-21.

50. Levin BR. Minimizing potential resistance: a population dynamics view. Clin Infect Dis. 2001;33:S161-9.

51. Heggelund L, Holm Samdal H, Eggum R, Jacobsen T, Bruun T, Elstrom P. Severe case of invasive community-acquired methicillin-resistant *Staphylococcus aureus* infection in Norway. Eurosurveillance. 2007;12:Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3304.

52. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA. 2007;298:1763-71.

53. Litster A, Moss SM, Honnery M, Rees B, Trott DJ. Prevalence of bacterial species in cats with clinical signs of lower urinary tract disease: recognition of *Staphylococcus felis* as a possible feline urinary tract pathogen. Vet Microbiol. 2007;121:182-8.

54. Chuang CY, Yang YL, Hsueh PR, Lee PI. Catheter-related bacteremia caused by *Staphylococcus pseudintermedius* refractory to antibiotic-lock therapy in a hemophilic child with dog exposure. J Clin Microbiol. 2010;48:1497-8.

55. Ebright JR, Penugonda N, Brown W. Clinical experience with *Staphylococcus lugdunensis* bacteremia: a retrospective analysis. Diagn Microbiol Infect Dis. 2004;48:17-21.

56. Pomba C, Couto N, Moodley A. Treatment of a lower urinary tract infection in a cat caused by a multi-drug methicillin-resistant *Staphylococcus pseudintermedius* and *Enterococcus faecalis*. J Feline Med Surg. 2010:802-6.

57. Riegel P, Jesel-Morel L, Laventie B, Boisset S, Vandenesch F, Prevost G. Coagulase-positive *Staphylococcus pseudintermedius* from animals causing human endocarditis. Int J Med Microbiol. 2010;301:237-9.

58. Van Hoovels L, Vankeerberghen A, Boel A, Van Vaerenbergh K, De Beenhouwer H. First case of *Staphylococcus pseudintermedius* infection in a human. J Clin Microbiol. 2006;44:4609-12.

59. Weese JS, Poma R, James F, Buenviaje G, Foster R, Slavic D. *Staphylococcus pseudintermedius* necrotizing fasciitis in a dog. Can Vet J. 2009;50:655-6.

60. Wettstein K, Descloux S, Rossano A, Perreten V. Emergence of methicillin-resistant *Staphylococcus pseudintermedius* in Switzerland: three cases of urinary tract infections in cats. Schweiz Arch Tierheilkd. 2008;150:339-43.

61. Penna B, Varges R, Martins R, Martins G, Lilenbaum W. In vitro antimicrobial resistance of staphylococci isolated from canine urinary tract infection. Can Vet J. 2010;51:738-42.

62. Higashide M, Kuroda M, Omura CT, Kumano M, Ohkawa S, Ichimura S, et al. Methicillin-resistant *Staphylococcus saprophyticus* isolates carrying staphylococcal cassette chromosome mec have emerged in urogenital tract infections. Antimicrob Agents Chemother. 2008;52:2061-8.

63. Finch R. Bacterial resistance-the clinical challenge. Clin Microbiol Infect. 2002;8:21-32.

64. Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res. 2005;36:697-705.

65. Martinez JL, Baquero F, Andersson DI. Predicting antibiotic resistance. Nat Rev Microbiol. 2007;5:958-65.

66. Falcone M, Serra P, Venditti M. Serious infections due to methicillin-resistant *Staphylococcus aureus*: an evolving challenge for physicians. Eur J Intern Med. 2009;20:343-7.

67. Berger-Bächi B, Rohrer S. Factors influencing methicillin resistance in staphylococci. Arch Microbiol. 2002;178:165-71.

68. Fuda C, Suvorov M, Vakulenko SB, Mobashery S. The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. J Biol Chem. 2004;279:40802-6.

69. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46:8-20.

70. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2000;44:1549-55.

71. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233-9.

72. Oliveira DC, Milheirico C, de Lencastre H. Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI. Antimicrob Agents Chemother. 2006;50:3457-9.

73. Huebner J, Goldmann DA. Coagulase-negative staphylococci: Role as pathogens. Annu Rev Med. 1999;50:223-36.

74. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev. 1994;7:117-40.

75. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of infections due to *Staphylococcus* species: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis. 2001;32:S114-S32.

76. Elston JW, Meigh J, Kearns AM, Jordan-Owers N, Newton A, Meigh RE, et al. Community-associated meticillin-resistant *Staphylococcus aureus*: epidemiology, microbiology and clinical impact in East Yorkshire, UK. J Hosp Infect. 2009;72:307-13.

77. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med. 2006;144:309-17.

78. Weese JS, van Duijkeren E. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol. 2010;140:418-29.

79. Cohn LA, Middleton JR. A veterinary perspective on methicillin-resistant staphylococci. J Vet Emerg Crit Care. 2010;20:31-45.

80. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet. 2006;368:874-85.

81. Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Korvick JA, et al. Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. Am J Med. 1993;94:313-28.

82. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9:978-84.

83. Berglund C, Molling P, Sjoberg L, Soderquist B. Predominance of staphylococcal cassette chromosome mec (SCCmec) type IV among methicillin-resistant *Staphylococcus aureus* (MRSA) in a Swedish county and presence of unknown SCCmec types with Panton-Valentine leukocidin genes. Clin Microbiol Infect. 2005;11:447-56.

84. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 2003;290:2976-84.

85. Petignat C, Attinger M. Enquête de prevalence des residents porteurs de MRSA dans le Canton de Vaud. Forum Hygiène. 2004.

86. Gaia V, De Benedetti A, Valsangiacomo C, Poloni C. Prevalenza di *Staphylococcus aureus* meticillino-resistente (MRSA) negli istituti a lunga degenza in canton Ticino: studio multicentrico 2008. Trib Med Tic. 2009;74:103-6.

87. Francioli P, Furrer H, Pittet D. Staphylocoques dorés résistants à la méthicilline: situation et enjeux. Swiss-Noso. 1995;4:25-9.

88. Zetola N, Francis JS, Nuermberger EL, Bishai WR. Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis. 2005;5:275-86.

89. Kumar D, Cawley JJ, Irizarry-Alvarado JM, Alvarez A, Alvarez S. Case of *Staphylococcus schleiferi* subspecies *coagulans* endocarditis and metastatic infection in an immune compromised host. Transpl Infect Dis. 2007;9:336-8.

90. Vandenesch F, Lebeau C, Bes M, Lina G, Lina B, Greenland T, et al. Clotting activity in *Staphylococcus schleiferi* subspecies from human patients. J Clin Microbiol. 1994;32:388-92.

91. Durdik P, Fedor M, Jesenak M, Hamzikova J, Knotkova H, Banovcin P. *Staphylococcus intermedius*-rare pathogen of acute meningitis. Int J Infect Dis. 2010;14:eS236-8.

92. Kelesidis T, Tsiodras S. *Staphylococcus intermedius* is not only a zoonotic pathogen, but may also cause skin abscesses in humans after exposure to saliva. Int J Infect Dis. 2010;14:e838-41.

93. Kempker R, Mangalat D, Kongphet-Tran T, Eaton M. Beware of the pet dog: a case of *Staphylococcus intermedius* infection. Am J Med Sci. 2009;338:425-7.

94. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J Clin Microbiol. 2007;45:2770-8.

95. Ghebremedhin B, Layer F, Konig W, Konig B. Genetic classification and distinguishing of *Staphylococcus* species based on different partial gap, 16S rRNA, hsp60, rpoB, sodA, and tuf gene sequences. J Clin Microbiol. 2008;46:1019-25.

96. Stackebrandt E, Goebel BM. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol. 1994;44:846-9.

97. von Eiff C, Proctor RA, Peters G. Coagulase-negative staphylococci - Pathogens have major role in nosocomial infections. Postgrad Med. 2001;110:63-76.

98. Piette G, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.

99. Von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect Dis. 2002;2:677-85.

100. Martin MA, Pfaller MA, Wenzel RP. Coagulase-negative staphylococcal bacteremia. Ann Intern Med. 1989;110:9-16.

101. Queck SY, Otto M. *Staphylococcus epidermidis* and other coagulase-negative staphylococci. In: Lindsay JA, editor. Staphylococcus molecular genetics. Norfolk, UK: Caister Academic Press; 2008. p. 227-55.

102. Oppenheim BA. The changing pattern of infection in neutropenic patients. J Antimicrob Chemother. 1998;41:7-11.

103. Pittet D, Wenzel RP. Nosocomial bloodstream infections. Secular trends in rates, mortality, and contribution to total hospital deaths. Arch Intern Med. 1995;155:1177-84.

104. Martins A, Cunha Mde L. Methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: epidemiological and molecular aspects. Microbiol Immunol. 2007;51:787-95.

105. Monsen T, Karlsson C, Wistrom J. Spread of clones of multidrug-resistant, coagulase-negative staphylococci within a university hospital. Infect Control Hosp Epidemiol. 2005;26:76-80.

106. Monsen T, Olofsson C, Ronnmark M, Wistrom J. Clonal spread of staphylococci among patients with peritonitis associated with continuous ambulatory peritoneal dialysis. Kidney Int. 2000;57:613-8.

107. Muldrew KL, Tang YW, Li H, Stratton CW. Clonal dissemination of *Staphylococcus epidermidis* in an oncology ward. J Clin Microbiol. 2008;46:3391-6.

108. Zadoks RN, Watts JL. Species identification of coagulase-negative staphylococci: genotyping is superior to phenotyping. Vet Microbiol. 2009;134:20-8.

109. Sivadon V, Rottman M, Chaverot S, Quincampoix JC, Avettand V, de Mazancourt P, et al. Use of genotypic identification by sodA sequencing in a prospective study to examine the distribution of coagulase-negative *Staphylococcus* species among strains recovered during septic orthopedic surgery and evaluate their significance. J Clin Microbiol. 2005;43:2952-4.

110. Safdar N, Bradley EA. The risk of infection after nasal colonization with *Staphylococcus aureus*. Am J Med. 2008;121:310-5.

111. Strausbaugh LJ, Crossley KB, Nurse BA, Thrupp LD. Antimicrobial resistance in long-term-care facilities. Infect Control Hosp Epidemiol. 1996;17:129-40.

112. Maree CL, Eells SJ, Tan J, Bancroft EA, Malek M, Harawa NT, et al. Risk factors for infection and colonization with community-associated methicillin-resistant *Staphylococcus aureus* in the Los Angeles County Jail: A case-control study. Clin Infect Dis. 2010;51:1248-57.

113. Santos HB, Machado DP, Camey SA, Kuchenbecker RS, Barth AL, Wagner MB. Prevalence and acquisition of MRSA amongst patients admitted to a tertiary-care hospital in Brazil. BMC Infect Dis. 2010;10:328.

114. Lucet JC, Chevret S, Durand-Zaleski I, Chastang C, Regnier B. Prevalence and risk factors for carriage of methicillin-resistant *Staphylococcus aureus* at admission to the intensive care unit: results of a multicenter study. Arch Intern Med. 2003;163:181-8.

115. Widerstrom M, Monsen T, Karlsson C, Wistrom J. Molecular epidemiology of meticillin-resistant coagulase-negative staphylococci in a Swedish county hospital: evidence of intra- and interhospital clonal spread. J Hosp Infect. 2006;64:177-83.

116. Vermont CL, Hartwig NG, Fleer A, de Man P, Verbrugh H, van den Anker J, et al. Persistence of clones of coagulase-negative staphylococci among premature neonates in neonatal intensive care units: Two-center study of bacterial genotyping and patient risk factors. J Clin Microbiol. 1998;36:2485-90.

117. Devriese LA, Hermans K, Baele M, Haesebrouck F. *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius*. Vet Microbiol. 2009;133:206-7.

118. Perreten V, Kadlec K, Schwarz S, Gronlund Andersson U, Finn M, Greko C, et al. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother. 2010;65:1145-54.

119. Hanselman BA, Kruth S, Weese JS. Methicillin-resistant staphylococcal colonization in dogs entering a veterinary teaching hospital. Vet Microbiol. 2008;126:277-81.

120. Schwarz S, Kadlec K, Strommenger B. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the BfT-GermVet monitoring programme 2004-2006 in Germany. J Antimicrob Chemother. 2008;61:282-5.

121. Weese JS. Antimicrobial resistance in companion animals. Anim Health Res Rev. 2008;9:169-76.

122. Ruscher C, Lubke-Becker A, Semmler T, Wleklinski CG, Paasch A, Soba A, et al. Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. Vet Microbiol. 2010;144:340-6.

123. Prevost G, Bouakham T, Piemont Y, Monteil H. Characterisation of a synergohymenotropic toxin produced by *Staphylococcus intermedius*. FEBS Lett. 1995;376:135-40.

124. Fitzgerald JR. The *Staphylococcus intermedius* group of bacterial pathogens: species re-classification, pathogenesis and the emergence of meticillin resistance. Vet Dermatol. 2009;20:490-5.

125. Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, et al. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol. 2010;48:765-9.

126. Hesselbarth J, Schwarz S. Comparative ribotyping of *Staphylococcus intermedius* from dogs, pigeons, horses and mink. Vet Microbiol. 1995;45:11-7.

127. Baron F, Cochet MF, Pellerin JL, Ben Zakour N, Lebon A, Navarro A, et al. Development of a PCR test to differentiate between *Staphylococcus aureus* and *Staphylococcus intermedius*. J Food Prot. 2004;67:2302-5.

128. Geoghegan JA, Smith EJ, Speziale P, Foster TJ. *Staphylococcus pseudintermedius* expresses surface proteins that closely resemble those from *Staphylococcus aureus*. Vet Microbiol. 2009;138:345-52.

129. Ruscher C, Lübke-Becker A, Wleklinski C-G, Soba A, Wieler LH, Walther B. Prevalence of Methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. Vet Microbiol. 2009;136:197-201.

130. De Lucia M, Moodley A, Latronico F, Giordano A, Caldin M, Fondati A, et al. Prevalence of canine methicillin resistant *Staphylococcus pseudintermedius* in a veterinary diagnostic laboratory in Italy. Res Vet Sci. 2010; doi:10.1016/j.rvsc.2010.09.014.

131. Abraham JL, Morris DO, Griffeth GC, Shofer FS, Rankin SC. Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* ssp. *schleiferi*. Vet Dermatol. 2007;18:252-9.

132. Bemis DA, Jones RD, Hiatt LE, Ofori ED, Rohrbach BW, Frank LA, et al. Comparison of tests to detect oxacillin resistance in *Staphylococcus intermedius*, *Staphylococcus schleiferi*, and *Staphylococcus aureus* isolates from canine hosts. J Clin Microbiol. 2006;44:3374-6.

133. Frank LA, Kania SA, Hnilica KA, Wilkes RP, Bemis DA. Isolation of *Staphylococcus schleiferi* from dogs with pyoderma. J Am Vet Med Assoc. 2003;222:451-4.

134. Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, Brunnberg L, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. Vet Microbiol. 2008;127:171-8.

135. Loeffler A, Pfeiffer DU, Lindsay JA, Soares-Magalhaes R, Lloyd DH. Lack of transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) between apparently healthy dogs in a rescue kennel. Vet Microbiol. 2010;141:178-81.

136. Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. Vet Microbiol. 2007;121:307-15.

137. van Duijkeren E, Box ATA, Heck M, Wannet WJB, Fluit AC. Methicillin-resistant staphylococci isolated from animals. Vet Microbiol. 2004;103:91-7.

138. Malik S, Peng H, Barton MD. Antibiotic resistance in staphylococci associated with cats and dogs. J Appl Microbiol. 2005;99:1283-93.

139. Soares Magalhaes RJ, Loeffler A, Lindsay J, Rich M, Roberts L, Smith H, et al. Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dogs and cats: a case-control study. Vet Res. 2010;41:55.

140. Faires MC, Traverse M, Tater KC, Pearl DL, Weese JS. Methicillin-resistant and -susceptible *Staphylococcus aureus* infections in dogs. Emerg Infect Dis. 2010;16:69-75.

141. Nienhoff U, Kadlec K, Chaberny IF, Verspohl J. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. Vet Microbiol. 2010;150:191-7.

142. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005;11:1965-6.

143. Wulf MW, Sorum M, van Nes A, Skov R, Melchers WJ, Klaassen CH, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinarians: an international study. Clin Microbiol Infect. 2008;14:29-34.

144. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol. 2008;128:298-303.

145. Wulf MWH, Tiemersma E, Kluytmans J, Bogaers D, Leenders ACAP, Jansen MWH, et al. MRSA carriage in healthcare personnel in contact with farm animals. J Hosp Infect. 2008;70:186-90.

146. Hanselman BA, Kruth SA, Rousseau J, Weese JS. Coagulase positive staphylococcal colonization of humans and their household pets. Can Vet J. 2009;50:954-8.

2. Aim and specific objectives of the thesis

Aim of this thesis is to gain insight in the microbiological and epidemiological relationship between humans and pets with regards to carriage of staphylococci. This will eventually allow better understanding of the potential role of cats and dogs as reservoirs of antibiotic resistant microorganisms.

Specific objectives of the thesis are:

1. To determine the prevalence of *Staphylococcus* spp. in cats, dogs and people in nursing homes of different Swiss regions;

2. To identify the isolated staphylococci at the species level and to assess their antimicrobial resistance pattern;

3. To assess whether people exposed to pets are at higher risk of carrying MDR staphylococci compared to people without close contact with pets;

4. To genotype the isolates to investigate the existence of epidemiological relationships between animal and human strains.

3. Overview of the manuscripts

Manuscripts issued from my PhD work are the result of different collaborations that allowed investigating microbiological and epidemiological aspects of MDR staphylococci. The work includes different approaches to the problem statement: challenges in the identification and discrimination of species belonging to the *Staphylococcus intermedius* Group (**RESEARCH PAPER 1)**, investigation on the distribution of MDR *Staphylococcus* spp. in pets and associated risk factors (**RESEARCH PAPER 2**), examination of MDR staphylococcal carriage in nursing homes residents in relation to the contact with pets (**RESEARCH PAPER 3**), assessment of physical closeness of pets with people in the household, emotional importance of this relationship and its implications for the carriage of staphylococci in pet owners (**WORKING PAPER 1**), clinical challenges related to the antibiotic treatment of methicillin-resistant *S. pseudintermedius* (MRSP) infections (**RESEARCH PAPER 4**).

4. Research paper 1

Identification of Staphylococcus intermedius Group by MALDI-TOF MS

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Abbreviations: SIG: *Staphylococcus intermedius* Group; MALDI-TOF MS: Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry; SARAMIS^{TM}: Spectral Archive And Microbial Identification System

hsp60 sequence data are accessible under the numbers FR731134 - FR731159

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Abstract

The *Staphylococcus intermedius* Group includes *S. intermedius*, *S. pseudintermedius* and *S. delphini*, coagulase-positive bacteria commonly isolated from animals. The identification of organisms belonging to this group is presently carried out using molecular methods. This study assessed the suitability of MALDI-TOF MS for their identification. 69 strains of different biological and geographic origins, identified by partial *hsp60* gene sequencing as *S. intermedius* (n = 15), *S. pseudintermedius* (n = 32) and *S. delphini* (n = 22), were analyzed by MALDI-TOF MS. The estimated sensitivity, specificity and efficiency were calculated. In addition we computed the agreement between the outcome of MALDI-TOF MS identification and partial *hsp60* gene sequencing. The sensitivity of MALDI-TOF MS was higher for *S. intermedius* [0.95 (95% CI: 0.68-0.99)], than for *S. pseudintermedius* [0.78 (95% CI: 0.60-0.90)] and *S. delphini* [0.64 (95% CI: 0.41-0.83)], whereas the specificity was 1 for *S. intermedius* and *S. delphini* and 0.97 (95% CI: 0.86-0.99) for *S. pseudintermedius*. The Cohen's kappa coefficient indicated almost perfect agreement between MALDI-TOF MS and *hsp60* gene sequencing for the identification of *S. intermedius* [0.96 (95% CI: 0.87-1.04)], and substantial agreement for *S. delphini* and *S. pseudintermedius* [0.70 (95% CI: 0.52-0.89) and 0.76 (95% CI: 0.62-0.92), respectively]. The overall efficiency of the proteomic identification ranged between 0.88 (95% CI: 0.78-0.95) for *S. pseudintermedius* and *S. delphini* and 0.99 (95% CI: 0.92-0.99) for *S. intermedius*. MALDI-TOF MS is thus a valuable and reliable tool for the rapid and accurate identification of bacteria belonging to the *Staphylococcus intermedius* Group.

KEY WORDS: MALDI-TOF MS; *hsp60;* Veterinary medicine; Identification; Sequencing; Taxonomy

Introduction

The *Staphylococcus intermedius* Group (SIG) includes *S. intermedius*, *S. pseudintermedius* and *S. delphini.* The denomination SIG was first used because the three species were indistinguishable by biochemical and morphological characters (1, 2). SIG are the most common coagulase-positive staphylococci (CPS) isolated from animals, in which they may act as opportunistic pathogens and cause a variety of infections such as otitis externa, pyoderma, abscesses, reproductive tract infections, mastitis, and wound infections (3). The identification of bacteria belonging to SIG is problematic. Phenotypic identification is unreliable, no commercial kits are available, and molecular identification is so far the only reliable tool (4). SIG strains share many phenotypic characteristics with *S. aureus* and this further complicates their identification (5). *S. pseudintermedius*, and not *S. intermedius* as previously thought, is the most common CPS species isolated from cats and dogs (6). Therefore, from a phylogenetic point of view, *S. pseudintermedius* is not a new emerging species among dogs, but rather a misidentified biotype of *S. intermedius* (7). In veterinary medicine, failure in treatments against staphylococcal infections might stem from inadequate species identification as for example in the case of methicillin-resistant CPS isolates, for which the MIC breakpoints of oxacillin differ with species (8, 9). Thus, a reliable and accurate method allowing a fast identification of staphylococci belonging to SIG is needed.

Many methods used to identify CPS were developed before the description of *S. pseudintermedius* in 2005 (10). Recently, Sasaki et al. (4), provided a first reliable molecular phylogenetic analysis and species identification based on partial *hsp60* gene sequences. Other genes already shown to be useful for the identification of staphylococcal species, e.g. the partial r*poB* gene (11), might also be used for the identification of SIG species, but their adequacy in identifying SIG species has not yet been proven.

In the last decade matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) has been increasingly used for the identification of microorganisms because of

its ease of use, the extremely small amount of sample needed and the possibility of simultaneous detection of analytes without previous isolation of bacterial strains (12). MALDI-TOF MS was shown to be highly accurate for bacterial classification and identification even in samples with low abundances and mixed flora (13). The technique produces a fingerprint spectrum of peptides and proteins of the analyzed microorganisms that allows an accurate identification of the bacterial species. In contrast to molecular biology, MALDI-TOF MS is a taxonomic tool with no direct phylogenetic component, being at least partly independent of the genomic features of the analyzed bacteria (14). This technique was already successfully applied to the identification of different staphylococcal isolates, both CPS and coagulase-negative species (CNS) (15, 16).

Our study aimed to assess the suitability of MALDI-TOF MS for the identification of members of the SIG complex. We calculated the estimated sensitivity, specificity and efficiency as well as the percentage of agreement in the identification of MALDI-TOF MS as compared to the sequencing of partial *hsp60* gene for the identification of strains belonging to the species *S. intermedius, S. pseudintermedius* and *S. delphini*.

Methods

Analyzed strains

We analyzed 69 strains belonging to the *Staphylococcus intermedius* Group (SIG) and identified them by sequencing of the partial *hsp60* gene as *S. intermedius* (n = 15), *S. pseudintermedius* (n = 32) and *S. delphini* (n = 22). The strains had different biological and geographic origins (Table 1). We included one reference strain and one type strain (**T**) each for *S. intermedius* (LMG19136, LMG13351- **T**) and *S. pseudintermedius* (LMG22221, LMG22219- **T**) from BCCM/LMG and the type strain for *S. delphini* (CCUG 30107- **T**) from the CCUG, Sweden. The BCCM/LMG reference

strain LMG19136 was identified as *S. pseudintermedius* by *hsp60* gene sequencing. All strains were stored in 7% skimmed milk at -80°C.

DNA extraction and genetic analysis

Pure cultures were grown on blood agar at 37°C for 24h and genomic DNA was extracted using the InstaGene™ kit (Bio-Rad, Cat. No. 732-6030) according to the manufacturer's instructions. Genetic analyses were performed using the partial heat shock protein (*hsp60*) gene sequences. The PCR mixture consisted of 20 µl of InstaGene DNA extract, 25 µl Taq PCR Master-Mix (Cat. No. 201445), 2 µl filtered (0.2 µm) and sterilized H_2O, 1.5 µl of a 10 µM primer forward and 1.5 µl of a 10 µM primer reverse solution. The positive control consisted of 20 µl of DNA extracted from the MRSA strain ATCC43300.

The primers for the amplification of the partial *hsp60* gene sequence were Staph H279 (nucleotide sequence 5'-GAATTCGAIIIIGCIGGIGA(TC)GGIACIACIAC-3') and Staph H280 (nucleotide sequence 5'-CGCGGGATCC(TC)(TG)I(TC)(TG)ITCICC(AG)AAICCIGGIGC(TC)TT -3'), which allowed the amplification of a 600bp DNA fragment (17, 18). The PCR thermal cycling conditions were 3 min at 95°C for 1 cycle, followed by 40 cycles of 1 min at 94°C, 2 min at 37°C, and 5 min at 72°C. The last cycle was performed at 72°C and lasted 10 min (18). DNA amplified fragments were stained on 0.8% agar gel with GelRed (Biotium, Cat. No. 41003). DNA purification was performed using NucleoSpin® (Cat. No. 740609.250) according to the instructions for direct purification of PCR products. We quantified the amplified and purified DNA fragments before the sequencing reaction using the software NANO DROP® ND-1000.

Sequencing reactions were carried out using Big Dye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) with a 15 µl total volume composed of 3 µl Big Dye® Terminator, 1.5 µl Big

Dye® buffer, 2.4 µl primer 1 µM, 7.1 µl H_2O and 1 µl DNA (~20 ng/µl) sample. Primers for the sequencing of the partial *hsp60* gene were the same as those used for the PCR reaction (18). The thermal cycling conditions were 1 min at 96°C for 1 cycle, followed by 25 cycles of 10 sec at 96°C, 5 sec at 50°C, and 4 min at 60°C. Sequence reactions were purified on a 0.025 µm membrane filter in a Tris-EDTA buffer solution pH 8 before sequencing with HiDi™ Formamide (Applied Biosystems, P/N: 4311320) on an ABI Prism™ 310-Genetic Analyzer (Perkin Elmer Instrument, Applied Biosystems).

Proteomic analysis

Pure cultures were grown on blood agar at 37°C for 24h. All samples were processed with a MALDI-TOF MS Axima Confidence™ spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan) in positive linear mode (m/z = 2,000-20,000). A small amount of a colony of each pure culture was transferred to a FlexiMass™ target well using a disposable loop, overlaid with 0.5 µl of 2,5-dihydroxybenzoic acid matrix solution (DHB; 10 mg/ml in acetonitrile / 0.1 % trifluoroacetic acid 1:1) and air-dehydrated within 1-2 min at 24-27 °C.

The reference strain *Escherichia coli* K12 (GM48 genotype) was used as a standard for calibration and as reference measurement for quality control. Sample information such as medium and grown conditions was imported into the software Shimadzu Biotech Launchpad™, v.2.8 (Shimadzu-Biotech Corp., Kyoto, Japan). Protein mass profiles were obtained with detection in the linear positive mode at a laser frequency of 50 Hz and within a mass range from 2,000-20,000 Da. Acceleration voltage was 20 kV, and the extraction delay time was 200 ns. A minimum of 20 laser shots per sample was used to generate each ion spectrum. For each bacterial sample, 50 protein mass fingerprints were averaged and processed. Spectra were analyzed using SARAMIS™ (Spectral Archive And Microbial Identification System, AnagnosTec GmbH) at default settings. We

created the reference spectra (SuperSpectra) on the basis of the most discriminating peaks for a given species and for each species we selected an amount of mass to charge ratios (m/z) that were genus specific, i.e. they were present in all SIG strains. Species specific peaks had to be present only in a given species. Mass to charge ratios that were species specific were given a larger relevance, as described in the SARAMIS™ user manual. Dendrograms were based on the peak patterns of all analyzed strains submitted to single-link clustering analysis using SARAMIS™ (0.08% error, range from m/z 2,000 to 20,000).

Data analysis

Genetic data were analyzed using the software ABI Prism™ 310 Collection Genetic Analyser (Applied Biosystems). Multiple alignments were performed using the BioNumerics software v.6.01 (Applied Maths). The modular microorganism identification system AnagnosTec AXIMA@SARAMIS was used to archive and evaluate MALDI-TOF MS data. SARAMIS™ was also used to construct dendrograms to show relationships among the strains.

We calculated the estimated sensitivity and specificity and the 95% confidence intervals (CI) compared to a constructed perfect standard (19), corresponding to the identification by partial *hsp60* gene sequences. We calculated the estimated sensitivity and specificity separately for the three species *S. intermedius*, *S. pseudintermedius* and *S. delphini*, defining a positive identification by MALDI-TOF MS when the identification confidence was ≥ 90%. The Cohen's kappa coefficient was also computed (20).

Table 1. Description of the investigated strains, name (T: type strain), source, biological origin, geographic origin (B: Belgium, CH: Switzerland, CZ: Czech Republic, DK: Denmark, F: France, I: Italy, J: Japan, PL: Poland, S: Spain, UK: United Kingdom, USA: United States of America) and *hsp60* identification.

Strain	Source	Biological origin	Geographic origin	*hsp60* identification
8086	Horse	N.D.	UK	*S. delphini*
8485	Horse	N.D.	UK	*S. delphini*
9106	Horse	N.D.	UK	*S. delphini*
AV8047	Pigeon	N.D.	J	*S. delphini*
AV8051	Pigeon	N.D.	J	*S. delphini*
AV8061	Pigeon	N.D.	J	*S. intermedius*
AV8063	Pigeon	N.D.	J	*S. intermedius*
AV8081	Pigeon	N.D.	F	*S. intermedius*
CCUG 30107-**T**	Dolphin	Purulent skin lesion	I	*S. delphini*
E021	Dog	Skin (pyoderma)	USA	*S. pseudintermedius*
G1	Badger	N.D.	S	*S. delphini*
Gi1	Dog	Skin (pyoderma)	Europe	*S. pseudintermedius*
h4A	Domestic horse	Nares	J	*S. delphini*
h6C	Domestic horse	Nares	J	*S. delphini*
h-9D	Domestic horse	Nares	J	*S. delphini*
HT20030674	Camel	N.D.	F	*S. delphini*
HT20030676	Camel	N.D.	F	*S. delphini*
HT20030677	Camel	N.D.	F	*S. delphini*
HT20030679	Camel	N.D.	F	*S. delphini*
HT20030680	Camel	N.D.	F	*S. delphini*
I0005	Dog	Ear	CH	*S. pseudintermedius*
I0008	Dog	Nares	CH	*S. pseudintermedius*
I0010	Dog	Nose	CH	*S. pseudintermedius*
I0048	Dog	Nares	CH	*S. pseudintermedius*
I0049	Dog	Nares	CH	*S. pseudintermedius*
I0057	Dog	Nares	CH	*S. pseudintermedius*
I0065	Dog	Nares	CH	*S. pseudintermedius*
I0073	Dog	Ear	CH	*S. pseudintermedius*
I0075	Dog	Nares	CH	*S. pseudintermedius*
KM241	Dog	Ear (otitis externa)	CH	*S. pseudintermedius*
KM337	Dog	Ear (otitis externa)	CH	*S. pseudintermedius*
KM1087	Dog	Vaginal mucosa (vaginitis)	CH	*S. pseudintermedius*
KM1250	Dog	Infected wound	CH	*S. pseudintermedius*
KM1381	Dog	Fistula after surgery	CH	*S. pseudintermedius*
KM1591	Dog	Pyoderma	CH	*S. pseudintermedius*
LMG13351-**T**	Pigeon	Nares	CZ	*S. intermedius*
LMG19136	Dog	Skin	B	*S. pseudintermedius*
LMG22219-**T**	Cat	Lung tissue	B	*S. pseudintermedius*
LMG22221	Dog	Ear (otitis)	B	*S. pseudintermedius*
M0612	Dog	Skin (pyoderma)	USA	*S. pseudintermedius*
M1	Mink	N.D.	DK	*S. delphini*
M86	Mink	N.D.	DK	*S. delphini*
NVAU02012	Dog	Wound pus from skin	J	*S. pseudintermedius*
NVAU02031	Dog	Wound pus from skin	J	*S. pseudintermedius*
NVAU02083	Cat	Wound pus from skin	J	*S. pseudintermedius*
P2A	Wild pigeon	Nares	J	*S. intermedius*
P4A	Wild pigeon	Nares	J	*S. intermedius*
P6A	Wild pigeon	Nares	J	*S. intermedius*
P9B	Wild pigeon	Nares	J	*S. intermedius*
P26	Domestic pigeon	Nares	J	*S. delphini*
P27B	Domestic pigeon	Nares	J	*S. delphini*
P30A	Domestic pigeon	Nares	J	*S. delphini*
P45A	Wild pigeon	Nares	J	*S. intermedius*
P46A	Wild pigeon	Nares	J	*S. intermedius*
P50	Wild pigeon	Nares	J	*S. delphini*
P52B	Wild pigeon	Nares	J	*S. intermedius*
P53	Wild pigeon	Nares	J	*S. intermedius*
P54A	Wild pigeon	Nares	J	*S. intermedius*
P69A	Wild pigeon	Nares	J	*S. intermedius*
P66A	Wild pigeon	Nares	J	*S. intermedius*
PL1	Dog	Ear (otitis)	PL	*S. pseudintermedius*
PL2	Dog	Skin of head	PL	*S. pseudintermedius*
PL3	Dog	Nose	PL	*S. pseudintermedius*
PL5	Dog	Skin (dermatosis)	PL	*S. pseudintermedius*
PL6	Dog	Skin	PL	*S. pseudintermedius*
RPC05C0284	Human	N.D.	F	*S. pseudintermedius*
S61H7	Horse	Skin (inflammation)	DK	*S. delphini*
SD1071	Dog	Nares	CH	*S. pseudintermedius*
TW6698	Human	Wound pus	J	*S. pseudintermedius*

Results

The estimated sensitivity of MALDI-TOF MS for the identification of *S. intermedius* was 0.95 (95% CI: 0.68-0.99), of *S. pseudintermedius* 0.78 (95% CI: 0.60-0.90), and of *S. delphini* 0.64 (95% CI: 0.41-0.83); the estimated specificity was 1.00 for *S. intermedius* and *S. delphini* and 0.97 (95% CI: 0.86-0.99) for *S. pseudintermedius*. The efficiency of identification was 0.99 (0.92-0.99) for *S. intermedius* and 0.88 (95% CI: 0.78-0.95) for *S. pseudintermedius* and *S. delphini*. The Cohen's kappa was 0.96 (95% CI: 0.87-1.04) for *S. intermedius*, 0.76 (95% CI: 0.62-0.92) for *S. pseudintermedius* and 0.70 (95% CI: 0.52-0.89) for *S. delphini*.

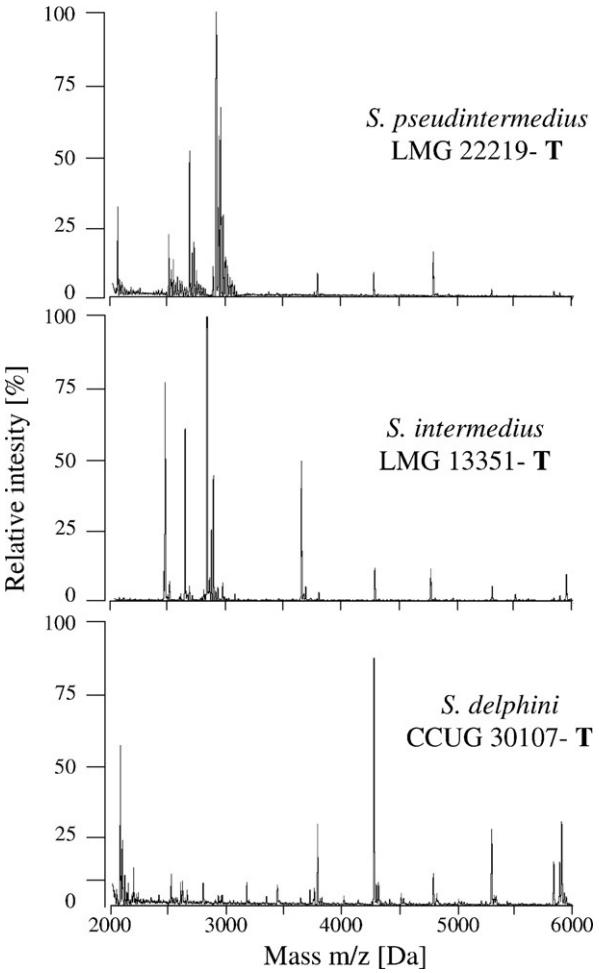

Figure 1. Representative spectra of the type strains *S. pseudintermedius* LMG 22219-T, *S. intermedius* LMG 13351-T and *S. delphini* CCUG 30107-T with relative intensity [%] of the protein profile peaks [m/z] ranging between 2000 and 6000 Da. T: type strain.

Spectra with specific peaks for the type strains of the three investigated species are shown in Figure 1. Spectra of strains belonging to the same species displayed a high level of similarity; within the same species, however, some variation in the pattern composition and the measured relative intensities were observed (e.g. *S. intermedius*, Figure 2). We created 2 new SuperSpectra for the identification of *S. intermedius*, 3 for *S. pseudintermedius* and 2 for *S. delphini*. The selected mass to charge ratios ranged from 2002.8 m/z to 19.883.7 m/z (error 0.08 %), with an average of 21.14±1.46 (SD) different mass to charge ratios used for the creation of each SuperSpectra. Details are reported in Table 2.

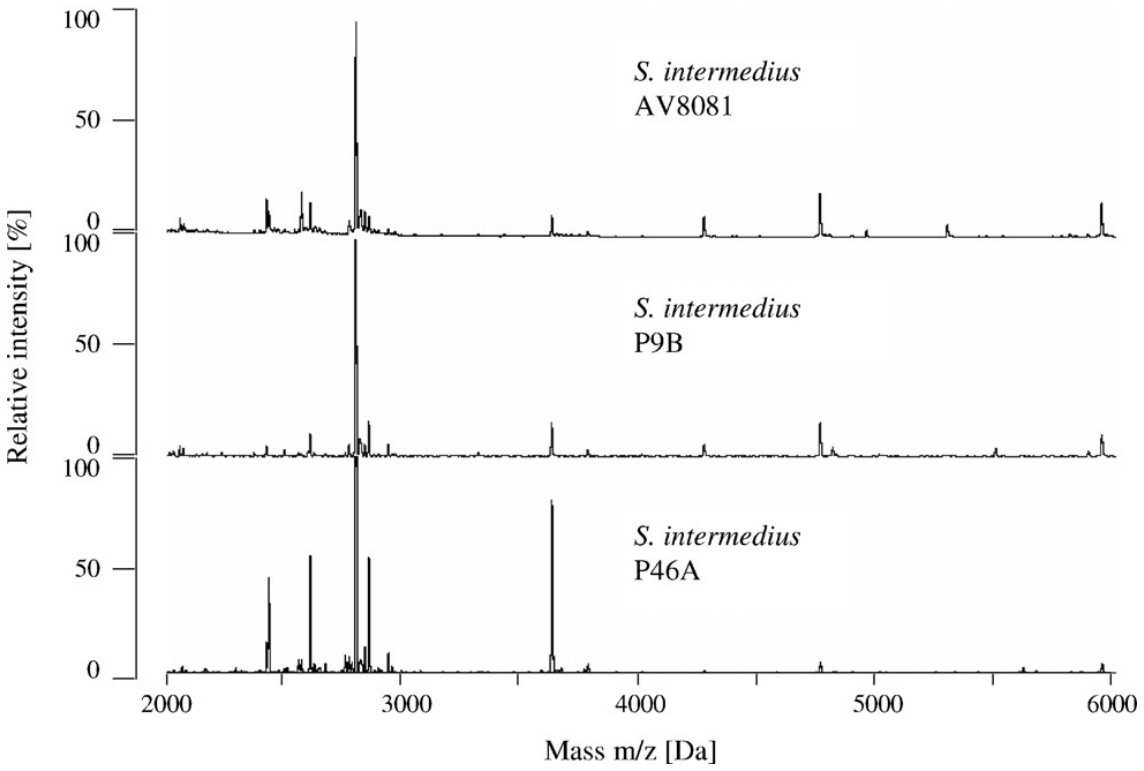

Figure 2. Spectra of different *S. intermedius* strains with relative intensity [%] of the protein profile peaks [m/z] ranging between 2000 and 6000 Da.

Table 2. Mass to charge ratios (m/z) used for the creation of the different SuperSpectra. Error 0.08%.

SuperSpectra_Staphylococcus_intermedius_ICM_13may 2009				
2226.1	2304.1	2580.6	2634.7	2810.6
2827.1	2849.0	2866.0	2904.1	2947.3
2963.5	3638.1	3676.3	3790.9	4771.5
5021.3	5627.5	5959.9	6663.0	7602.2
8012.7				
SuperSpectra_Staphylococcus_intermedius_ICM_12may 2009				
2024.8	2036.0	2042.0	2067.8	2091.4
2226.4	2304.3	2327.2	2569.0	2634.6
2651.0	2681.7	2849.1	2865.9	3084.0
3195.6	3654.2	4282.1	5022.1	6662.9
SuperSpectra_Staphylococcus_delphini_ICM_12may 2009				
2024.8	2042.1	2068.1	2363.8	2495.0
2538.5	3416.6	4146.5	4185.4	4624.6
5790.7	6057.1	6619.7	8076.4	8620.7
9955.9	10,006.3	10,843.5	14,809.2	19,883.7
SuperSpectra_Staphylococcus_delphini_ICM_27may 2009				
2041.6	2362.9	2433.9	2593.2	2609.2
2689.2	2922.0	3342.0	3438.1	4829.0
5849.8	5920.5	5945.6	6123.0	6248.6
6717.5	7299.7	8066.4	9039.5	9084.3
9679.0				
SuperSpectra_Staphylococcus_pseudintermedius_ICM_12may 2009				
2137.0	2188.8	2510.6	2697.1	2713.0
2732.2	2917.3	3785.1	3820.8	4183.9
4275.3	5302.4	5845.4	6343.9	6731.2
6751.1	6767.5	7443.3	9035.7	9626.9
SuperSpectra_Staphylococcus_pseudintermedius_ICM_14may 2009				
2132.7	2163.4	2180.5	2239.0	2357.8
2379.9	2489.2	2527.7	2541.8	2564.5
2587.6	2669.8	2845.7	2910.7	3361.1
3431.8	3757.1	3784.6	4165.3	4275.5
7205.0	8479.7			
SuperSpectra_Staphylococcus_pseudintermedius_ICM_14may 2009-2				
2002.8	2005.4	2006.6	2010.8	2066.5
2083.0	2120.2	2471.3	2493.8	2516.2
2553.6	2567.7	2570.2	2589.3	2610.6
2674.7	2693.0	2751.4	2779.2	2876.4
2917.4	6732.3	6767.2	7545.8	

Within the strains identified as *S. pseudintermedius* with the created SuperSpectra 65.6% (21/32) strains were correctly identified with a confidence of 99.9%, 12.5% (4/32) with a confidence between 99.8% and 90%, and 21.9% (7/32) with a confidence lower than 90%. For *S. intermedius* 93.3% (14/15) of the strains were correctly identified with a confidence of 99.9% and 6.7% (1/15) with a confidence lower than 90%. For *S. delphini*, 31.8% (7/22) of the strains were correctly identified with a confidence of 99.9%, 31.8% (7/22) with a confidence between 99.8% and 90%, and 36.4% (8/22) with a confidence lower than 90%.

The dendrogram showed two main clusters, one including all *S. intermedius* and the other *S. pseudintermedius* and *S. delphini*. The latter two where closer in the dendrogram but all the strains identified as *S. delphini* formed a cluster distinct from all *S. pseudintermedius* isolates (Fig. 3). All 7 strains identified as *S. pseudintermedius* with a confidence lower than 90% were included in the cluster of *S. pseudintermedius*. Isolate P2A, one of the two identified as *S. intermedius* with a confidence lower than 90%, was included in the *S. intermedius* and isolate P66A in the *S. delphini* cluster. All strains identified as *S. delphini* with a confidence lower than 90%, with the exception of isolate P27B, belonged to the *S. delphini* cluster (Fig. 3).

Discussion

MALDI-TOF MS can be used to reliably identify bacterial species belonging to SIG. The estimated sensitivity of MALDI-TOF in the identification of the SIG species was higher for *S. intermedius* than for *S. pseudintermedius* and *S. delphini*, whereas the estimated specificity was 1 for *S. intermedius* and *S. delphini* and 0.97 for *S. pseudintermedius*. The Cohen's kappa coefficient indicated almost perfect agreement between MALDI-TOF MS and *hsp60* gene sequencing in the identification of *S. intermedius* and substantial agreement for *S. delphini* and *S. pseudintermedius*. The overall efficiency of the proteomic identification was quite high and ranged between 88% and 99% for *S. pseudintermedius* - *S. delphini* and *S. intermedius* respectively.

We based the choice of the constructed standard used in this study, the *hsp60* gene, for the calculation of the estimated specificity, sensitivity, efficacy and agreement on the results of the work carried out by Sasaki et al. (4). The choice of another constructed standard (e.g. the *nuc, gap*, or *sodA* genes, (4, 21)) might have led to slightly different agreement values between the two identification methods, but overall the identification with MALDI-TOF MS showed to be robust enough to allow the creation of reliable SuperSpectra.

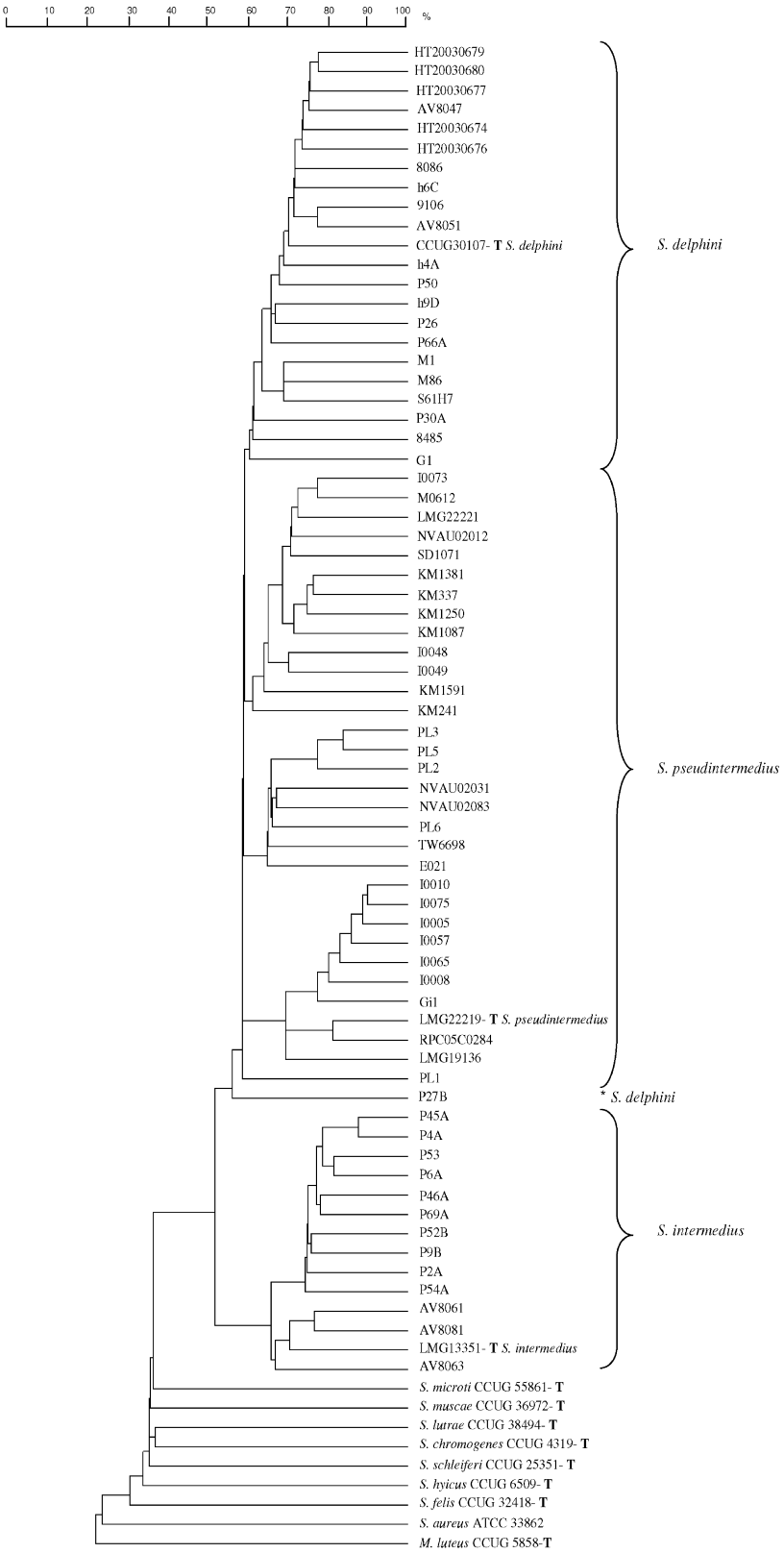

Figure 3. Dendrogram resulting from single-link clustering analysis (SARAMIS^{TM} database software) of MALDI-TOF MS on *Staphylococcus intermedius* Group strains. Error 0.08%; range of m/z from 2000 to 20,000 Da. T: type strain.

In the last year new genetic tests have been described for the identification of CPS species. For example, the PCR-RFLP method based on *pta* gene allows accurate differentiation of *S. pseudintermedius* from the other SIG species and also from other important human and animal pathogenic staphylococcal species such as *S. schleiferi* and *S. aureus* (22). This approach, however, does not allow conclusive identification of other SIG species. Recently, Blaiotta et al. have described the same methodological approach but based on another housekeeping gene, the *kat* gene, which allows unambiguous identification of CPS, including *S. pseudintermedius* and *S. delphini* (23). A multiplex-PCR method based on the *nuc* gene was also shown to reliably identify CPS (24). All these methods are based on the analyses of genetic components of the investigated bacteria and thus need the classic approach of culture of the organism followed by DNA extraction, PCR amplification and gel staining for the detection of amplified fragments. This genetic approach allows reliably identification of the investigated species but it is time-consuming if compared with the MALDI-TOF MS proteomic approach which shows equivalent efficiency.

MALDI-TOF MS allows rapid and accurate identification of SIG bacteria within 24h, provided a reliable comparison database is available. This means that for each group of microorganisms careful phylogenetic characterization of a sufficient number of geographically and genetically diverse isolates of the species under consideration is needed before they can be used to construct SuperSpectra. In fact, SuperSpectra for *S. intermedius* previously present in the database led to erroneous identification of strains in the present study. This relates most probably to an insufficient characterization of the strains used for the creation of these SuperSpectra. It is therefore crucial to use only fingerprints of strains well characterized by phylogenetic studies (e.g. by analysis of at least two different genes) for the creation of SuperSpectra. The SuperSpectra for *S. pseudintermedius, S. intermedius* and *S. delphini* described in Table 2 have been constructed according to these criteria and have been shown to be highly reliable.

The created SuperSpectra were able to identify only 31.8% of the *S. delphini* strains with a confidence greater than 99%. This reflects the high heterogeneity within the *S. delphini* group which has been already described in a molecular study suggesting the presence of a new species within the *S. delphini* group and dividing this species in two groups, A and B (4). In particular the strain P27B was identified as *S. delphini* by partial *hsp60* gene sequence and by MALDI-TOF with a confidence of 79.5%; in the dendrogram resulting from the MALDI-TOF analysis, however, it was not included in the cluster of *S. delphini* strains but was closer to the *S. pseudintermedius* strains. The position of strain P27B, known to belong to *S. delphini* group B (24), might be explained by the fact that strains of group B are more closely related to those of *S. pseudintermedius* than to *S. delphini* group A (4).

This study has provided evidence of the validity and usefulness of MALDI-TOF MS for a rapid, comparatively cheap and reliable identification of bacterial isolates belonging to the *Staphylococcus intermedius* Group. Additional work with MALDI-TOF MS, coupled with corresponding phylogenetic analyses, may allow better insight in the ongoing speciation within *S. delphini* as well as the geographic validation of the newly created SuperSpectra for *S. pseudintermedius* and *S. intermedius* with a larger number of collected strains.

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References

1. Devriese LA, Vancanneyt M, Baele M, Vaneechoutte M, De Graef E, Snauwaert C, et al. *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int J Syst Evol Micr. 2005;55:1569-73.

2. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol. 2007;45:1118-25.

3. Ruscher C, Lübke-Becker A, Wleklinski C-G, Soba A, Wieler LH, Walther B. Prevalence of Methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. Vet Microbiol. 2009;136:197-201.

4. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J Clin Microbiol. 2007;45:2770-8.

5. Baron F, Cochet MF, Pellerin JL, Ben Zakour N, Lebon A, Navarro A, et al. Development of a PCR test to differentiate between *Staphylococcus aureus* and *Staphylococcus intermedius*. J Food Prot. 2004;67:2302-5.

6. Devriese LA, Hermans K, Baele M, Haesebrouck F. *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius*. Vet Microbiol. 2009;133:206-7.

7. Hesselbarth J, Schwarz S. Comparative ribotyping of *Staphylococcus intermedius* from dogs, pigeons, horses and mink. Vet Microbiol. 1995;45:11-7.

8. Bemis DA, Jones RD, Hiatt LE, Ofori ED, Rohrbach BW, Frank LA, et al. Comparison of tests to detect oxacillin resistance in *Staphylococcus intermedius*, *Staphylococcus schleiferi*, and *Staphylococcus aureus* isolates from canine hosts. J Clin Microbiol. 2006;44:3374-6.

9. Pottumarthy S, Schapiro JM, Prentice JL, Houze YB, Swanzy SR, Fang FC, et al. Clinical isolates of *Staphylococcus intermedius* masquerading as methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol. 2004;42:5881-4.

10. Roberson JR, Fox LK, Hancock DD, Besser TE. Evaluation of methods for differentiation of coagulase-positive staphylococci. J Clin Microbiol. 1992;30:3217-9.

11. Mellmann A, Becker K, von Eiff C, Keckevoet U, Schumann P, Harmsen D. Sequencing and staphylococci identification. Emerg Infect Dis. 2006;12:333-6.

12. Bizzini A, Greub G. MALDI-TOF MS, a revolution in clinical microbial identification. Clin Microbiol Infect. 2010;16:1614–9.

13. Claydon M, Davey S, Edwards-Jones V, Gordon D. The rapid identification of intact microorganisms using mass spectrometry. Nat Biotechnol. 1996;14:1584-6.

14. Petersen CE, Valentine NB, Wahl KL. Characterization of microorganisms by MALDI mass spectrometry. Methods Mol Biol. 2009;492:367-79.

15. Carbonnelle E, Beretti JL, Cottyn S, Quesne G, Berche P, Nassif X, et al. Rapid identification of staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2007;45:2156-61.

16. Dubois D, Leyssene D, Chacornac JP, Kostrzewa M, Schmit PO, Talon R, et al. Identification of a variety of *Staphylococcus* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2010;48:941-5.

17. Goh S, Potter S, Wood J, Hemmingsen S, Reynolds R, Chow A. HSP60 gene sequences as universal targets for microbial species identification: studies with coagulase-negative staphylococci. J Clin Microbiol. 1996;34:818-23.

18. Kwok AYC, Su SC, Reynolds RP, Bay SJ, Av-Gay Y, Dovichi NJ, et al. Species identification and phylogenetic relationships based on partial HSP60 gene sequences within the genus *Staphylococcus*. Int J Syst Evol Micr. 1999;49:1181-92.

19. FDA. Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests. 2007.

20. Attermann J. On the agreement between two diagnostic methods with binary outcomes. Scand J Clin Lab Inv. 2003;63:525-8.

21. Ghebremedhin B, Layer F, Konig W, Konig B. Genetic classification and distinguishing of *Staphylococcus* species based on different partial gap, 16S rRNA, hsp60, rpoB, sodA, and tuf gene sequences. J Clin Microbiol. 2008;46:1019-25.

22. Bannoehr J, Franco A, Iurescia M, Battisti A, Fitzgerald JR. Molecular diagnostic identification of *Staphylococcus pseudintermedius*. J Clin Microbiol. 2009;47:469-71.

23. Blaiotta G, Fusco V, Ercolini D, Pepe O, Coppola S. Diversity of *Staphylococcus* species strains based on partial kat (catalase) gene sequences and design of a PCR-restriction fragment length polymorphism assay for identification and differentiation of coagulase-positive species (*S. aureus*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans*). J Clin Microbiol. 2010;48:192-201.

24. Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, et al. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol. 2010;48:765-9.

5. Research paper 2

Prevalence and risk factors for carriage of multi-drug resistant staphylococci in healthy cats and dogs

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Abstract

We investigated the distribution of commensal staphylococcal species and determined the prevalence of multi-drug resistance in healthy cats and dogs. Risk factors associated to the carriage of multi-drug resistant strains were explored. Isolates from 256 dogs and 277 cats were identified at the species level using MALDI-TOF mass spectrometry. Diversity of coagulase-negative staphylococci (CNS) was high, with 22 species in dogs and 24 in cats. Multi-drug resistance was frequent (17%) and not always associated with the presence of the *mecA* gene. A stay in a veterinary clinic in the last year was associated with an increased risk of colonisation by multi-drug resistant staphylococci (OR = 2.4, 95 % CI: 1.1-5.2, p-value LRT = 0.04). In finding new efficient control strategies against antibiotic resistance, the presence of mechanisms other than methicillin resistance, and the possible role of CNS in the spread of resistance determinants should be considered.

Background

Staphylococci resistant against methicillin and other antibiotics have been frequently reported in pets worldwide (1, 2). These microorganisms are opportunistic pathogens that may colonise the skin and mucosae of humans and other animals. Bacteria belonging to the genus *Staphylococcus* are currently divided into coagulase-positive (*S. aureus, S. pseudintermedius, S. hyicus, S. intermedius, S. delphini, S. schleiferi* subsp. *coagulans*, *S. lutrae*) and coagulase-negative species (e.g. *S. epidermidis, S. hominis, S. warneri, S. felis*). For long time the pathogenicity of coagulase-negative staphylococci (CNS) has been underestimated, because these species were associated with more chronic or subacute infections when compared with coagulase-positive staphylococci (CPS) (3). Presently, however, the etiological role of CNS in prosthesis and foreign body infections is being recognised in human medicine (4-6). In pets, the pathogenic potential of these microorganisms has not yet been clearly defined, although we can find some reports of infections related to methicillin-resistant CNS in cats and dogs (7, 8).

Few studies have addressed the composition of staphylococcal populations of the mucosae of healthy cats and dogs (9, 10). Previous investigations on the staphylococcal species diversity in these animals have focused on clinical isolates (11), mainly CPS (12), or described the distribution of well defined antibiotic resistances within a limited number of staphylococcal species (13, 14). Moreover these studies were carried out before 2005 when *S. pseudintermedius* had not yet been described: In fact, this species had probably been reported in all previous studies as *S. intermedius*, leading thus to confusion as to its real occurrence in pets (15-19). Recently *S. pseudintermedius* has been suggested to be the most relevant and prevalent CPS colonising dogs, with an increasing amount of reports on its pathogenic and methicillin resistance characteristics in this host (20).

To date, the carriage of CNS strains in pets has been neglected. Recently the development of new molecular techniques has allowed accurate identification of CNS (21, 22). This will eventually lead to a better understanding and knowledge on these bacteria species. More knowledge on CNS

carriage in animals will be of benefit, because these bacteria might represent a genetic pool of antibiotic resistance for CPS species; in fact, horizontal gene transfer of staphylococcal chromosome cassette *mec* (SCC*mec*) has been documented between CPS and CNS species (23).

In the last decade several authors have suggested that pets may be reservoirs of antibiotic resistant bacteria (24-26). This assumption was mainly based on studies reporting antibiotic resistance in clinical CPS isolates from dogs and humans being in close contact (27, 28). A clear picture of the distribution, diversity and multi-drug resistance (MDR) of both CPS and CNS species in pets, however, is lacking and thus the role of cats and dogs as reservoirs of antibiotic resistance hardly known.

The purpose of the present study was to gain insight into the distribution of commensal staphylococcal species of healthy cats and dogs of Switzerland and to determine the occurrence of MDR in both CNS and CPS. In addition, we explored risk factors associated with the carriage of these microorganisms in pets.

Methods

Study design and settings

Samples were collected between March 2008 and December 2009 in four different Swiss cantons (Berne, Ticino, Vaud and Zurich). Only healthy pets with no overt acute disease at the time of sample collection were enrolled in the study. The pets either lived in or visited nursing homes for pet-therapy or lived in households. The selection strategy differed between community and nursing homes. Pets in the community were included in the study based on a convenience sampling in households (n = 196) in four Swiss cantons representing the northern, southern, central and western part of Switzerland. Additional pets (n=239) were recruited from cats and dogs visiting a total of 12 veterinary practices in the same regions for routine vaccinations. Nursing homes were selected by a

two-stage random cluster sampling from an exhaustive list of nursing homes located in the four Swiss cantons. In randomly selected nursing homes, all pets matching the inclusion criteria and present at the time of sample collection (n=98) were enrolled in the study. An informed written consent was given by all pet owners. The study received the approval for animal experimentation by the Cantonal and Swiss Federal Veterinary Offices (authorisation reference number 01/2008-02/2008).

Sample collection

Nasal and ear swab samples were collected by means of cotton swabs (Amies agar gel 108C and 110C, Copan, Italy) previously soaked in a physiological 0.9 % NaCl solution. A swab was introduced for 1-2 cm in the nostril and a second one as deeply as possible in the ear channel of each animal. The collected samples were conserved in the transport medium at room temperature and analyzed for the presence of staphylococci within 24-48 h of collection. A questionnaire collecting information on the demographic and health status of the pets had to be filled in by the owners (available from the corresponding author on request).

Sample analyses

Both swabs were streaked on Mannitol Salt Agar (Chapman 2 – MSA 2, bioMérieux® SA, France), then incubated for 48 h at 37 °C, enriched in MRSA broth supplemented with 6 mg/L of oxacilllin (48 h at 37 °C) and cultured on Gelose ChromID *S. aureus* (SAID, bioMérieux® SA, France) for 48 h at 37 °C. All morphologically different colonies were isolated and catalase positive, Gram positive coccal bacteria were frozen in skimmed milk at -80 °C until further analyses.

Isolates were grown on blood agar during 24 h and identified by matrix-assisted laser desorption ionisation - time of flight mass spectrometry (MALDI-TOF MS) using an Axima Confidence™ spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan) in positive linear mode (m/z = 2,000 to 20,000) (29). Identity of isolates that could not be identified by MALDI-TOF MS (24%) was confirmed by sequencing of the amplified partial *rpoB* gene (21).

Phenotypic antibiotic resistance to 24 different drugs was assessed by the Kirby-Bauer method on Mueller-Hinton blood agar (MHS2, bioMérieux® SA, France). The following antibiotics were tested: penicillin (10 units), ampicillin (10 µg), oxacillin (1 µg), cefazolin (30 µg), gentamicin (10 µg), tetracycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), trimethoprim-sulfamethoxazole (1.25+23.75 µg), ciprofloxacin (5 µg), amoxicillin and clavulanic acid (20+10 µg), ceftazidim (30 µg), imipenem (10 µg), tobramycin (10 µg), fusidic acid (10 µg), rifampicin (30 µg), chloramphenicol (30 µg), cefoxitin (30 µg), kanamycin (30 µg), doxycyclin (30 µg), mupirocin (5 µg), linezolid (30 µg) and quinopristin-dalfopristin (15 µg). Inducible clindamycin resistance test ("D-zone" test) was also carried out for all isolates. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (30, 31); for the purpose of this study, intermediate results were classified as resistant. Multi-drug resistance (MDR) was defined as resistance to at least 3 drugs belonging to 3 different antibiotic classes. Additionally, the presence of the *mec*A gene, which confers methicillin resistance, was investigated by polymerase chain reaction (PCR) on all isolates that showed phenotypic resistance to oxacillin (32, 33).

We considered isolates from the same animal as being different strains if they belonged to different staphylococcal species or their phenotypic antibiotic resistance profile differed.

Statistical analyses

Sample size calculation was based on the assumption that 5 % of pets carried at least one MDR staphylococcal strain and that the intra-class correlation coefficient (rho) was 0.15. We used the cluster sample equation of Bennett et al. (34) for all calculations. Estimating that each nursing home with pets owned or was visited by three animals on average; the sample collection in 42 different nursing homes would have provided 126 pets. The expected precision for the prevalence estimate of MDR in pets would subsequently have a standard error of 2.2 %, and a 95 % confidence interval (CI) = 0.68-9.3 %.

Characteristics of the cats and dogs were compared to check for consistent differences in the demographics and health status of the different population sampled. Chi-square test (Fisher's exact test when expected observations < 5) and 95 % CI were used for this comparison. We reported the prevalence of staphylococci and MDR staphylococci and the distribution of antibiotic resistance among the different staphylococcal species together with the median number of resistances to different antibiotic classes. Univariable logistic regression models, with MDR staphylococcal carriage status of pet as the outcome variable of interest, were applied to explore risk factors. Unadjusted odds ratios (OR with 95% CI) were calculated as a measure of association. Statistical significance of each explanatory variable was determined by a likelihood-ratio test (LRT). We included in a multivariable model all variables with LRT p-values ≤0.2 from the univariable analysis. All statistical analyses were performed with STATA 9.0 (Stata Corporation, College Station, TX, USA).

Results

Demographics and staphylococcal carriage in pets

We collected samples from 533 healthy pets (277 cats and 256 dogs). Ninety-eight lived in or visited nursing homes at least once a week and 435 lived in the community. The demographics of the two population studied are reported in Table 1. Parameters such as sex, age, sterilisation, otitis in the last year, and antibiotic treatment showed different distributions between the nursing home and community settings, but the 95% CI estimates of these parameters overlapped (Table 1). We did not carry out stratified analyses of the samples because the overall frequencies of MDR in nursing homes (15/98) and in the community (76/435) did not differ significantly ($\chi^2 = 0.27$, $p = 0.6$).

Staphylococci were detected in 60% (320/533) of pets; 17 % (92/533) of all animals carried at least one MDR strain. There were no significant differences in MDR carriage between pet species [14.8 % (95 % CI: 11.0-19.5) in cats and 20.0 % (95 % CI: 15.5-25.4) in dogs; $\chi^2 = 2.1$, $p = 0.14$] (Table 2). In cats, most CNS were MDR (39/41), whereas the proportion of MDR in CPS was small (1/41). In dogs, on the other hand, MDR CPS (20/51) and MDR CNS (28/51) carriage was almost equal (Table 2). We observed species-specific differences ($\chi^2 = 63.69$, $p < 0.001$) in the proportion of *S. pseudintermedius* carriage, with 27 % (70/256) of dogs and 3 % of cats (8/277) harbouring this species. No difference in *S. aureus* carriage was seen between the two pet species (13/256 dogs and 14/277 cats, respectively).

Table 1. Demographics of investigated cats and dogs with proportions (%) and 95 % confidence intervals (95 % CI).

Characteristics	Nursing home(n = 98)			Community (n = 435)			p-value [1)]
	n	%	95% CI	n	%	95% CI	
Cats	53/98	54	44-63	224/435	51	47-56	0.64
Female	63/98	64	54-73	226/434	52	47-57	0.03
Sterilized	80/98	82	73-88	306/433	71	66-75	0.03
Age							0.006
< 3 years	19/98	19	12-27	156/435	36	31-40	
3-10 years	56/98	57	47-67	189/435	43	39-48	
> 10 years	23/98	23	15-32	90/435	21	17-24	
Cantons							0.14
Berne	32/98	33	23-42	95/435	22	18-26	
Ticino	26/98	27	18-35	140/435	32	28-37	
Vaud	20/98	20	12-28	107/435	25	21-29	
Zurich	20/98	20	12-28	93/435	21	18-25	
Visit to veterinary clinics in the last year	3/97	3	1-9	28/431	7	5-9	0.19
Stay in animal home in the last year	8/98	8	4-15	22/434	5	3-8	0.23
Pyoderma in the last year	3/96	3	1-9	22/423	5	3-8	0.60 [2)]
Urinary affections in the last year	4/93	4	2-11	17/421	4	3-6	1.00 [2)]
Otitis in the last year	3/93	3	1-10	39/425	9	7-12	0.06
Antibiotic treatment in the last 3 months	5/94	5	2-12	59//429	14	11-17	0.02
Immunosuppressant in the last 3 months	3/92	3	1-10	20/423	5	3-7	0.78 [2)]

[1)] $\chi 2$ test

[2)] Fischer's exact test applied because expected frequency in at least one cell <5

Table 2. Proportion (%) of staphylococcal and MDR staphylococcal carriage in pets (cats and dogs). 95 % CI = 95 % confidence interval, CPS = coagulase-positive staphylococci, CNS = coagulase-negative staphylococci.

	Staphylococcal carriage			Multi-drug resistance carriage					
				Staphylococci			CPS	CNS	CPS & CNS
	n	%	95%CI	n	%	95%CI	n	n	n
Total	320/533	60.0	55.8-64.1	92/533	17.3	14.3-20.7	21/92	67/92	4/92
Cats	164/277	59.2	53.3-64.8	41/277	14.8	11.0-19.5	1/41	39/41	1/41
Dogs	156/256	60.9	54.8-66.7	51/256	20.0	15.5-25.4	20/51	28/51	3/51

Staphylococcal isolates

We isolated 284 staphylococcal strains (176 from the nostrils and 108 from the ears) from dogs (Table 3) and 300 (153 from the nostrils and 147 from the ears) from cats (Table 4). We could identify 94.5 % (552/584) of all isolates at the species level. Two *S. schleiferi* isolates from two cats were identified only at the species level. CNS species accounted for 60 % (172/284) of all isolates in dogs and 86 % (258/300) in cats (Table 3 and Table 4).

In cats, the total number of CPS strains was lower (22/300) as compared to dogs (98/284). Among the CPS strains *S. pseudintermedius* was more frequently isolated from dogs [(85/98), 87 %] than from cats [(8/22), 36 %], whereas *S. aureus* was more frequent in cats [(14/22), 63 %] than dogs [(13/98), 13 %]. No other CPS were isolated.

The diversity of CNS was high, with 22 different species in dogs and 24 in cats (Table 3 and Table 4). *S. felis* was isolated only from cats, in particular from their nostrils, and it represented 31 % of all CNS isolates (41/132). Other CNS recovered in relevant proportions from both pets were *S. epidermidis, S. warneri, S. hominis, S. xylosus* and *S. equorum* (Table 3 and Table 4).

Antibiotic resistance

Presence of the *mecA* gene was observed in 6 % (11/172) of dog and 3 % (7/258) of cat isolates. We did not recover any MDR *S. aureus* (Table 3 and Table 4). MDR, with a few strains showing resistance up to eight different antibiotic classes, was detected in bacteria at proportions of 21 % (36/172) in dogs and 16 % (42/258) in cats. MDR was observed in *S. pseudintermedius* isolated from both pet species with resistances to up to six different antibiotic classes, but no methicillin resistance was seen (Table 3 and 4).

About 50% of all isolates in dogs and 30% in cats showed phenotypic resistance to penicillin and ampicillin (Table 5). Fusidic acid and erythromycin resistance were detected in 31% and 25% of dog and 28% and 19% of cat isolates, respectively. 15% of all strains isolated from dogs were resistant to tetracycline and 11% to kanamycin. Clindamycin resistance was reported from 16% of dog and 15% of cat isolates (Table 5).

Exploratory analysis of risk factors

Univariable analysis revealed that the stay in a veterinary clinic in the last year was associated with an increased risk of colonisation by MDR staphylococci in pets (unadjusted OR = 2.4, 95 % CI: 1.1-5.2, p-value LRT = 0.04) (Table 6). We included following variables in the multivariable analysis according to criteria mentioned in the method section: species, canton, stay in veterinary clinic in last year and antibiotic treatment in last 3 months. A total of 465 records had no missing data for these variables. When accounting for other variables, we could see an influence of the investigated cantons on the carriage of MDR staphylococci (p-value LRT = 0.02). Cats had a lower risk of being carriers of MDR staphylococci, whereas staying in veterinary clinic in the last year and antibiotic treatment in the last 3 months showed higher risk, although none of them were statistically significant (Table 6).

Table 3. Staphylococcal strains isolated from the nostril and the ear of 256 dogs and distribution of antibiotic resistance among the different species. Q1 = 1st quartile, Q3 = 3rd quartile, Max. = maximum, *mecA* = gene encoding methicillin resistance, MDR = multi-drug resistance.

Identified isolates	**Isolates**		**No. different resistant antibiotic classes**				**mecA**		**MDR isolates**	
	Nose	**Ear**	**Nose**		**Ear**		**Nose**	**Ear**	**Nose**	**Ear**
	n	**n**	**Median (Q1;Q3)**	**Max**	**Median (Q1; Q3)**	**Max**	**n**	**n**	**n**	**n**
Coagulase positive	69	29	1 (1; 2)	6	2 (1; 5)	6	0/69	0/29	15/69	9/29
S. aureus	10	3	1 (0; 2)	2	1 (1; 2)	2	0/10	0/3	0/10	0/3
S. pseudintermedius	59	26	1 (0; 3)	6	2 (1; 5)	6	0/59	0/26	15/59	9/26
Coagulase negative	97	75	1 (0; 2)	8	1 (0; 2)	8	8/97	3/75	22/97	14/75
S. arlettae	1	-	3 (3; 3)	3	-	-	0/1	-	1/1	-
S. auricularis	1	1	0 (0; 0)	0	0 (0; 0)	0	0/1	0/1	0/1	0/1
S. capitis	3	1	0 (0; 0)	0	0 (0; 0)	0	0/3	0/1	0/3	0/1
S. caprae/capitis	5	3	2 (1; 2)	2	1 (0; 1)	1	0/5	0/3	0/5	0/3
S. cohnii	4	1	2.5 (1; 4)	5	1 (1; 1)	1	0/4	0/1	2/4	0/1
S. devriesei	-	3	-	-	0 (0; 0)	0	-	0/3	-	0/3
S. epidermidis	15	12	1 (0; 2)	7	0.5 (0; 2)	3	2/15	1/12	2/15	1/12
S. equorum	9	3	1 (0; 1)	4	0 (0; 0)	0	1/9	0/3	2/9	0/3
S. haemolyticus	8	5	1 (0.5; 2.5)	8	2 (0; 3)	8	2/8	0/5	2/8	2/5
S. hominis	9	11	2 (0; 2)	3	2 (1; 2)	3	2/9	1/11	2/9	1/11
S. kloosi	-	1	-	-	1 (1; 1)	1	-	0/1	-	0/1
S. lentus	-	1	-	-	3 (3; 3)	3	-	0/1	-	1/1
S. lugdunensis	-	1	-	-	0 (0; 0)	0	-	0/1	-	0/1
S. pasteuri	2	1	0.5 (0; 1)	1	1 (1; 1)	1	0/2	0/1	0/2	0/1
S. pettenkoferi	2	-	0 (0; 0)	0	-	-	0/2	-	0/2	-
S. saprophyticus	4	5	1 (1; 1.5)	2	2 (2; 3)	3	0/4	0/5	0/4	1/5
S. sciuri	6	1	3 (3; 3)	4	4 (4; 4)	4	1/6	0/1	6/6	1/1
S. simulans	1	-	1 (1; 1)	1	-	-	0/1	-	0/1	-
S. succinus	3	1	0 (0; 2)	2	0 (0; 0)	0	0/3	0/1	0/3	0/1
S. vitulinus	6	2	1 (1; 1)	1	1 (1; 1)	1	0/6	0/2	0/6	0/2
S. warneri	9	13	1 (0; 2)	3	1 (1; 3)	5	0/9	1/13	2/9	5/13
S. xylosus	9	9	2 (2; 3)	4	2 (2; 2)	4	0/9	0/9	3/9	2/9
Other staphylococci	10	4								
Staphylococcus spp.	10	4	0 (0; 2)	3	0.5 (0; 1)	1	0/10	0/4	2/10	0/4

Table 4. Staphylococcal strains isolated from the nostril and the ear of 277 cats and the distribution of antibiotic resistance among the different species. Q1 = 1st quartile, Q3 = 3rd quartile, Max. = maximum, *mecA* = gene encoding methicillin resistance, MDR = multi-drug resistance.

Identified isolates	**Isolates**		**No. different resistant antibiotic classes**				**mecA**		**MDR isolates**	
	Nose	**Ear**	**Nose**		**Ear**		**Nose**	**Ear**	**Nose**	**Ear**
	n	**n**	**Median (Q1; Q3)**	**Max**	**Median (Q1; Q3)**	**Max**	**n**	**n**	**n**	**n**
Coagulase positive	17	5	0 (0; 1)	1	1 (1; 3)	5	0/17	0/5	0/17	2/5
S. aureus	11	3	1 (0; 1)	1	1 (0; 1)	1	0/11	0/3	0/17	0/3
S. pseudintermedius	6	2	0 (0; 0)	0	4 (3; 5)	5	0/6	0/2	0/7	2/2
Coagulase negative	132	126	1 (0; 2)	7	1 (0; 2)	7	3/132	4/126	23/132	19/126
S. arlettae	1	-	6 (6; 6)	6	-	-	0/1	-	1/1	-
S. auricularis	4	10	0 (0; 0)	0	0 (0; 1)	4	0/4	0/10	0/4	1/10
S. capitis	1	6	3 (3; 3)	3	1 (0; 2)	3	0/1	0/6	1/1	1/6
S. caprae	-	1	-	-	1 (1; 1)	1	-	0/1	-	0/1
S. caprae/capitis	4	5	1.5 (1; 2)	2	1 (1; 1)	1	0/4	0/5	0/4	0/5
S. cohnii	2	3	0.5 (0; 1)	1	1 (1; 4)	4	0/2	0/3	0/2	1/3
S. epidermidis	16	20	1 (0.5; 3)	4	1 (1; 2)	7	1/16	4/20	5/16	4/20
S. equorum	11	10	0 (0; 1)	7	0 (0; 1)	2	0/11	0/10	1/11	0/10
S. felis	41	28	0 (0; 0)	3	0 (0; 0)	3	0/41	0/28	4/41	2/28
S. haemolyticus	5	1	1 (1; 3)	3	1 (1; 1)	1	0/5	0/1	2/5	0/1
S. hominis	5	4	1 (0; 2)	5	0 (0; 0.5)	1	1/5	0/4	1/5	0/4
S. lentus	3	1	2 (2; 4)	4	3 (3; 3)	3	0/3	0/1	1/3	1/1
S. lugdunensis	-	2	-	-	2.5 (2; 3)	3	-	0/2	-	1/2
S. nepalensis	2	-	2.5 (1; 4)	4	-	-	0/2	-	1/2	-
S. pasteuri	-	1	-	-	2 (2; 2)	2	-	0/1	-	0/1
S. pettenkoferi	4	4	0 (0; 0)	0	0 (0; 0)	0	0/4	0/4	0/4	0/4
S. saprophyticus	2	4	1.5 (1; 2)	2	1.5 (1; 2.5)	3	0/2	0/4	0/2	1/4
S. sciuri	5	3	2 (1; 3)	3	2 (1; 3)	3	1/5	0/3	2/5	1/3
S. simulans	3	8	1 (1; 1)	2	1 (1; 1)	3	0/3	0/8	0/3	1/8
S. succinus	-	1	-	-	0 (0; 0)	0	-	0/1	-	0/1
S. vitulinus	1	-	1 (1; 1)	1	-	-	0/1	-	0/1	-
S. warneri	12	6	1 (0.5; 1.5)	4	1.5 (1; 3)	4	0/12	0/6	1/12	2/6
S. xylosus	10	8	2 (2; 3)	4	1.5 (1; 3)	3	0/10	0/8	3/10	3/8
Coagulase n.d.	4	16								
S. schleiferi sp.	1	1	0 (0; 0)	0	0 (0; 0)	0	0/1	0/1	0/1	0/1
Staphylococcus spp.	3	15	0 (0; 2)	2	0 (0; 1)	4	0/3	0/15	0/3	1/15

Table 5. In vitro antibiotic resistance. Proportion (%) on all staphylococcal isolates of the in vitro antibiotic resistance against tested drugs.

	Resistance in dogs isolates (n = 284)		**Resistance in cat isolates (n = 300)**	
	n	**%**	**n**	**%**
Beta-lactams				
Penicillin	140	49	90	30
Ampicillin	131	46	77	26
Cefazolin	1	0.3	0	0
Cefoxitin	9	3	7	2
Ceftazidim	13	5	8	3
Co-amoxicillin	1	0.3	1	0.3
Imipenem	0	0	0	0
Oxacillin	13	5	7	2
Aminoglycosides				
Gentamicin	5	2	1	0.3
Kanamycin	31	11	4	1
Tobramycin	1	0.3	0	0
Tetracyclines				
Doxycyclin	35	12	12	4
Tetracycline	44	15	17	6
Macrolides				
Erythromycin	72	25	58	19
Lincosamides				
Clindamycin	45	16	39	15
Glycopeptides				
Vancomycin	0	0	0	0
Fluoroquinolones				
Ciprofloxacin	4	1	5	2
Folate pathway inhibitors				
Trimethoprim-sulfamethoxazole	10	3	4	1
Ansamycin				
Rifampicin	0	0	0	0
Phenicols				
Chloramphenicol	22	8	4	1
Oxazolidinones				
Linezolid	0	0	0	0
Streptogramins				
Quinopristin-dalfopristin	8	3	10	3
Other classes				
Fusidic acid	87	31	85	28
Mupirocin	0	0	1	0.3

Table 6. Risk factors. Univariable logistic regressions with odds ratio (OR) and 95 % confidence interval (95 % CI) as measure of possible association with the carriage of multi-drug resistant (MDR) staphylococci and multivariable logistic regression with adjusted odds ratio (AOR); p-value of the likelihood-ratio test (LRT) was considered statistically significant if ≤0.05.

			Univariable analysis			Multivariable model		
Variable level		**MDR**	**OR**		**LRT**	**AOR**		**LRT**
	N	**n (%)**		**95 % CI**	**p-value**		**95 % CI**	**p-value**
Origin								
Nursing homes	98	15 (15)	baseline					
Community setting	434	76 (18)	1.1	0.6-2.0	0.6		not included	
Species								
Dog	255	50 (20)	baseline			baseline		
Cat	277	41 (15)	0.7	0.4-1.1	0.1	0.7	0.4-1.1	0.1
Sex								
Male	243	42 (17)	baseline					
Female	288	49 (17)	1.0	0.6-1.5	0.9		not included	
Age								
0-3 years	175	36 (21)	baseline					
3-10 years	245	40 (16)	0.8	0.5-1.2				
10-20 years	112	15 (13)	0.6	0.3-1.2	0.3		not included	
Sterilised								
No	145	27 (19)	baseline					
Yes	385	64 (17)	0.9	0.5-1.4	0.6		not included	
Canton								
Bern	126	20 (16)	baseline			baseline		
Ticino	166	20 (12)	0.7	0.4-1.4		0.6	0.3-1.2	
Vaud	127	27 (21)	1.4	0.8-2.7		1.5	0.8-3.0	
Zurich	113	24 (21)	1.4	0.7-2.8	0.1	1.5	0.8-3.0	0.02
Stay in veterinary clinic in last year								
No	496	81 (16)	baseline			baseline		
Yes	31	10 (32)	2.4	1.1-5.4	0.04	1.3	0.4-3.8	0.6
Stay in animal home in last year								
No	501	84 (17)	baseline					
Yes	30	7 (23)	1.5	0.6-3.6	0.4		not included	
Pyoderma in last year								
No	493	85 (17)	baseline					
Yes	25	5 (20)	1.2	0.4-3.3	0.7		not included	
Urinary infection in last year								
No	492	88 (18)	baseline					
Yes	21	2 (10)	0.5	0.1-2.1	0.3		not included	
Otitis in last year								
No	475	84 (18)	baseline					
Yes	42	7 (17)	0.9	0.4-2.2	0.9		not included	

Variable level		MDR	Univariable analysis			Multivariable model		
			OR		LRT	AOR		LRT
	N	n (%)		95 % CI	p-value		95 % CI	p-value
Antibiotic treatment in last 3 months								
No	458	73 (16)	baseline			baseline		
Yes	64	15 (23)	1.6	0.9-3.0	0.1	1.3	0.6-2.8	0.4
Immunosuppressant in last 3 months								
No	491	79 (16)	baseline					
Yes	23	6 (26)	1.8	0.7-4.8	0.24	not included		

Discussion

This study provides, for the first time since the description of *S. pseudintermedius*, detailed information on staphylococcal carriage in healthy cats and dogs and on drug resistance of these bacteria to different antibiotic classes. We showed that *S. pseudintermedius* was recovered from the mucosae of healthy dogs more frequently than from those of healthy cats. Previous hospitalisation (at least one night in a veterinary clinic) was a risk factor for the carriage of MDR staphylococci in pets using the univariable approach. The multivariable model showed that the Swiss Cantons in which the animals lived had an influence on the carriage of MDR staphylococci. This finding most likely reflects different prescription practices of veterinarians in different regions of Switzerland.

Identification of the staphylococci was carried out by MALDI-TOF MS, which provides a reliable and rapid identification of the taxa in the *S. intermedius* group (*S. delphini*, *S. intermedius* and *S. pseudintermedius*) (29). Previous studies on the staphylococcal population of the mucosae of cats and dogs were based on phenotypic characterisation of the isolates, which may have led to misidentification of some closely related staphylococcal species (17, 35).

We have isolated MDR staphylococcal strains from healthy cats and dogs: MDR, however, was not always associated with the presence of the *mecA* gene. In this study, resistance of strains to different antibiotic classes ranged from very low proportions, as for resistance to ciprofloxacin (1-2%) in cats

and dogs, to high values as for kanamycin resistance in dogs (11%). Methicillin resistance is of particular interest, because it confers resistance to all beta-lactams and is also often linked to resistance to other antibiotic classes, but in clinical settings other resistances are also relevant; in fact, infections resulting from MDR opportunistic pathogens are a critical problem to clinicians because they limit the choice of active antibiotic treatments (36).

Our study has some limitations. The exploratory analysis of risk factors was carried out by combining all staphylococcal species and information on pet-therapy animals as well as household pets, although the risk associated to the carriage of MDR staphylococci belonging to several species might differ between the two groups. This approach was necessary because the numbers for given combinations of investigated risk factors and animals carrying different MDR staphylococcal species were small. Pet management factors in the three months preceding the study were reported by the owners: therefore, a recall bias might be present, but we do not think it important because one can reasonably expect pet owners to recall whether or not a pet had visited a veterinary clinic during the preceding three months. We did not collect data on the number of different antibiotic treatment and the length of treatment: analysis of these data could have revealed other risk factors. Despite the limitations of an exploratory univariable approach, our results confirm findings from published studies on factors associated with the carriage of MDR staphylococci in pets, and in particular the importance of previous hospitalisation, which was already reported as a risk factor for acquisition of both MRSA and MRSP in pets (37, 38).

Conclusions

Our study has shown that carriage of multi-drug resistant staphylococci in healthy cats and dogs is common. Thus, clinical therapy guidelines would benefit from an approach that is not only focused on methicillin resistance, neglecting the presence of other resistances. The monitoring of antibiotics use in veterinary clinics could provide an overview on the possible future trends of antibiotic

resistance in pets. In veterinary medicine, further studies investigating the dissemination of antibiotic resistance determinants would benefit from considering the possible role of reservoir of CNS for their spread.

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References

1. Cohn LA, Middleton JR. A veterinary perspective on methicillin-resistant staphylococci. J Vet Emerg Crit Care. 2010;20:31-45.

2. Malik S, Peng H, Barton MD. Antibiotic resistance in staphylococci associated with cats and dogs. J Appl Microbiol. 2005;99:1283-93.

3. Huebner J, Goldmann DA. Coagulase-negative staphylococci: Role as pathogens. Annu Rev Med. 1999;50:223-36.

4. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev. 1994;7:117-40.

5. von Eiff C, Proctor RA, Peters G. Coagulase-negative staphylococci - Pathogens have major role in nosocomial infections. Postgrad Med. 2001;110:63-76.

6. Von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect Dis. 2002;2:677-85.

7. van Duijkeren E, Box ATA, Heck M, Wannet WJB, Fluit AC. Methicillin-resistant staphylococci isolated from animals. Vet Microbiol. 2004;103:91-7.

8. Litster A, Moss SM, Honnery M, Rees B, Trott DJ. Prevalence of bacterial species in cats with clinical signs of lower urinary tract disease: recognition of *Staphylococcus felis* as a possible feline urinary tract pathogen. Vet Microbiol. 2007;121:182-8.

9. Cox HU, Hoskins JD, Newman SS, Foil CS, Turnwald GH, Roy AF. Temporal study of staphylococcal species on healthy dogs. Am J Vet Res. 1988;49:747-51.

10. Cox HU, Hoskins JD, Newman SS, Turnwald GH, Foil CS, Roy AF, et al. Distribution of staphylococcal species on clinically healthy cats. Am J Vet Res. 1985;46:1824-8.

11. Penna B, Varges R, Martins R, Martins G, Lilenbaum W. In vitro antimicrobial resistance of staphylococci isolated from canine urinary tract infection. Can Vet J. 2010;51:738-42.

12. Griffeth GC, Morris DO, Abraham JL, Shofer FS, Rankin SC. Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. Vet Dermatol. 2008;19:142-9.

13. Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L. Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. Vet Microbiol. 2007;121:307-15.

14. Yoo JH, Yoon JW, Lee SY, Park HM. High prevalence of fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius* isolates from canine pyoderma and otitis externa in veterinary teaching hospital. J Microbiol Biotechnol. 2010;20:798-802.

15. Fazakerley J, Nuttall T, Sales D, Schmidt V, Carter SD, Hart CA, et al. Staphylococcal colonization of mucosal and lesional skin sites in atopic and healthy dogs. Vet Dermatol. 2009;20:179-84.

16. Igimi S, Atobe H, Tohya Y, Inoue A, Takahashi E, Konishi S. Characterization of the most frequently encountered *Staphylococcus* sp. in cats. Vet Microbiol. 1994;39:255-60.

17. Jousson O, Di Bello D, Vanni M, Cardini G, Soldani G, Pretti C, et al. Genotypic versus phenotypic identification of staphylococcal species of canine origin with special reference to *Staphylococcus schleiferi* subsp. *coagulans*. Vet Microbiol. 2007;123:238-44.

18. Medleau L, Long RE, Brown J, Miller WH. Frequency and antimicrobial susceptibility of *Staphylococcus* species isolated from canine pyodermas. Am J Vet Res. 1986;47:229-31.

19. Medleau L, Blue JL. Frequency and antimicrobial susceptibility of *Staphylococcus* spp isolated from feline skin lesions. J Am Vet Med Assoc. 1988;193:1080-1.

20. Fitzgerald JR. The *Staphylococcus intermedius* group of bacterial pathogens: species re-classification, pathogenesis and the emergence of meticillin resistance. Vet Dermatol. 2009;20:490-5.

21. Mellmann A, Becker K, von Eiff C, Keckevoet U, Schumann P, Harmsen D. Sequencing and staphylococci identification. Emerg Infect Dis. 2006;12:333-6.

22. Carbonnelle E, Beretti JL, Cottyn S, Quesne G, Berche P, Nassif X, et al. Rapid identification of staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2007;45:2156-61.

23. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46:8-20.

24. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

25. Lloyd DH. Reservoirs of antimicrobial resistance in pet animals. Clin Infect Dis. 2007;45:S148-52.

26. Loeffler A, Lloyd DH. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect. 2010;138:595-605.

27. Manian FA. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis. 2003;36:26-8.

28. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, et al. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol. 2006;115:148-55.

29. Decristophoris P, Fasola A, Benagli C, Tonolla M, Petrini O. Identification of *Staphylococcus intermedius* Group by MALDI-TOF MS. Syst Appl Microbiol. 2011;34:45-51.

30. NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Second edition M 31-A2. 2004;22.

31. CLSI. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement M 100-S19. 2010;29.

32. Couto I, Sanches IS, Sa-Leao R, de Lencastre H. Molecular characterization of *Staphylococcus sciuri* strains isolated from humans. J Clin Microbiol. 2000;38:1136-43.

33. Cotter L, Lynch M, Cryan B, Greer P, Fanning S. Investigation of a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak in an Irish hospital: triplex PCR and DNA amplification fingerprinting. J Hosp Infect. 1997;36:37-47.

34. Bennett S, Woods T, Liyanage WM, Smith DL. A simplified general method for cluster-sample surveys of health in developing countries. World Health Stat Q. 1991;44:98-106.

35. Zadoks RN, Watts JL. Species identification of coagulase-negative staphylococci: genotyping is superior to phenotyping. Vet Microbiol. 2009;134:20-8.

36. Weese JS. Antimicrobial resistance in companion animals. Anim Health Res Rev. 2008;9:169-76.

37. Soares Magalhaes RJ, Loeffler A, Lindsay J, Rich M, Roberts L, Smith H, et al. Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dogs and cats: a case-control study. Vet Res. 2010;41:55.

38. Nienhoff U, Kadlec K, Chaberny IF, Verspohl J. Methicillin-resistant *Staphylococcus pseudintermedius* among dogs admitted to a small animal hospital. Vet Microbiol. 2010;150:191-7.

6. Research paper 3

Evaluation of pet contact as a risk factor for carriage of multi-drug resistant staphylococci in nursing home residents

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Abstract

Background: Pets, often used as companionship and for psychological support in the therapy of nursing home residents, have been implicated as reservoirs for antibiotic-resistant bacteria. We investigated the importance of pets as reservoirs of multidrug-resistant (MDR) staphylococci in nursing homes.

Methods: We assessed the carriage of MDR staphylococci in pets and in 2 groups of residents, those living in nursing homes with pets and those living without pet contacts. We collected demographic, health status, and human–pet contact data by means of questionnaires. We assessed potential bacteria transmission pathways by investigating physical resident-to-pet contact.

Results: The observed prevalence of MDR staphylococci carriage was 84/229 (37%) in residents living with pets and 99/216 (46%) in those not living with pets (adjusted odds ratio [AOR], 0.6; 95% confidence interval [CI], 0.4-0.9). Active pet contact was associated with lower carriage of MDR staphylococci (AOR, 0.5; 95% CI, 0.4-0.8). Antibiotic treatment during the previous 3 months was associated with significantly increased risk for MDR carriage in residents (AOR, 3.1; 95% CI, 1.8-5.7).

Conclusions: We found no evidence that the previously reported benefits of pet contact are compromised by the increased risk of carriage of MDR staphylococci in residents associated with interaction with these animals in nursing homes. Thus, contact with pets, always under good hygiene standards, should be encouraged in these settings.

Key Words: Companion animals; antibiotics; social network analyses; exchange of strains; ‘‘One Health’’.

Background

Multidrug resistance (MDR) in microorganisms poses a serious public health challenge. In nursing homes, MDR in opportunistic pathogens is of concern because people often suffer from infections or are immunocompromised and thus at increased risk for disease after infection by MDR microorganisms (1, 2). Methicillin resistant *Staphylococcus aureus* (MRSA) is a major problem in human medicine (3, 4), and coagulase-negative staphylococci (CNS) also have emerged as a major cause of nosocomial infections (5). Patients with foreign bodies such as catheters or prostheses are usually at risk for CNS infections (6). Unlike MRSA, the clinical manifestations of MDR CNS, are subtle, with a subacute or even chronic clinical course and no immediate signs of infection (7, 8). Nonetheless, the role of these pathogens and their increasing importance and incidence have been recognized (6).

Pets have been described as potential reservoirs for drug-resistant bacteria (9-11). Companion animals represent potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in veterinary practices that treat small animals (12, 13). Staphylococci, which are part of the normal skin and mucosal bacterial community of cats and dogs, have developed resistance to antibiotics (14). The transmission of MDR staphylococci between pets and humans has been documented in different settings (15-17), and MRSA has been reported in a pet therapy dog that visits aged-care wards in the United Kingdom (18).

Pets are increasingly used as companions and for psychological support in the therapy of chronically diseased or elderly patients, but their zoonotic potential is a matter of debate (19, 20). Studies of elderly persons residing in institutional settings have reported a general health benefit from pet-assisted therapy, along with reduced feelings of loneliness and isolation (21, 22). People who interact with animals may have improved physical health and psychological and social well being; moreover, actively caring for a pet also might serve as an incentive to maintain at least a

moderate level of physical activity in elderly people, thereby reducing the risk of being overweight (23).

The present study aimed to quantify the importance of pets as reservoirs of MDR staphylococci in nursing homes by assessing the carriage of these microorganisms in residents and in pets living in or regularly visiting the nursing homes, and by recording physical contact between residents and pets.

Methods

Study design and setting

This was a retrospective study of the carriage of MDR staphylococci in pets and in 2 groups of residents: those living in nursing homes in which pets were present and those in nursing homes without pets. Sample collection was performed between March 2008 and September 2009. The nursing homes were located in 4 different Swiss cantons (Berne, Ticino, Vaud, and Zurich). Nasal and ear swabs from pets and nasal swabs from residents in the same home were collected. A questionnaire eliciting information on demographic data, health status, and human–pet contact was completed by each participant and for each animal investigated. A nursing home was classified as hosting pets if at least 1 cat or dog was owned by the nursing home or by a resident, or a pet therapist was active in the home at least once a week. The study received ethical clearance by the 4 responsible cantonal Ethical Committees and authorization for animal experimentation from the cantonal and federal veterinary offices.

Selection of participants

This study included adult residents who either provided informed consent or had informed consent provided by relatives. For pets, consent had to be given by the owner. Residents and pets with any acute disease or currently already participating in another clinical trial were excluded. We randomly selected 315 nursing homes from an exhaustive list of 827 eligible homes provided by canton health authorities. Among these, 195 nursing homes reported keeping pets and 120 nursing homes reported having no pets. We then selected 68 nursing homes from each group and invited them to participate. A total of 59 homes declined to participate; thus, we enrolled 39 nursing homes with pets and 38 without pets in the study. Within each nursing home, we randomly selected 6 residents from an alphabetical list of residents provided by management. Residents who declined to participate were replaced by another randomly selected resident living in the same home.

Sample collection and laboratory analyses

Samples were collected with swabs (Amies Agar Gel 108C and 110C; Copan, Murrieta, CA), conserved in the transport medium at room temperature, and analyzed for the presence of staphylococci within 24-48 hours of collection. Swabs were first streaked on Mannitol Salt Agar (Chapman 2–MSA 2; bioMérieux® SA, Craponne, France) and incubated for 48 hours at 37°C, and then enriched in MRSA broth, supplied with 6 mg/L of oxacilllin (48 hours at 37°C), followed by culture on Gelose ChromID *S aureus* (SAID bioMérieux® SA) for 48 hours at 37°C. Colonies were isolated, and catalase-positive, gram-positive coccal bacteria isolates were frozen in skimmed milk at -80°C until further analyses.

Identification of pure colonies grown on blood agar at 37 °C for 24 h was carried out with a matrix assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) using an Axima Confidence™ spectrometer (Shimadzu-Biotech Corp., Kyoto, Japan) in positive linear mode

(m/z = 2,000 to 20,000) as described by Decristophoris et al. (24). Cultures not identifiable by MALDI-TOF MS were identified by sequencing of the amplified *rpoB* gene fragments (25).

Phenotypic antibiotic resistance was assessed using the Kirby-Bauer method on Mueller-Hinton blood agar (MHS2, bioMérieux® SA, France). Antibiotics tested included penicillin (10 units), ampicillin (10 µg), oxacillin (1 µg), cefazolin (30 µg), gentamicin (10 µg), tetracycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), trimethoprim-sulfamethoxazole (1.25+23.75 µg), ciprofloxacin (5 µg), amoxicillin and clavulanic acid (20+10 µg), ceftazidim (30 µg), imipenem (10 µg), tobramycin (10 µg), fusidic acid (10 µg), rifampicin (30 µg), chloramphenicol (30 µg), cefoxitin (30 µg), kanamycin (30 µg), doxycyclin (30 µg), mupirocin (5 µg), linezolid (30 µg) and quinopristin-dalfopristin (15 µg). Strains were classified as susceptible, intermediately resistant, or resistant to the drug according to Clinical and Laboratory Standards Institute (CLSI) guidelines (26, 27). A strain was defined as MDR if it was resistant to at least 3 antibiotics of different classes. Polymerase chain reaction analysis was used to investigate for the presence of the *mecA* gene, which confers methicillin resistance (28).

Analysis of physical contact and typing of strains

We investigated the arrangement and the intensity of physical contact between pets and residents (time spent with pets), and thus the potential zoonotic bacteria transmission pathways, by means of questionnaires. Resident–pet contacts were described graphically using Pajek version 1.28. (29). To reveal potential transmission scenarios of MDR staphylococci in residents and pets, molecular typing was carried out when the same bacterial species that was MDR and harbored the *mecA* gene was identified contemporaneously in at least 1 pet and 1 resident in contact within the same nursing home. Strains were typed by multilocus sequence typing following Thomas et al. (30) and by pulsed field gel electrophoresis following MacKenzie et al. (31), using *Salmonella* serotype Braenderup H9812 as a universal standard for the analysis. The staphylococcal cassette chromosome mec

(*SCCmec*) harbouring the *mecA* gene was typed by polymerase chain reaction following Milheirico et al. and Zhang et al. (32, 33).

Statistical analysis

Sample size. The intra-class correlation coefficient (rho) was estimated to be 0.15, with an estimated prevalence of about 10 % of people and 5 % of pets carrying at least one MDR staphylococcal strain. For estimating prevalence with a precision of a standard error of 2.5 % (upper 95 % CI = 14.9 %), 252 people clustered in 42 different nursing homes of each category were needed. We estimated that each nursing home with pets owned or was visited by three animals on average; thus, a total of 126 pets could have been sampled in 42 different nursing homes. The expected precision for the prevalence in pets would have been within a standard error of 2.2 % (upper 95 % CI = 9.3 %).

Univariable and multivariable analyses. We used generalized estimating equation (GEE) models with correlated binomial outcome, a logit link function, an exchangeable correlation matrix, and robust standard errors to account for clustering at the nursing home level. Subsequent models were adjusted for age, sex, and antibiotic treatment within 3 months before the study. The model used to explore possible risk factors was adjusted for age, sex, and contact with pets. All statistical analyses were performed with Stata 9.0 (StataCorp, College Station, TX).

Results

We collected samples from 216 residents living in 38 nursing homes where pets were not allowed, as well as from 229 residents and 98 pets in 39 nursing homes in which cats and dogs were present. Thirtyfour residents declined to participate and were replaced by randomly selected alternatives; 17 others also declined to participate, but no alternatives were available. A total of 135 animals were

present in 39 nursing homes; exclusions included 2 that did not meet the inclusion criteria, 2 in which it was not possible to collect nasal and ear swabs, and 33 that were not present at the time of sample collection. The median age of the residents living with pets was 86.6 years and that of those without pets was 85.2 years; the proportion of women in the 2 groups was 75% and 69%, respectively. Approximately 60% of the residents of nursing homes with pets and 9% of those in homes without pets had contact with cats and dogs at least once a week. Cats represented 54% of all investigated pets; dogs, 46%. Demographic data for residents and pets are summarized in Tables 1 and 2. In terms of the sociopsychological component of the human–pet relationship, 63% (281/445) of all investigated residents reported considering contact with pets of great importance to quality of life.

Table 1. Demographic characteristics of residents living with and without pets.

	With pets	Without pets
Age, years, median ± interquartile range	86.6 ± 10.8	85.2 ± 11.4
Female	75% (172/229)	69% (148/216)
History of MRSA	5% (12/229)	3% (7/216)
Catheter in the last year	16% (36/229)	14% (30/216)
Urinary infection in the last year	22% (50/226)	26% (55/212)
Surgical intervention in the last year	14% (32/227)	6% (14/216)
Stay in another care center in the last year	23% (53/228)	22% (47/215)
Antibiotic treatment the last 3 months	23% (52/228)	21% (45/214)
Contact with pets at least once a week	60% (137/229)	9% (20/216)

Table 2. Demographic characteristics of pets living in or regularly visiting nursing homes.

Owner	Cats (n = 53), n (%)	Dogs (n = 45), n (%)
Nursing homes	43 (81)	0
Residents	9 (17)	5 (11)
Pet-assisted therapy	0	11 (24)
Visitors	0	11 (24)
Care staff	0	14 (31)
Others	1 (2)	4 (9)

The adjusted prevalence of MDR staphylococci carriage was 36 % (95 % CI: 29-44) in the 229 residents living with pets, 46 % (95 % CI: 39-53) in the 216 residents living without pets, and 16 % (95 % CI: 10-25) in the 98 pets. A total of 815 staphylococcal strains were isolated from the nostrils of the residents, and 109 strains were isolated from the nostrils and ears of the animals. MDR strains (n=127 in residents without pets; n=108 in residents with pets; n=28 in pets) belonged to various staphylococcal species, including *S. aureus, S. pseudintermedius, S. epidermidis, S. hominis, S. haemolyticus, S. xylosus, S. warneri, S. capitis, S. simulans, S. pettenkoferi, S. fleuretti, S. saprophyticus, S. pasteuri, S. cohnii, S. auricularis, and S. croceolyticus*, in different proportions. *S. epidermidis* represented > 50% of the MDR strains recovered from residents and about 30% of those recovered from pets. The *mecA* gene was harbored by 17% (140/815) of all staphylococcal strains from residents and 7% (8/109) of those strains from pets. We isolated 9 MRSA strains from 8 residents, but found no methicillin-resistant *S. pseudintermedius*. No MRSA or methicillin-resistant *S. pseudintermedius* was isolated from pets.

Living in nursing homes in which pets were present was associated with a decreased prevalence of MDR staphylococci carriage (AOR = 0.6; 95 % CI: 0.4-0.9; Table 3). We also analyzed the exposure to pets by defining it as active contact at least once a week, and found a negative

association here as well (AOR = 0.5; 95 % CI: 0.4-0.8) (Table 3). The frequency of the contact with pets was not associated with an increased risk of MDR staphylococci carriage (Table 4).

Table 3. Stay in a nursing home with pets and active contact of nursing home residents with pets as risk factors for staphylococcal carriage using GEE models to account for within-home clustering.

Organism			Crude RR (95% CI)	Crude OR (95% CI)	aOR (95% CI)
	Homes with pets (n = 229), n (%)	**Homes without pets (n = 216), n (%)**			
Residents					
Staphylococcus spp	216 (94)	208 (96)	1.0 (0.9-1.0)	0.6 (0.3-1.5)	0.7 (0.3-1.6)
MDR *Staphylococcus* spp	84 (37)	99 (46)	0.8 (.6-1.0)	0.7 (0.5-1.0)	0.6 (0.4-0.9)
Staphylococci with *mecA*	55 (24)	64 (30)	0.8 (.6-1.1)	0.8 (0.5-1.2)	0.7 (0.4-1.1)
MRSA	2 (1)	6 (3)	0.3 (.1-1.5)	0.3 (0.1-1.6)	ND
Pets	(n = 98)				
Staphylococcus spp	56 (57)	NA	NA	NA	NA
MDR *Staphylococcus* spp	16 (16)	NA	NA	NA	NA
Staphylococci with *mecA*	6 (6)	NA	NA	NA	NA
MRSA	0 (0)	NA	NA	NA	NA
	Contact (n =157), n (%)	**No contact (n = 288), n (%)**			
Residents					
Staphylococcus spp	146 (93)	278 (97)	1.0 (0.9-1.0)	0.5 (0.2-1.2)	0.5 (0.2-1.2)
MDR *Staphylococcus* spp	54 (34)	129 (45)	0.8 (0.6-1.0)	0.7 (0.4-1.0)	0.5 (0.4-0.8)
Staphylococci with *mecA*	32 (20)	87 (30)	0.7 (0.5-1.0)	0.6 (0.4-1.0)	0.5 (0.3-0.9)
MRSA	2 (1)	6 (2)	0.6 (0.1-3.0)	0.6 (0.1-2.9)	ND

NOTE. Active contact was defined as self-reported physical contact to a pet at least once a week.
aOR, OR adjusted for age, sex, and antibiotic treatment in the last 3 months; *GEE*, generalized estimating equation; *NA*, not applicable; *ND*, not determined; *RR*, relative risk.

Table 4. Association between carriage of MDR staphylococci in residents and contact intensity with pets (time spent with pets) using GEE models to account for within-home clustering.

Time spent with pets	MDR yes (n = 84), n (%)	MDR no (n = 145), n (%)	Crude OR (95% CI)	aOR (95% CI)
No contact	39 (46)	62 (43)	Reference	Reference
>12 hours/day	3 (4)	9 (6)	0.6 (0.2-2.3)	0.5 (0.1-2.0)
1-12 hours/day	9 (11)	5 (3)	3.3 (0.9-12.8)	3.6 (1.1-12.1)
<1 hour/day	12 (14)	16 (11)	1.2 (0.6-2.3)	0.9 (0.4-2.0)
1 visit per week	21 (25)	53 (37)	0.6 (0.3-1.2)	0.5 (0.2-1.1)

NOTE. Reference group, no contact.
aOR, OR adjusted for age, sex, and antibiotic treatment in the last 3 months; *GEE*, generalized estimating equation.

In only 1 nursing home was the same MDR staphylococcal species carried by 1 pet and at least 1 resident who had been in physical contact. In this home, 1 cat and 1 resident, linked by a low-frequency contact (1 visit per week), carried MDR *S. epidermidis* strains with the *mecA* gene (Figure 1). The 2 strains belonged to the same multilocus sequence type ST5, harbored the same type of SCCmec IVa, and had identical pulsed field gel electrophoresis profiles, indicating a clonal origin (34). Both strains were phenotypically resistant to penicillin, ampicillin, oxacillin, gentamicin, tetracycline, erythromycin, ciprofloxacin, fusidic acid, cefoxitin, and kanamycin. In addition, strain p1 was resistant to cefazolin, and strain r1 was resistant to clindamycin.

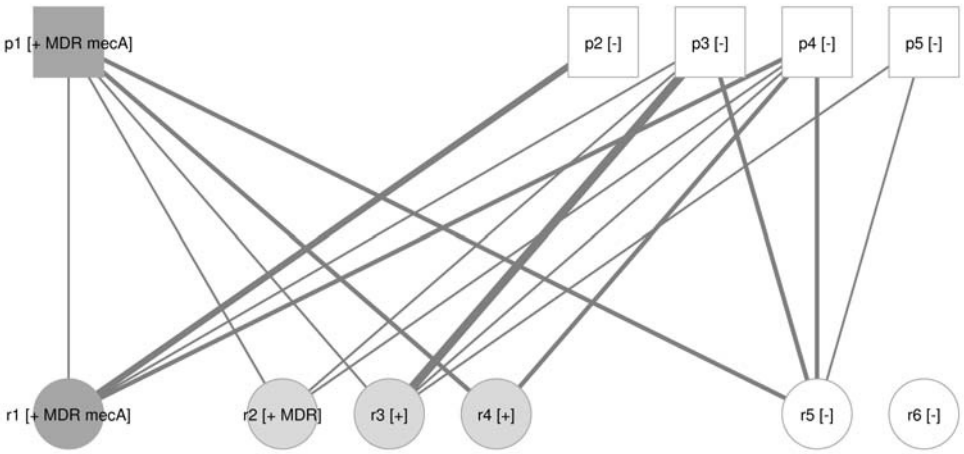

Figure 1. Weighted graph illustrating the network of contacts in a nursing home setting, with pets (p) represented by boxes and residents (r) represented by circles. Edges represent physical contacts between pets and persons, with line thickness increasing with higher reported contact frequency. Individuals carrying *S. epidermidis* [1] are depicted as gray vertices, those not carrying this staphylococcal species [-] in white. *mecA* is the gene encoding for methicillin resistance in staphylococci.

Additional univariable exploratory analysis of risk factors related to the carriage of MDR staphylococci in residents showed that antibiotic treatment within 3 months before the start of the investigation (OR = 3.5; 95 % CI: 2.2-5.5), presence of a catheter (OR = 2.3; 95 % CI: 1.3-4.1), urinary infections (OR = 2.6; 95 % CI: 1.6-4.2) and hospitalization in the last year (OR = 1.7; 95 % CI: 1.1-2.6) all increased the risk of carrying MDR staphylococci in residents (Table 5). However, in the multivariate analysis, only antibiotic treatment within 3 months before the investigation remained a significant factor in increasing the risk of MDR carriage (AOR = 3.1; 95 % CI: 1.8-5.7).

Table 5. Exploratory analysis of potential risk factors for the carriage of MDR staphylococci in residents using GEE models to account for within-home clustering.

Characteristics	MDR yes	MDR no	Crude OR (95% CI)	aOR (95% CI)
Catheter	21% (39/183)	10% (27/262)	2.3 (1.3-4.1)	1.9 (1.0-3.7)
Urinary tract infections	34% (61/178)	17% (44/260)	2.6 (1.6-4.2)	1.4 (0.8-2.5)
Surgical intervention	10% (18/182)	11% (28/261)	0.9 (0.4-1.9)	0.4 (0.2-1.1)
Hospitalization	28% (51/182)	19% (49/261)	1.7 (1.1-2.6)	1.5 (1.0-2.5)
Antibiotic treatment	34% (62/180)	13% (35/262)	3.5 (2.2-5.5)	3.1 (1.8-5.7)

aOR, OR adjusted for age, sex, and contact with pets; *GEE*, generalized estimating equation.

Discussion

In this study, living in a nursing home in which pets were present or residents had regular contact with pets at least once a week did not increase the risk for MDR staphylococci carriage. In fact, residents living in nursing homes with pets and with regular pet contact had a lower prevalence of MDR staphylococci carriage. This might not indicate a causal protective effect of pets, however; rather, compared with residents without contact with pets, residents with contact might be more active and healthier, and thus more immunocompetent and less likely to be colonized by MDR staphylococci.

The only risk factor identified in the multivariate analysis as associated with MDR staphylococci carriage was antibiotic treatment within 3 months before the investigation. Factors reported to increase the risk of colonization or infection by MDR staphylococci include age >60 years, previous colonization, hospitalization in the previous year, antibiotic use in the previous 3 months, the presence of wounds, and recent skin infection (1, 35-37).

Previous reports have suggested that pets may be reservoirs of MDR microorganisms, and cases of possible exchange of staphylococcal strains between animals and humans have been described (15-17, 38, 39). In this study, we found a lower risk of MDR staphylococci carriage in residents living with pets, suggesting that strain transmission is rather limited. We identified only 1 case in which an exchange of strains most likely had occurred. Whether the carriage of these microorganisms originated from a common source of colonization (e.g., surrounding environment) or from direct exchange between humans and animals remains to be clarified, however. Our findings seem to indicate that for most residents, the previously reported benefits of pet contact might outweigh the potential risk of infection.

Limitations of this study include a potential recall bias in elderly persons who had to self-report their contact with pets and the time that they regularly spent with the animals. The study focused on the role of pets in nursing homes and did not generate information on person-to-person contacts, which would have completed the contact network. Thus, we could not fully explore the relative importance of resident-to-resident versus resident-to-pet contacts for the MDR staphylococci carriage. Ideally, to uncover potential transmission scenarios, the totality of human and pet bacterial isolates should be typed to reveal molecular epidemiologic patterns and their congruence with physical contact patterns. But strain molecular typing is expensive and time-consuming, and social network analyses can be a useful approach that may help keep the focus only on situations that are of interest and determinant for an exchange of microorganisms. We investigated the carriage of staphylococci because MDR in these microorganism is of concern in health care settings, but we did

not explore the potential for pets to act as reservoirs of other MDR microorganisms, such as extended-spectrum b-lactamase–producing enterobacteriaceae (40). In addition, we defined MDR as resistance of a strain to at least 3 antibiotics of different classes. Unfortunately, official guidelines (eg, Clinical and Laboratory Standards Institute and EUCAST; European Committee on Antimicrobial Susceptibility Testing) lack a clear and standard definition of the criteria needed to define a staphylococcal strain as MDR. This reduces the possibility of carrying out meaningful comparisons with published data.

To the best of our knowledge, this is the first study that has investigated pets as potential reservoirs of MDR staphylococci in the nursing home setting. The study's limitations notwithstanding, we have shown how the complementary analysis of epidemiologic links via physical contacts and molecular typing are useful for targeting the investigation of the complexity of possible exchange of MDR microorganisms. We reported a case of possible exchange of methicillin-resistant *S. epidermidis* strains between a resident and a cat. In addition, we explored possible risk factors for the carriage of MDR staphylococci in nursing home residents. Future studies are needed to assess risk factors for the carriage of these microorganisms in pets.

Public health investigations may benefit from an increased awareness of the potential for cooperation among different sectors (eg, human medicine, veterinary medicine, social science). Investigations under the ‘‘One Health’’ paradigm are important to understanding the complexity of public health challenges (41, 42). Evaluating the presence of pets in nursing homes must take into account various social, emotional, and psychological components and factors. More than 60% of the residents surveyed reported that they considered contact with pets of great importance to their quality of life. We found no evidence that the previously reported benefits of pet contact are compromised by the increased risk of MDR staphylococci carriage in residents with pet contact. Thus, we suggest that contact with pets be encouraged in nursing home settings: Published

guidelines for animal-assisted interventions in health care facilities may be important for defining applicable hygiene standards (43, 44).

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References

1. Strausbaugh LJ, Crossley KB, Nurse BA, Thrupp LD. Antimicrobial resistance in long-term-care facilities. Infect Control Hosp Epidemiol. 1996;17:129-40.

2. Rogers MA, Mody L, Chenoweth C, Kaufman SR, Saint S. Incidence of antibiotic-resistant infection in long-term residents of skilled nursing facilities. Am J Infect Control. 2008;36:472-5.

3. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA. 2007;298:1763-71.

4. ECDC/EMEA. The bacterial challenge: time to react. Joint technical report [http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf]. 2009;doi 10.2900/2518.

5. von Eiff C, Proctor RA, Peters G. Coagulase-negative staphylococci - Pathogens have major role in nosocomial infections. Postgrad Med. 2001;110:63-76.

6. Piette G, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.

7. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev. 1994;7:117-40.

8. Huebner J, Goldmann DA. Coagulase-negative staphylococci: Role as pathogens. Annu Rev Med. 1999;50:223-36.

9. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

10. Lloyd DH. Reservoirs of antimicrobial resistance in pet animals. Clin Infect Dis. 2007;45:S148-52.

11. Loeffler A, Lloyd DH. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect. 2010;138:595-605.

12. Prescott JF. Antimicrobial use in food and companion animals. Anim Health Res Rev. 2008;9:127-33.

13. Regula G, Torriani K, Gassner B, Stucki F, Muntener CR. Prescription patterns of antimicrobials in veterinary practices in Switzerland. J Antimicrob Chemother. 2009;63:805-11.

14. Weese JS. Antimicrobial resistance in companion animals. Anim Health Res Rev. 2008;9:169-76.

15. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, et al. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol. 2006;115:148-55.

16. van Duijkeren E, Wolfhagen MJ, Heck ME, Wannet WJ. Transmission of a Panton-Valentine leucocidin-positive, methicillin-resistant *Staphylococcus aureus* strain between humans and a dog. J Clin Microbiol. 2005;43:6209-11.

17. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol. 2007;45:1118-25.

18. Enoch DA, Karas JA, Slater JD, Emery MM, Kearns AM, Farrington M. MRSA carriage in a pet therapy dog. J Hosp Infect. 2005;60:186-8.

19. Guay DR. Pet-assisted therapy in the nursing home setting: potential for zoonosis. Am J Infect Control. 2001;29:178-86.

20. Brodie SJ, Biley FC, Shewring M. An exploration of the potential risks associated with using pet therapy in healthcare settings. J Clin Nurs. 2002;11:444-56.

21. Colombo G, Buono MD, Smania K, Raviola R, De Leo D. Pet therapy and institutionalized elderly: A study on 144 cognitively unimpaired subjects. Arch Gerontol Geriat. 2006;42:207-16.

22. Brodie SJ, Biley FC. An exploration of the potential benefits of pet-facilitated therapy. J Clin Nurs. 1999;8:329-37.

23. Cutt H, Giles-Corti B, Knuiman M, Timperio A, Bull F. Understanding dog owners' increased levels of physical activity: results from RESIDE. Am J Public Health. 2008;98:66-9.

24. Decristophoris P, Fasola A, Benagli C, Tonolla M, Petrini O. Identification of *Staphylococcus intermedius* Group by MALDI-TOF MS. Syst Appl Microbiol. 2011;34:45-51.

25. Mellmann A, Becker K, von Eiff C, Keckevoet U, Schumann P, Harmsen D. Sequencing and staphylococci identification. Emerg Infect Dis. 2006;12:333-6.

26. CLSI. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement M 100-S19. 2010;29.

27. NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Second edition M 31-A2. 2004;22.

28. Cotter L, Lynch M, Cryan B, Greer P, Fanning S. Investigation of a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak in an Irish hospital: triplex PCR and DNA amplification fingerprinting. J Hosp Infect. 1997;36:37-47.

29. Batagelj V, Mrvar A. Pajek – Program for large network analysis. Home page: http://vladofmfuni-ljsi/pub/networks/pajek/.

30. Thomas JC, Vargas MR, Miragaia M, Peacock SJ, Archer GL, Enright MC. Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. J Clin Microbiol. 2007;45:616-9.

31. MacKenzie FM, Greig P, Morrison D, Edwards G, Gould IM. Identification and characterization of teicoplanin-intermediate *Staphylococcus aureus* blood culture isolates in NE Scotland. J Antimicrob Chemother. 2002;50:689-97.

32. Milheirico C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant *Staphylococcus aureus*: 'SCCmec IV multiplex'. J Antimicrob Chemother. 2007;60:42-8.

33. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol. 2005;43:5026-33.

34. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233-9.

35. Safdar N, Bradley EA. The risk of infection after nasal colonization with *Staphylococcus aureus*. Am J Med. 2008;121:310-5.

36. Maree CL, Eells SJ, Tan J, Bancroft EA, Malek M, Harawa NT, et al. Risk factors for infection and colonization with community-associated methicillin-resistant *Staphylococcus aureus* in the Los Angeles County Jail: A case-control study. Clin Infect Dis. 2010;51:1248-57.

37. Santos HB, Machado DP, Camey SA, Kuchenbecker RS, Barth AL, Wagner MB. Prevalence and acquisition of MRSA amongst patients admitted to a tertiary-care hospital in Brazil. BMC Infect Dis. 2010;10:328.

38. Morris DO, Boston RC, O'Shea K, Rankin SC. The prevalence of carriage of meticillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets. Vet Dermatol. 2010;21:400-7.

39. Loeffler A, Pfeiffer DU, Lloyd DH, Smith H, Soares-Magalhaes R, Lindsay JA. Meticillin-resistant *Staphylococcus aureus* carriage in UK veterinary staff and owners of infected pets: new risk groups. J Hosp Infect. 2010;74:282-8.

40. Oteo J, Perez-Vazquez M, Campos J. Extended-spectrum [beta]-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. Curr Opin Infect Dis. 2010;23:320-6.

41. Zinsstag J, Schelling E, Bonfoh B, Fooks AR, Kasymbekov J, Waltner-Toews D, et al. Towards a 'One Health' research and application tool box. Vet Ital. 2009;45:121-33.

42. Zinsstag J, Schelling E, Wyss K, Mahamat MB. Potential of cooperation between human and animal health to strengthen health systems. Lancet. 2005;366:2142-5.

43. Friedmann E, Son H. The human-companion animal bond: how humans benefit. Vet Clin North Am Small Anim Pract. 2009;39:293-326.

44. Lefebvre SL, Golab GC, Christensen E, Castrodale L, Aureden K, Bialachowski A, et al. Guidelines for animal-assisted interventions in health care facilities. Am J Infect Control. 2008;36:78-85.

7. Working paper 1

Relationship between pets and people in Swiss households: implications for the carriage of staphylococci in pet owners

Abstract

Background: In the modern society the relationship between pets and their owners has become very close, with cats and dogs being integrated in the family network. On one hand the relationship with pets positively influences human physical and mental health, on the other this interaction may raise some concerns due to the possible role of pets as reservoir of antibiotic resistant microorganisms. In this study we aimed at investigating the relationship between cats, dogs and their owners in Swiss households and to evaluate the importance of these contacts for the carriage of staphylococci in people.

Results: We investigated 356 pet owners and 177 people not owning any cat or dog since at least one year. Among the owners, 114 held only dogs and 141 only cats; the remaining 101 had both. 70 % allowed their pets to touch their faces. 34.5 % affirmed that their pet slept regularly on the bed. Contacts with animals other than cats and dogs were recorded for 33 % of owners and 30 % of people not owning any cat or dog. The same proportion of multi-drug resistant staphylococcal carriage (20 %) was found in pet owners and in the control group. *S. pseudintermedius* was carried by eight owners but by none of the non-owners.

Conclusions: Although we did not find any evident impact of pets on multi-drug resistance carriage in the staphylococci isolated from their owners, the intensity of the contact between pets and humans deserve to be considered when investigating the exchange of multi-drug resistant strains under an interdisciplinary "One Health" approach.

Background

Human and pets are living together since ancient times. The evolution of cooperation with dogs led to their domestication (1). Earliest archaeological evidence of a "true" domestic dog is dated to 14,000 years ago and the emergence of dog keeping seems to coincide with the evolutionary appearance of altruism and empathy among humans (2). Domestication of cats has a more recent history. It suggested that cats were fully domesticated only during the last 150 years, although the history of *Felis catus* is characterised by the animal drifting unpredictably in and out of various states of domestication, semi-domestication and feralness (3). First archaeological evidence of cat keeping stems from Cyprus where, in human settlements dating from 6,000 years ago, remains of cat's jawbone were found (3).

In modern societies pets have taken up an important companion role as they probably never previously had in the human history. The relationship between pets and their owners has become closer with time: cats and dogs can now be considered regular members of the family network. Statistics from 2008 show that 500,000 dogs and 1,4 million of cats live in 385,000 respectively 780,000 Swiss households. According to the Society for the food of domestic animals, in the last years the number of Swiss households keeping cats and dogs is increasing, reaching 12 % for dogs and 25 % for cats in 2008 (4).

The World Health Organisation (WHO) defined health as a state of complete physical, mental and social well-being and not merely as the absence of disease or infirmity. In this context, the relationship with pets is an important component of human health. It has been shown that people interacting with animals may benefit from improved physical health and psychological and social well-being (5). Pets can help people by providing them much needed companionship, thus giving them a feeling of security (6). Serpell (7) showed long term befits of pet ownership on people's health, behaviour and general well-being. A positive short term influence of the presence and interaction with pets on the indicators of stress (e.g. blood pressure and anxiety) were also

demonstrated (8). Additionally, actively looking after pets might be an incentive to keep a moderate level of physical activity (9).

In the last decade the relationship with cats and dogs has been suggested to be of potential concern to public health due to the possible role of pets as reservoir of antibiotic resistant microorganisms (10-13). In fact, studies reported probable exchange of multi-drug resistant (MDR) microorganisms between pets and humans (14-17). Meanwhile there is raising awareness of the added value that an interdisciplinary approach would have in the investigation of the spread of antibiotic resistance (18). To this aim, not only knowledge on bacterial resistance processes, but also a better understanding of the emotional and physical relationship between human and their pets is of great interest.

This study aimed at investigating the relationship between cats, dogs and their owners in the Swiss household and to evaluate the importance of these contacts for the carriage of staphylococci in people.

Methods

Study design and setting

Samples were collected between November 2008 and December 2009 in four different Swiss cantons (Berne, Ticino, Vaud and Zurich). Only healthy people older than 18 years old, with no overt acute disease at the time of sample collection, were enrolled in the study. We investigated Swiss cat and dog owners, including people not owning these pets since at least one year as control group. People were sampled based on random selection as well as on convenience. Pet owners were enrolled both by selecting them from a list of dog owners provided by the Cantonal Veterinary Offices and by recruitment at veterinary clinics that they visited for routine vaccination of their animals. People not owning pets were mainly contacted in their household as well as in travel

medicine centres. An informed written consent had to be given by each participant. The study received ethical clearance from the corresponding Cantonal Ethical Committees.

Data collection

A questionnaire with demographic, health status information and description of emotional and physical relationship with cats and dogs was filled in by each participant. People were asked to answer closed questions. Relevance and intensity of the relationship the owners had with their pets was self-reported by the participants.

Sample collection

Nasal swabs were collected by means of a cotton swab (Amies agar gel 108C, Copan, Italy) previously soaked in a physiological 0.9 % NaCl solution. The swab was introduced for 1-2 cm in the nostril. The collected samples were conserved in the transport medium at room temperature and analysed for the presence of staphylococci within 24-48 h of collection.

Sample analyses

Swabs were both streaked on Mannitol Salt Agar (Chapman 2 – MSA 2, bioMérieux® SA, France), incubated for 48 h at 37 °C, and enriched in MRSA broth supplied with 6 mg/L of oxacilllin (48 h at 37 °C) and cultured on Gelose ChromID *S. aureus* (SAID, bioMérieux® SA, France) for 48 h at 37 °C. Colonies were isolated and catalase positive, Gram positive coccal bacteria isolates were frozen in skimmed milk at -80 °C until further analyses.

Strains were grown on blood agar during 24 h and identified by mass assisted laser desorption ionisation - time of flight (MALDI-TOF MS) using an Axima Confidence™ spectrometer

(Shimadzu-Biotech Corp., Kyoto, Japan) in positive linear mode (m/z = 2,000 to 20,000) (19). Sequencing of the amplified partial *rpoB* gene was carried out on isolates that could not be identified by MALDI-TOF MS (20).

Phenotypic antibiotic resistance to 24 different drugs was assessed by the Kirby-Bauer method on Mueller-Hinton blood agar (MHS2, bioMérieux® SA, France). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (21, 22); for the purpose of this work, intermediate results were considered resistant. In this investigation MDR was defined as resistance to at least 3 drugs belonging to different antibiotic classes. Additionally, the presence of the *mec*A gene which confers methicillin resistance was investigated by polymerase chain reaction (PCR) (23, 24).

Statistical analysis

Medians were reported with interquartile range (IQR) and with 1st quartile (Q1) and 3rd quartile (Q3); proportions (%) with 95 % confidence interval (95 % CI). Statistical analyses were performed with STATA 9.0 (Stata Corporation, College Station, TX, USA).

Results

Demographic of investigated people

533 people living in the Swiss community of the Cantons of Bern (n=132), Ticino (n=135), Vaud (n=131) and Zürich (n=135) were included in this study; 356 were pet owners and 177 did not own any cat or dog since at least one year. Median age of the pet owners was 46, of people without pets 45. Demographic characteristics of the two groups were similar; details are reported in Table 1.

Table 1. Demographics of investigated people with median age and proportions (%) of the different characteristics. IQR = interquartile range, 95 % CI = 95 % confidence intervals.

Characteristics	**Pet owners (n = 356)**			**No pet owners (n = 177)**		
	n	**Median**	**IQR**	**n**	**Median**	**IQR**
Age	353	45	20	176	45	26
	n	**%**	**95% CI**	**n**	**%**	**95% CI**
Women	263/354	74	70-79	67/177	62	57-67
MRSA history	7/355	2	0.9-4	4/176	2	0.8-6
Antibiotic treatment in the last 3 months	13/350	13	10-17	17/176	10	6-15
Immunosuppressant in the last 3 months	7/349	2	1-4	3/174	2	0.5-5
Surgical intervention in the last year	43/355	12	9-16	14/177	8	5-13
Stay in an hospital in the last year	25/355	7	5-10	13/177	7	4-12
Diabetes	3/355	0.8	0.3-3	1/177	0.6	0.8-4
Catheter	16/355	5	3-8	6/177	3	2-7
Urinary affections in the last year	32/354	9	6-12	9/176	5	3-9

Relationship with cats, dogs and other animals

On average the relationship between owners and their pets lasted since 6 years, with a minimum of 5 days and a maximum of 18 years. About 96 % (343/356) of the owners rated pets to have a positive influence on their quality of life (QoL) whereas 54 % (95/177) of people not owning a pet acknowledged that the presence of a cat or a dog could possibly have a positive impact on their QoL (Table 2). Among the 356 owners, 114 held only dogs and 141 only cats; the remaining 101 kept both species within their household. The median number of dogs per household was 1 (IQR = 1) dog, with 10 households reporting 5 or more of these animals. For cats, the median value was 1 (IQR = 2), with 21 households where 5 or more cats were present. Two owners reported that more than 5 cats and more than 5 dogs where present within the same household. Contact with animals other than cats and dogs was reported in 33 % (95 % CI: 28-38) of owners and 30 % (95 % CI: 23-37) of control people.

Table 2. Influence of pets on the QoL (quality of life), self-evaluated by the investigate people. 95 % CI = 95 % confidence interval; N.D. = not determined.

	Pet owners (n = 356)			Controls (n = 177)		
Influence of pets on QoL	**n**	**%**	**95%CI**	**n**	**%**	**95%CI**
Positive	343	96	94-98	95	54	46-61
Indifferent	9	2.5	0.9-4	39	22	16-28
Negative	3	1	0-2	42	24	17-30
Do not know	-	-	-	1	0.5	0-1.6
N.D.	1	0.5	0-0.8	-	-	-

Intensity of the contact between cats, dogs and their owners in the household

About 29 % (104/356) of the respondents declared to have only hand contact with their pets, whereas 70 % (248/356) allowed their animals to touch also their face. In one case the holder declared not having any physical contact with the dog and the cat present at home (Table 3). In 49 % (173/355) of the household, pets slept regularly (minimum of 4 nights per week) in the room of the owner. Additionally, 34.5 % (122/354) of the respondents declared that their pet slept regularly on their bed (Table 3). In at least 31 of the 122 documented situations [9 % (31/354) of all households] the pet sleeping regularly on the bed was a dog.

Table 3. Descriptive statistic of the physical contact between owners and their pets in the households. 95 % CI = 95 % confidence interval.

	n	**%**	**95%CI**
Type of Contact			
No contact	1/356	0.3	0-0.8
Only hands	104/356	29	24-34
Hands and face	248/356	70	65-74
Other	3/356	0.8	0-1.7
Pet sleep in the room	173/355	49	44-54
Pet sleep on the bed	122/354	34.5	30-40

Staphylococcal carriage in healthy people in the household

Staphylococci were recovered from 92 % (326/356) of the owner and 97 % (172/177) of the non-owner nostrils. *S. epidermidis* was isolated at highest frequency, with 54 % (488/905) of all recovered strains belonging to this species. *S. pseudintermedius* was carried by eight owners but by none of the people in the control group (Table 4). The same proportion of MDR staphylococcal carriage (20 %: 71/356 and 35/177 respectively) was observed in owners and in control group. The *mecA* gene encoding for methicillin resistance was identified in 6 % (95 % CI: 4-8; 20/356) of the owners and 11 % (95 % CI: 7-17; 20/177) of the other people; with 93 % (38/41) of all *mecA* positive strains belonging to the species *S. epidermidis*. No methicillin-resistant *S. aureus* (MRSA) or methicillin-resistant *S. pseudintermedius* (MRSP) were isolated (Table 4). About 28 % (147/533) of the investigated people carried *S. aureus* in their nostril. Some strains belonging to the species *S. epidermidis* (n = 7) and *S. pseudintermedius* (n = 1) were found to be phenotypically resistant to six or more different antibiotic classes, with this latter species present only in pet owners (Table 4).

Table 4. Staphylococcal strains (n = 905) isolated from the nostril of 533 people (356 pet owners and 177 controls) and the distribution of antibiotic resistance among the different species. Q1 = 1st quartile, Q3 = 3rd quartile, Max. = maximum, *mecA* = gene encoding methicillin resistance, MDR = multi-drug resistance.

Identified isolates	**Pet owners (n = 607)**				**Controls (n = 298)**			
	Isolates	**Median (Q1; Q3; Max.)**	**mecA**	**MDR**	**Isolates**	**Median (Q1; Q3; Max.)**	**mecA**	**MDR**
	n		**n (%)**	**n (%)**	**n**		**n (%)**	**n (%)**
Coagulase positive	**112**	**1 (1; 1; 6)**	**0 (0)**	**8 (7)**	**48**	**1 (1; 2; 4)**	**0 (0)**	**4 (8)**
S. aureus	102	1 (0; 1; 4)	0 (0)	5 (5)	48	1 (1; 2; 4)	0 (0)	4 (8)
S. delphini	1	0 (0; 0; 0)	0 (0)	0 (0)	-	-	-	-
S. intermedius	1	1 (1; 1; 1)	0 (0)	0 (0)	-	-	-	-
S. pseudintermedius	8	2 (2; 5; 6)	0 (0)	3 (38)	-	-	-	-
Coagulase negative	**494**	**1 (0; 2; 8)**	**20 (4)**	**78 (16)**	**250**	**1 (0; 2; 7)**	**21 (8)**	**32 (13)**
S. arlettae	1	4 (4; 4; 4)	0 (0)	1 (100)	-	-	-	-
S. capitis	21	0 (0; 1; 3)	0 (0)	1 (5)	8	0.5 (0; 1; 1)	0 (0)	0 (0)
S. caprae	1	0 (0; 0; 0)	0 (0)	0 (0)	-	-	-	-
S. caprae/capitis	4	0 (0; 1; 2)	0 (0)	0 (0)	5	0 (0; 0; 2)	0 (0)	0 (0)
S. cohnii	6	2.5 (2; 4; 4)	0 (0)	3 (50)	2	0.5 (0; 1; 1)	0 (0)	0 (0)
S. devriesei	-	-	-	-	1	3 (3; 3; 3)	0 (0)	1 (100)
S. epidermidis	325	1 (1; 2; 8)	18 (6)	57 (17)	163	1 (0; 2; 7)	20 (12)	26 (16)
S. equorum	4	1 (0; 2; 2)	0 (0)	0 (0)	3	1 (0; 1; 1)	0 (0)	0 (0)
S. fleuretti	1	2 (2; 2; 2)	0 (0)	0 (0)	-	-	-	-
S. haemolyticus	16	1 (1; 2; 5)	1 (6)	2 (13)	10	2 (1; 3; 4)	1 (10)	3 (30)
S. hominis	7	2 (1; 2; 3)	0 (0)	1 (14)	5	1 (1; 1; 2)	0 (0)	0 (0)
S. kloosii	1	2 (2; 2; 2)	0 (0)	0 (0)	-	-	-	-
S. lugdunensis	13	0 (0; 0; 2)	0 (0)	0 (0)	4	0 (0; 0.5; 1)	0 (0)	0 (0)
S. pasteuri	13	0 (0; 1; 3)	0 (0)	1 (8)	2	0.5 (0; 1; 1)	0 (0)	0 (0)
S. pettenkoferi	2	2.5 (1; 4; 4)	0 (0)	1 (50)	-	-	-	-
S. saprophyticus	14	2 (1; 2; 4)	0 (0)	3 (21)	6	2 (2; 2; 2)	0 (0)	0 (0)
S. sciuri	3	3 (1; 4; 4)	1 (33)	2 (67)	1	2 (2; 2; 2)	0 (0)	0 (0)
S. simulans	1	1 (1; 1; 1)	0 (0)	0 (0)	1	0 (0; 0; 0)	0 (0)	0 (0)
S. succinus	2	0.5 (0; 1; 1)	0 (0)	0 (0)	4	0.5 (0;1.5; 2)	0 (0)	0 (0)
S. vitulinus	2	1 (1; 1; 1)	0 (0)	0 (0)		-	-	-
S. warneri	43	0 (0; 1; 4)	0 (0)	4 (9)	25	1 (0; 1; 2)	0 (0)	0 (0)
S. warneri/pasteuri	2	0 (0; 0; 0)	0 (0)	0 (0)	3	1 (1; 2; 2)	0 (0)	0 (0)
S. xylosus	11	2 (2; 2; 3)	0 (0)	2 (18)	7	2 (1; 3; 3)	0 (0)	2 (29)
Staphylococcus spp.	1	1 (1; 1; 1)		0 (0)	-	-	-	-

Discussion

This study has demonstrated the physical closeness of pets with people and the emotional importance of this relationship. The majority of the owners reported pets to positively influence their QoL. No impact of the animals on the MDR carriage in owners was evident. MDR and methicillin resistance were identified in staphylococci recovered from both people in contact and people not owning any cat or dog. No MRSA or MRSP were detected. *S. pseudintermedius* was isolated only from people belonging to the owner's group.

We observed a very close contact between owners and their pets, with a large number of owners allowing their pets a direct contact with their face. The number of pets sleeping on the bed of their owners is also quite large. It could be expected that cats can hardly be forbidden this sleeping practice, but we can reasonably assume that dogs reported to sleep on the owner's bed were consciously allowed to do so by their owners. This suggests that a large number of people are actively seeking a very close contact with their pets.

In a previous study, where 30 % of healthy dogs were found to be carriers of *S. pseudintermedius,* we suggested host specificity of this staphylococcal species for dogs (25). Results of the present study, however, suggest that carriage of this microorganism only in pet owners may imply transmission of strains from animals to owners. An additional investigation in nursing homes, where people had contacts with cats and dogs did not allow to recover any *S. pseudintermedius* isolate (26). This might be explained by the fact that in institutionalised settings people usually have no such intense and close contact with pets as we could document it in the households.

There is increasing awareness on the importance of the interactions between the different hosts (e.g. human and animals) when evaluating spreading dynamics of microorganisms. For example Alvarez et al. (27) proposed the use of network analysis as a valuable approach for the modelling of bovine *S. aureus* spread between farms. However the analysis of contact networks using cattle-tracing data

alone did not adequately capture the disease dynamics and it was suggested that consideration also of human contacts (i.e. farmers and farm visitors) might allow a better evaluation of strain spread (27). In our investigation we did not explore the relationship between different pets within the same household. This information, together with human contacts would eventually present a more exhaustive picture of the potential spreading dynamics of MDR staphylococci within household.

Conclusion

We did not find any evident impact of the close relationship between pet and owners on MDR carriage in people. Nevertheless, we suggest considering the intensity of the contact between pets and humans as an important component when investigating the possible exchange of MDR staphylococci between different hosts under the interdisciplinary "One Health" approach.

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References

1. Bradshaw JWS, Brown SL. Behavioural adaptations of dogs to domestication. In: Burger IH, editor. Waltham Symposium 20 - Pets, benefits and practice; Harrogate, UK: BVA Publications; 1990.

2. Steven L. Origins and domestication. In: Steven L, editor. Handbook of applied dog behavior and training - Vol 1 - Adaptation and learning. Iowa: Iowa State Press - Blackwell Publishing Company; 2000. p. 3-30.

3. Serpell JA. Domestication and history of the cat. In: Turner DC, Bateson P, editors. The domestic cat: the biology of its behaviour, 2nd edition. Cambridge, UK: Cambridge University Press; 2000. p. 179-92.

4. Société pour l'alimentation des animaux familiers. http://www.vhn.ch/zahlen-heimtiermarkt.php.

5. Serpell JA. Beneficial effects of pet ownership on some aspects of human health and behaviour. J R Soc Med. 1991;84:717-20.

6. Katcher AH, Friedmann E. Potential health value of pet ownership. Compendium of continuing education practice veterinarian. 1980;2:117-21.

7. Serpell JA. Evidence for long term effects of pet ownership on human health. In: Burger IH, editor. Waltham Symposium 20 - Pets, benefits and practice; Harrogate, UK: BVA Publications; 1990.

8. Friedmann E. The value of pets for health and recovery. In: Burger IH, editor. Waltham Symposium 20 - Pets, benefits and practice; Harrogate, UK: BVA Publications; 1990.

9. Cutt H, Giles-Corti B, Knuiman M, Timperio A, Bull F. Understanding dog owners' increased levels of physical activity: results from RESIDE. Am J Public Health. 2008;98:66-9.

10. Damborg P, Top J, Hendrickx AP, Dawson S, Willems RJ, Guardabassi L. Dogs are a reservoir of ampicillin-resistant *Enterococcus faecium* lineages associated with human infections. Appl Environ Microbiol. 2009;75:2360-5.

11. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

12. Loeffler A, Pfeiffer DU, Lloyd DH, Smith H, Soares-Magalhaes R, Lindsay JA. Meticillin-resistant *Staphylococcus aureus* carriage in UK veterinary staff and owners of infected pets: new risk groups. J Hosp Infect. 2010;74:282-8.

13. Loeffler A, Lloyd DH. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect. 2010;138:595-605.

14. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, et al. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol. 2006;115:148-55.

15. Kempker R, Mangalat D, Kongphet-Tran T, Eaton M. Beware of the pet dog: a case of *Staphylococcus intermedius* infection. Am J Med Sci. 2009;338:425-7.

16. Simoons-Smit AM, Savelkoul PHM, Stoof J, Starink TM, Vandenbroucke-Grauls CMJ. Transmission of *Staphylococcus aureus* between humans and domestic animals in a household. Eur J Clin Microbiol Infect Dis. 2000;19:150-2.

17. Hanselman BA, Kruth SA, Rousseau J, Weese JS. Coagulase positive staphylococcal colonization of humans and their household pets. Can Vet J. 2009;50:954-8.

18. Buckley M. Antibiotic resistance: an ecological perspective on an old problem. Colloquium on antibiotic resistance; Annecy, France: A report from the american academy of microbiology; 2009.

19. Decristophoris P, Fasola A, Benagli C, Tonolla M, Petrini O. Identification of *Staphylococcus intermedius* Group by MALDI-TOF MS. Syst Appl Microbiol. 2011;34:45-51.

20. Mellmann A, Becker K, von Eiff C, Keckevoet U, Schumann P, Harmsen D. Sequencing and staphylococci identification. Emerg Infect Dis. 2006;12:333-6.

21. NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Second edition M 31-A2. 2004;22.

22. CLSI. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement M 100-S19. 2010;29.

23. Couto I, Sanches IS, Sa-Leao R, de Lencastre H. Molecular characterization of *Staphylococcus sciuri* strains isolated from humans. J Clin Microbiol. 2000;38:1136-43.

24. Cotter L, Lynch M, Cryan B, Greer P, Fanning S. Investigation of a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak in an Irish hospital: triplex PCR and DNA amplification fingerprinting. J Hosp Infect. 1997;36:37-47.

25. Decristophoris P, Regula G, Petrini O, Zinsstag J, Schelling E. Prevalence and risk factors for carriage of multi-drug resistant staphylococci in healthy cats and dogs. In: Epidemiology of multi-drug resistant staphylococci in cats, dogs and people in Switzerland. PhD thesis, University Basel 2011.

26. Decristophoris P, De Benedetti A, Petignat C, Attinger M, Guillaume J, Fiebig L, et al. Evaluation of pet contact as a risk factor for carriage of multi-drug resistant staphylococci in nursing home residents. Am J Infect Control. 2011; doi:10.1016/j.ajic.2011.04.007.

27. Alvarez LG, Webb CR, Holmes MA. A novel field-based approach to validate the use of network models for disease spread between dairy herds. Epidemiol Infect. 2011;139:1863-74.

8. Research paper 4

Antibiotic treatments of a methicillin-resistant *Staphylococcus pseudintermedius* infection in a dog: a case presentation

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Summary

We report the antibiotic treatments administered to a female dog with mastitis and successive pyoderma. Microbiological investigations allowed the identification of *Staphylococcus pseudintermedius* after 54 days of various antibiotic treatments. The isolate carried the *mecA* gene and was resistant to 9 out of 15 tested antibiotics. Consistent antibiotic treatment of the infection was possible only after an accurate microbiological diagnosis.

Key words: pyoderma, microbiological investigation, *Staphylococcus pseudintermedius*, antibiogram, antibiotic treatment

Antibiotische Behandlungen einer Methicillin-resistenten *Staphylococcus pseudintermedius* Infektion bei einem Hund: eine Fallvorstellung

Wir berichten über die antibiotischen Behandlungen einer Hündin mit Mastitis und folgender Pyodermie. Die mikrobiologische Untersuchung erlaubte die Identifizierung von *Staphylococcus pseudintermedius* nach 54 Tagen mit verschiedenen antibiotischen Behandlungen. Der isolierte Stamm beinhaltete das *mecA*-Gen und war gegen 9 der 15 getesteten Antibiotika resistent. Eine angemessene antibiotische Behandlung der Infektion war erst nach einer genauen mikrobiologischen Diagnose möglich.

Schlüsselwörter: Pyodermie, mikrobiologische Untersuchung, *Staphylococcus pseudintermedius*, Antibiogramm, antibiotische Behandlung

Introduction

Staphylococcus pseudintermedius is an opportunistic pathogen of various animal species, particularly dogs and cats, and causes skin and soft tissue infections (1). This pathogen was first described in 2005 from four clinical specimens from a cat, a dog, a horse and a parrot (2). Phylogenetic analyses showed that this was not a new emerging species among dogs, but rather a misidentified biotype of *Staphylococcus intermedius* (3). Phenotypic identification methods can lead to incorrect identification of *S. pseudintermedius*, whereas molecular methods (e.g. sequencing of the partial *hsp60* gene, MALDI-TOF MS) allow reliable identification of this species (3, 4). Methicillin resistance, encoded by the *mecA* gene, was described in *S. pseudintermedius* and multidrug resistance has been reported with increasing frequency in veterinary settings (5, 6). This is of concern for the treatment of animal diseases and may carry potential public health consequences (7). To effectively manage antibiotic resistant bacteria and subsequent infections, it is mandatory to prevent inappropriate use of drugs and to improve the rapid prescription of appropriate antibiotics to a patient (8). We report the antibiotic treatments administered to a female dog before an infection by *S. pseudintermedius* had been diagnosed and a consistent antibiotic therapy was administered to the dog.

Case history

A 5-year-old, mixed breed, female dog with an ongoing mastitis was brought to an Italian veterinary practice. The dog had an ovariectomy 3 months before the visit because of recurrent pseudopregnancies. For treatment of mastitis the antibiotic first choosen consisted of Synulox (amoxicillin-clavulanic acid, 14.3 mg/kg bw and 3.6 mg/kg bw respectively, bid for 7 days) together with Stomorgyl (spiramycin-metronidazole, 17.9 mg/kg bw, sid for 7 days). Due to the lack of efficacy, treatment with these antibiotics was stopped and the dog was given Baytril (enrofloxacin, 10.7 mg/kg bw, sid orally for 2 days) and Rocefin (ceftriaxone, sc, 35.7 mg/kg bw,

sid for 31 days). At that stage the dog weighed 14 kg, was anaemic with a haematocrit of 31.9 % (37-55 %), had raised liver values (AP = 472 U/L; 23-212 U/L) and was febrile (40.8°C). A complete blood cell count (CBC) revealed a leukocytosis of 23.47 K/µL (5.50-16.90 K/µL) characterized by a neutrophilia of 18.56 K/µL (2.00-12.00 K/µL) and a monocytosis of 3.55 K/µL (0.30-2.00 K/µL).

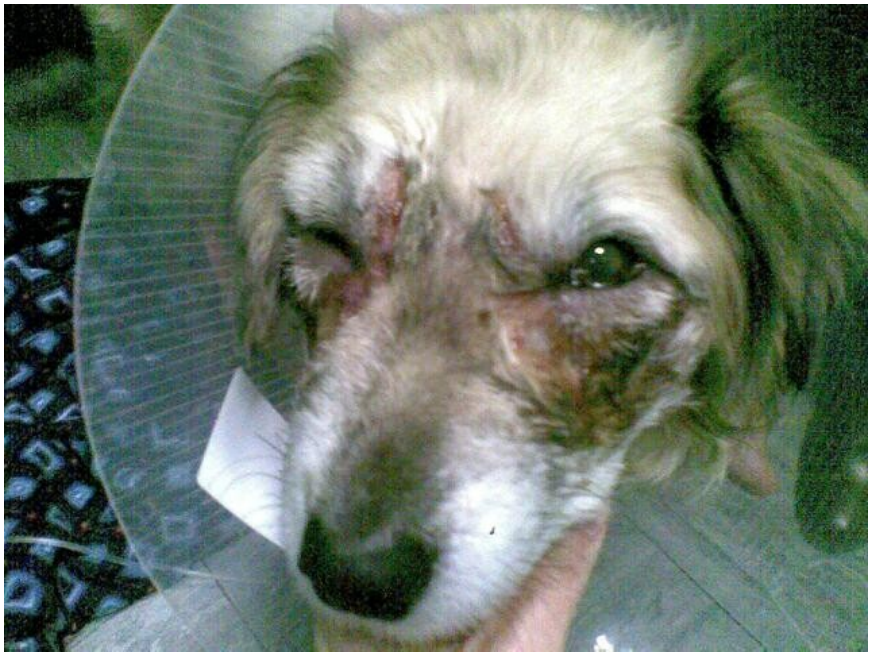

Figure 1. Pyoderma with exudates and blood appearing around the occipital bone.

Enrofloxacin was stopped after 2 days and treatment was continued with ceftriaxone (sc, 35.7 mg/kg bw, sid) alone. Skin lesions with purulent and sanguineous exudates appeared on the back and around the occipital bone (Fig. 1). An Elizabethan collar was used to hinder scratching, because the dog was very pruritic. Diagnosis of deep pyoderma was made on the basis of the clinical appearance of the skin, but no skin tests were carried out. Due to the lack of efficacy of ceftriaxone alone, Antirobe (clindamycin; 10.7 mg/kg bw, bid for 5 days) was added to the ongoing treatment together with methylprednisolone tablets (0.3 mg/kg bw, bid for 30 days) to control the pruritus. The mastitis resolved but the pyoderma persisted and the owner decided to bring the dog to another

veterinary clinic, where clindamycin treatment was stopped and the antifungal Sporanox (itraconazole, 7.1 mg/kg bw, sid for 5 days) was added to the ongoing ceftriaxone and corticosteroid treatment; also in this case no skin scraping or other cytological tests were performed. Pyoderma persisted and blood analysis revealed anaemia with a haematocrit of 24.5% and Hb = 9.8 g/dL (12.0-18.0 g/dL) and eosinophilia (2.51 K/µL; 0.10-1.49 K/µL), together with elevated liver enzyme values (ALT = 187 U/L, 10-100 UL; AP value = 2400 U/L). Ceftriaxone treatment resulted in no skin improvement, therefore after 31 days of treatment the owner went to a third veterinary clinic where the drug was replaced by doxycycline (7.1 mg/kg bw, sid for 9 days). Progressive decreasing methylprednisolone doses and treatment with doxycycline were continued with no significant improvement of the skin, leading to the decision to carry out a microbiological analysis. A sterile swab (Amies agar gel 108C; Copan, Italy) was taken directly from the eyebrow arch lesion, plated out on blood agar with nalidixic acid within 24h after collection, and incubated during 24h at 35°C.

Bacteriology

Heavy growth of Gram positive haemolytic, catalase positive cocci was observed. The strain was identified as *Staphylococcus aureus* by RapiDEC Staph biochemical tests (bioMérieux® SA, France), but this result was not confirmed by latex agglutination test (Pastorex®; Bio-Rad, France). The strain was eventually identified as *S. pseudintermedius* by MALDI-TOF MS analysis (matrix assisted laser desorption ionisation – time of flight mass spectrometry) with a confidence of 99.9% (4). MALDI-TOF MS produces a fingerprint spectrum of peptides and proteins of the analyzed microorganism. The diagnosis was later confirmed by sequencing of the partial *hsp60* gene (9). According to the disk diffusion method (Clinical and Laboratory Standards Institute, previously NCCLS guidelines) (10), the isolated methicillin-resistant *S. pseudintermedius* (MRSP) was phenotypically resistant to 9 antibiotics of 15 tested, but sensitive to minocycline, a long acting tetracycline (minimal inhibitory concentration assessed by E-test method: 0.19 µg/mL) (Tab. 1).

Table 1. Phenotypic antibiotic test with the Kirby-Bauer method.

Antibiotic	Phenotypic behaviour	Measured diameters (mm)	Clinical breakpoints (mm)		Possible therapeutic options after (8, 11)
			S	R	Lyold 2010 and Weese 2006
Oxacillin	R [1]	6	≥18	≤17	No
Tobramycin	R	11	≥15	≤12	No
Doxycycline	R	8.5	≥16	≤12	No
Chloramphenicol	R	6	≥18	≤12	No
Ciprofloxacin	R	6	≥21	≤15	No
Clindamycin	R	6	≥21	≤14	No
Erythromycin	R	6	≥23	≤13	No
Fusidic acid	R	18	≥20	≤19	No
Trimethoprim-sulfamethoxazole	R	6	≥16	≤10	No
Minocycline	S	0.19 *	≤4 **	≥16 **	First line
Rifampicin	S	31.5	≥20	≤16	restricted
Linezolid	S	26.5	≥21	-	restricted
Quinupristin-dalfopristin	S	21	≥19	≤15	restricted
Mupirocin	S	22	≥14	≤13	restricted
Vancomycin	S	16	≥15	≤14	restricted

[1] when resistant to oxacillin and presenting the *mecA* gene, the strain is considered resistant also to all other beta-lactams (i.e. penicillins, cephalosporins and carbapenems). Cefoxitin breakpoints are not predictive of *mecA*-mediated resistance to methicillin/oxacillin in *Staphylococcus pseudintermedius* (12). R = resistant, S = susceptible

[*] in µg/mL

[**] human interpretative criteria of the E-test for the assessment of the minimal inhibitory concentration (MIC) measured in µg/mL

Oxacillin susceptibility test, used to predict *mecA*-mediated resistance in *S. pseudintermedius*, was evaluated according to Bemis and co-workers (13). The *mecA* gene responsible for methicillin resistance was detected by PCR (14). Minocycline (14.29 mg/kg bw, bid orally for 7 days) was administered based on dosage recommendation of CliniPharm/Clinitox-Datenbanken from the Institute of Veterinary Pharmacology and Toxicology, Zürich (www.vetpharm.uzh.ch). The dog's general clinical condition improved, the areas of pyoderma decreased in size and the sanguineous secretions diminished. Leukocytosis disappeared (11.18 k/µL). Topical treatment with a cream

containing allicin, a garlic extract, was administered to help healing of the lesions. Despite of their improvement, two weeks later, the dog had a febrile attack which was clinically traced back to the *S. pseudintermedius* and the dog was therefore given rifampicin (21.4 mg/kg bw, sid orally for 7 days). After the administration of this antibiotic the pyoderma continued to improve, the liver values returned to the normal range (ALT = 33 U/L) and the fever disappeared. Since the treatment, the animal has been suffering from an undiagnosed articular pain.

Discussion

Infections due to *S. pseudintermedius* and MRSP have been reported with increased frequency in dogs since the species was described in 2005. *S. pseudintermedius* was isolated as colonising agent from the ear, perineum and nasal mucosae of both healthy dogs and dogs with atopic dermatitis (15). Moreover, MRSP was isolated as urinary tract infection agent also in cats (5), and *S. pseudintermedius* infections in humans were documented (16, 17). Lack of the use of molecular identification methods might lead to incorrect characterisation of *S. pseudintermedius*. The RapiDEC Staph is a test based on biochemical reactions for the identification of the main staphylococci isolated from human specimens (*S. aureus, S. epidermidis* and *S. saprophyticus*) but also for the presumptive identification of *S. intermedius*, a staphylococcal species of animal origin. The use of RapiDEC Staph for the diagnosis of the dog isolate led to incorrect identification of the recovered microorganism. This observation suggest that the use of this phenotypic test can entail misidentification of *S. pseudintermedius*, because this species shares many phenotypic characteristics and mechanisms of antibiotic resistance with *S. aureus* (2, 6).

The described clinical manifestations due to *S. pseudintermedius* in pets mainly consist of pyoderma and skin infections (18). Here we report a MRSP strain isolated from a pyoderma lesion in a female dog previously affected by mastitis. The origin of the infection is unclear. The dog might have contracted the MRSP infection during the ovariectomy. Correspondingly, cases of

nosocomial wound infections are reported for methicillin-resistant *S. aureus* (MRSA) in humans after surgery (19). On the other hand, it is also possible that the dog carried the *S. pseudintermedius* strain as part of its normal skin bacterial community, so that the multi-drug resistant strain was selected by the antibiotics. Humans and other in-contact pets could also have been a source of MRSP transmission to the dog, but this possibility was not further explored.

The eosinophilia present in the blood could have been an indicator for an allergic or parasitic origin particularly because of the pruritus observed. Skin scraping for parasites can be easily carried out in any practice together with a Fungassay to rule out any fungal infections, as well as an impression smears to confirm diagnosis by showing intracytoplasmic bacteria on cytological examination. If underling systemic disease is suspected, a full blood workup has to be done taking care to include also differential count of the WBC and eventually also a skin biopsy. These clinical diagnostic methods should have been applied also in the described case before the administration of different antibiotics. In any case bacterial culture and sensitivity test should be carried out if antibiotic treatment does not yield the expected results.

Treatment of MRSP is critical because of its resistance to all beta-lactams and, often, other classes of antibiotics. As for the antibiotic therapy of other bacterial infections, the administration of antibiotics until completion of full treatment is of primary importance. An effective antimicrobial therapy depends on several parameters such as bacterial susceptibility, pharmacokinetic characteristics of the drug, and dosage regime. Lack of treatment completion or prolonged antibiotics use may result in selection for resistant strains (20). Delayed or inadequate prescriptions can reduce the efficacy of treatment and favour the spread of the infection, both in human and in veterinary medicine (21). As in human medicine, treatment of animal infections should rely on a stepwise approach that includes successive use of first, second and third line antibiotics (8). In this case, the first therapy consisted of amoxicillin-clavulanic acid and spiramycin-metronidazole. Due to lack of efficacy of this empiric approach, enrofloxacin was administered together with

ceftriaxone. These drugs, however, belong to antibiotic classes that are usually employed as third line treatment in human medicine: thus, they should be used in animals only for a limited number of cases, i.e. where all other antibiotics fail (22). Minocycline is a semi-synthetic, long acting tetracycline which has been suggested to be effective against staphylococci resistant to semisynthetic penicillins and cephalosporins (23). In Japan, minocycline is commonly used in veterinary dermatological practices (24), whereas in other countries, such as Switzerland, drugs containing this antimicrobial principle are not approved for use in animals (www.vetpharm.uzh.ch).

If the infection persists after a first line empiric treatment, the choice of a more specific, active drug to be administered should rely on an accurate microbiological analysis and antibiogram, which allow to evaluate the susceptibility of the pathogen against different drugs (8). In the described case, the antibiotic treatment of pyoderma included the administration of six different antibiotics, one corticosteroid and one antifungal agent, during a total of 54 days, before a microbiological investigation was eventually carried out. The microbiological analysis allowed determination of the antibiotic susceptibility profile of the MRSP strain within 48 h.

Possible therapeutic options after failure of the treatment with minocycline and successive relapse were limited (Tab. 1). These consisted of antibiotic agents for which the use in veterinary medicine should be restricted to life-threatening infections, when culture and susceptibility testing indicate no other options (8). The dog was treated with rifampicin, even if this drug is rarely used in dogs, at the recommended doses of 10-15 mg/kg orally sid. This is a bactericidal staphylococcal agent which inhibits the bacterial RNA polymerase activity resulting in a block of the protein synthesis. Normally, with deep pyoderma antibiotic treatment is given for at least six weeks, extended to no less than two weeks after clinical resolution. However as rifampicin is very hepatotoxic and plasma liver enzymes should be monitored weekly, the veterinary surgeon preferred to give a higher dose for only seven days. Topical treatment was hardly used in this case; only an ointment containing allicin was applied toward to the end of the treatment, although it is very useful in the treatment of

pyoderma. It helps removing debris and bacteria and favours drainage of exudative and deep lesions. There are many products on the market, such as soaks with chlorhexidine or iodine and special shampoos containing benzoyl peroxide or ethyl lactate. Creams tend to be used for localised lesions. In this case the frequent change of veterinary practices did not permit a continuity of the dog follow up and this had a deleterious effect on the outcome.

Conclusion

This clinical case emphasizes the importance of a rapid and accurate microbiological diagnosis, based on the identification of the pathogen and an antibiogram for an effective treatment of severe cutaneous infections in dogs, especially when an empiric first line treatment is not successful. This is particularly important in the case of infections that might result from microorganisms such as *S. pseudintermedius*, known to develop multidrug resistance (7).

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References

1. Bannoehr J, Ben Zakour NL, Waller AS, Guardabassi L, Thoday KL, van den Broek AHM, et al. Population genetic structure of the *Staphylococcus intermedius* Group: Insights into agr diversification and the emergence of methicillin-resistant strains. J Bacteriol. 2007;189:8685-92.

2. Devriese LA, Vancanneyt M, Baele M, Vaneechoutte M, De Graef E, Snauwaert C, et al. *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int J Syst Evol Micr. 2005;55:1569-73.

3. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. J Clin Microbiol. 2007;45:2770-8.

4. Decristophoris P, Fasola A, Benagli C, Tonolla M, Petrini O. Identification of *Staphylococcus intermedius* Group by MALDI-TOF MS. Syst Appl Microbiol. 2011;34:45-51.

5. Wettstein K, Descloux S, Rossano A, Perreten V. Emergence of methicillin-resistant *Staphylococcus pseudintermedius* in Switzerland: three cases of urinary tract infections in cats. Schweiz Arch Tierheilkd. 2008;150:339-43.

6. Weese JS, van Duijkeren E. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudointermedius* in veterinary medicine. Vet Microbiol. 2010;140:418-29.

7. Cohn LA, Middleton JR. A veterinary perspective on methicillin-resistant staphylococci. J Vet Emerg Crit Care. 2010;20:31-45.

8. Lloyd D. Recognising and controlling risk factors for antimicrobial resistance. Schweiz Arch Tierheilkd. 2010;152:131-4.

9. Kwok AYC, Su SC, Reynolds RP, Bay SJ, Av-Gay Y, Dovichi NJ, et al. Species identification and phylogenetic relationships based on partial HSP60 gene sequences within the genus *Staphylococcus*. Int J Syst Evol Micr. 1999;49:1181-92.

10. NCCLS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Second edition M 31-A2. 2004;22.

11. Weese JS. Prudent use of antimicrobials. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM, editors. Antimicrobial therapy in veterinary medicine. Iowa: Blackwell Publishing; 2006. p. 437-48.

12. Papich MG. Proposed changes to Clinical Laboratory Standards Institute interpretive criteria for methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs. J Vet Diagn Invest. 2010;22:160.

13. Bemis DA, Jones RD, Frank LA, Kania SA. Evaluation of susceptibility test breakpoints used to predict mecA-mediated resistance in *Staphylococcus pseudintermedius* isolated from dogs. J Vet Diagn Invest. 2009;21:53-8.

14. Cotter L, Lynch M, Cryan B, Greer P, Fanning S. Investigation of a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak in an Irish hospital: triplex PCR and DNA amplification fingerprinting. J Hosp Infect. 1997;36:37-47.

15. Fazakerley J, Nuttall T, Sales D, Schmidt V, Carter SD, Hart CA, et al. Staphylococcal colonization of mucosal and lesional skin sites in atopic and healthy dogs. Vet Dermatol. 2009;20:179-84.

16. Van Hoovels L, Vankeerberghen A, Boel A, Van Vaerenbergh K, De Beenhouwer H. First case of *Staphylococcus pseudintermedius* infection in a human. J Clin Microbiol. 2006;44:4609-12.

17. Chuang CY, Yang YL, Hsueh PR, Lee PI. Catheter-related bacteremia caused by *Staphylococcus pseudintermedius* refractory to antibiotic-lock therapy in a hemophilic child with dog exposure. J Clin Microbiol. 2010;48:1497-8.

18. Vincze S, Paasch A, Walther B, Ruscher C, Lubke-Becker A, Wieler LH, et al. Multidrug- and methicillin resistant *Staphylococcus pseudintermedius* as a cause of canine pyoderma: a case report. Berl Munch Tierarztl Wochenschr. 2010;123:353-8.

19. Sisirak M, Zvizdic A, Hukic M. Methicillin-resistant *Staphylococcus aureus* (MRSA) as a cause of nosocomial wound infections. Bosn J Basic Med Sci. 2010;10:32-7.

20. Prescott JF. Antimicrobial drug resistance and its epidemiology. In: Prescott JF, Baggot JD, Walker RD, editors. Antimicrobial therapy in veterinary medicine. 3th ed. Iowa: Blackwell Publishing Professional; 2000. p. 27-49.

21. Buckley M. Antibiotic resistance: an ecological perspective on an old problem. Colloquium on antibiotic resistance; Annecy, France: A report from the american academy of microbiology; 2009.

22. Ungemach FR, Muller-Bahrdt D, Abraham G. Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. Int J Med Microbiol. 2006;296:S33-8.

23. Minuth JN, Holmes TM, Musher DM. Activity of tetracycline, doxycycline, and minocycline against methicillin-susceptible and -resistant staphylococci. Antimicrob Agents Chemother. 1974;6:411-4.

24. Kawakami T, Shibata S, Murayama N, Nagata M, Nishifuji K, Iwasaki T, et al. Antimicrobial susceptibility and methicillin resistance in *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* subsp. *coagulans* isolated from dogs with pyoderma in Japan. J Vet Med Sci. 2010;72:1615-9.

9. Discussion and conclusion

9.1. The inter- and multidisciplinary approach

Resistance to antibiotics is a multifactorial, complex problem, with the occurrence of several factors (e.g. antibiotic administration, the success in the transmission of specific resistant clones and the fitness cost related to resistance mechanisms) affecting its evolution in time and space. Multi-drug resistance affects human, animal and environmental health, thus asking for a multi-faceted approach towards understanding and management of resistance evolution.

I believe that the "One Health" interdisciplinary approach, with investigations at the interface between veterinary and human medicine, will be helpful to understand the role of pets in the spread of antibiotic resistance, considering the socio-emotional context of the human-pet relationship. I tried, therefore, to use a multidisciplinary approach in my work and I explored the complexity of the problem at several levels. I first examined the diagnostic and microbiological perspective, studying then the risk factors for epidemiological spread of multi-drug resistant (MDR) bacteria and the behavioural characteristics of the relationship between human and pets, to finish with a study on the clinical implications of antibiotic resistance.

9.2. Overview of findings

Matrix assisted laser desorption ionisation – time of flight mass spectrometry (MALDI-TOF MS) allowed rapid and reliable identification of staphylococcal species also for phylogenetically close related taxa such as *S. delphini, S. intermedius, S. pseudintermedius* (**REARCH PAPER 1)**. The analysis of the staphylococcal population composition of healthy cats and dogs revealed that *S. pseudintermedius* was present in 27 % of healthy dogs and 3 % of healthy cats, whereas *S. felis* was isolated only from cats and represented 31 % of their CNS isolates. About 17 % of the pets carried at least one MDR *Staphylococcus* spp. strain. Previous hospitalisation (stay in a veterinary

clinic during at least one night) was identified as a risk factor for the carriage of MDR staphylococci in nostrils and ears of cats and dogs (**RESEARCH PAPER 2).** Although a relevant proportion of pets and nursing home residents carried MDR staphylococci, people had no increased risk of being carriers of MDR strains when living in homes with pets or having contact with them at least once a week compared to people living in homes without pets (**RESEARCH PAPER 3**). Our findings on MDR staphylococcal carriage in residents living with pets suggested that strain transmission between pets and humans within nursing homes is limited. Two genetically identical strains of methicillin-resistant *S. epidermidis* were isolated from a nostril of a nursing resident and a cat with which the resident had contact once a week; however it was not possible to infer the origin and direction of the possible strain exchange (**RESEARCH PAPER 3**). Physical closeness of pets with their owners was very frequent in the household, also indicating the emotional importance of this relationship, but we did not find any evident impact of pets on carriage of MDR staphylococci in their owners (**WORKING PAPER 1**). Clinical implications of methicillin-resistant *S. pseudintermedius* (MRSP) infection were then discussed in **RESEARCH PEPER 4**, using as an example the isolation of *S. pseudintermedius* from a pyoderma lesion in a dog that underwent various antibiotic treatments before the correct diagnosis was made and an appropriate antibiotic treatment was administered**.**

9.3. Contact to pets and risk of transmission of staphylococci

The emotional importance of pets for Swiss people was evident during the whole investigation. People and pets had a close and intense interaction within their household; in nursing homes the physical closeness between residents and pets was, as a general rule, less strong. This suggests different probabilities of microorganism transmission between the two settings.

I did not observe any increased carriage of MDR staphylococci in people due to pet contact in nursing homes or in the households. This might stem from the fact that nursing home residents,

basically more susceptible to get MDR staphylococci because often immunosuppressed, had less intense physical contact to pet and thus a low probability to acquire staphylococci from the animals. People in the community, on the average, had intense physical contact with their pets, but, being immunocompetent, their chances of acquisition of MDR staphylococci from their companions are presumably not very high.

9.4. Identification of staphylococci

My work benefited from the use of rapid, accurate and relatively inexpensive microorganism identification by MALDI-TOF MS. The correct and exhaustive identification of both coagulase-positive and coagulase-negative staphylococci was a pre-requisite to understand the distribution of *Staphylococcus* spp. in people and pets. Our results suggested the presence of host-specific staphylococcal species, with *S. pseudintermedius* being the most prevalent species in dogs, *S. felis* in cats and *S. epidermidis* in humans. This information, coupled with typing methods (e.g. pulsed field gel electrophoresis, multilocus sequence typing) might be of value also in future works dealing with the direction of possible exchange of strains among the different hosts.

9.5. Antibiotic administration and surveillance

The correct identification of isolates is also of primary importance for the assessment of antibiotic resistance and subsequent correct antibiotic administration, as highlighted in the MRSP case report. Antibiotic treatment is known to be one of the factors influencing the acquisition of resistance in microorganisms (1). Thus, antibiotic stewardship is important to reduce the rapidity at which microorganisms develop resistance and to control the occurrence of co-selection between drugs used in different sectors (e.g. veterinary and human medicine) (2). In this context there is a need to increase the awareness of veterinarians and medical doctors on the challenge deriving from antibiotic resistance; this global challenge cannot be dealt with a sectorial approach. Joined

antibiotic surveillance programs such as the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (3) should be promoted everywhere. In Switzerland, a recent investigation highlighted the potential for synergic actions between veterinary and human medicine (4). Also in this context antibiotic resistance management would benefit from a joint surveillance program.

9.6. Fitness cost of antibiotic resistance

Fitness cost is one of the key parameters influencing establishment of antibiotic resistance (5). I reported MDR in staphylococci carried by healthy cats, dogs and people. The absence of risk related to the contact with pets for the carriage of these microorganisms in nursing home residents indicated a relatively low rate of strain exchange between different hosts. This, together with the presence of host-specific staphylococcal species, suggests different fitness costs associated to the colonisation of different hosts. There is therefore a need to investigate the fitness costs of antibiotic resistance associated to the different staphylococcal species in different hosts. This would eventually allow evaluating the epidemic potential of antibiotic resistant staphylococci of different origins and understanding their real impact on human and animal health.

9.7. Management of pets in nursing homes

I did not observe any increased risk of MDR staphylococcal carriage in nursing home residents in contact with cats and dogs; nevertheless, guidelines for a correct keeping of pets would be of importance. During sample collection, I had the impression that the decision to allow the presence of pets in nursing homes was left to the initiative of individuals, with each nursing home taking up independent decisions. To my knowledge, so far no official guidelines have been developed at the Federal or Cantonal level. Better awareness of benefits and risks linked to the presence of pets in nursing home is pivotal to help administrations in taking informed decisions on the opportunity and

methods to keep pets in the institutions. Allergy to pets should also be taken into account, for example by leaving "pet-free" areas for allergic residents.

9.8. Conclusions

MDR staphylococci were recovered in relevant proportions from healthy pets and people in nursing homes and in the community. I documented the potential for exchange of strains as a consequence of close physical contact between the different hosts. My results, however, indicated negligible rates of MDR staphylococcal transmission between human and pets. I also document the importance of considering different antibiotic resistances (not merely methicillin resistance), all staphylococcal species (not just coagulase-positive) and the socio-cultural context of the study when investigating the possible exchange of staphylococcal strains between different hosts.

Future studies are now needed to assess the public health impact of MDR in people and in pets in other contexts by combining, under the "One Health" approach, investigations on fitness costs of antibiotic resistance in different staphylococcal species recovered from different hosts and modelling of multi-drug resistance carriage data within contact networks.

9.9. References

1. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10:S122-9.

2. Andersson DI, Hughes D. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol. 2010;8:260-71.

3. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). http://www.phac-aspc.gc.ca/cipars-picra/index-eng.php.

4. Meisser A. One Health in der Schweiz: Ein visionäres Konzept am Scheideweg? Davos: Master Thesis of Public Health Universitäten Basel, Bern und Zürich; 2010.

5. Andersson DI. The biological cost of mutational antibiotic resistance: any practical conclusions? Curr Opin Microbiol. 2006;9:461-5.

10. Appendices

10.1. Posters

During the 3-years of my PhD thesis I had the opportunity to attend national and international meetings and conferences were I could present posters on the progresses of my work and to exchange opinions on my PhD project with researchers in the field of microbiology, antimicrobial resistance and public health.

In particular I attended and I presented posters at the following conferences:

- Sympo Staph, Lyon, France, 13th-15th of October 2008 (**POSTER 1**)

- ASM (American Society of Microbiology) Conference – Antimicrobial resistance in zoonotic bacteria and foodborne pathogens in animals, humans and the environment, Toronto, Canada, 8th-11th of June 2010 (**POSTER 2** and **POSTER 3**)

- Annual Congress of the SSM (Swiss Society of Microbiology), Zürich, Switzerland, 24th-25th of June 2010 (**POSTER 4**)

- EcoHealth 2010 – Global Ecohealth Challenges; Multiple Perspectives, London, United Kingdom, 18th-20th of August 2010 (**POSTER 5**)

10.1.1. POSTER 1 - Portage de staphylocoque antibiorésistants chez les chiens et chats présents dans des établissements de long séjour – étude pilote

Presented at the: Sympo Staph, Lyon, France, 13th-15th of October 2008

By: P. Decristophoris, E. Papin, G. Regula, O. Petrini

Portage de staphylocoques antibiorésistants chez les chiens et chats présents dans des établissements de long séjour – étude pilote

[1] Decristophoris P. M. A., [2] Papin E., [2] Regula G., [1] Petrini O.

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Bundesamt für Veterinärwesen BVET
Office vétérinaire fédéral OVF
Ufficio federale di veterinaria UFV

Introduction

Chiens et chats sont employés de plus en plus dans la thérapie des personnes âgées ou malades chroniques résidents dans des établissements médico-sociaux (EMS).

- ***Résidents des EMS***: personnes immunosupprimées exposées à un risque potentiel d'infections par des bactéries multirésistantes aux antibiotiques;
- ***EMS***: jusqu'à 10% des résidents porteurs de MRSA (Méthicillin Resistant *Staphylococcus Aureus*);
- ***Chats et chiens***: porteurs de staphylocoques qui peuvent développer des résistances vis – à – vis de divers types d'antibiotiques.

Animaux décrits comme potentiels réservoirs pour les bactéries résistantes aux antibiotiques.

But

Déterminer le taux de portage de staphylocoques résistants aux antibiotiques chez les chats et chiens présents dans des EMS.

Méthode

Population source: chats et chiens dans EMS des Cantons Bern et Tessin, Suisse (chats n=22; chiens n=21).

Écouvillons ouatés: au nez (une seule narine) et à l'oreille (une seule oreille) de chaque animal (Amies agar gel 108C et 110C, Copan) humidifiés avec NaCl 0.9%, analysés dans les 48h.

Questionnaire: récolte d'informations générales, de permanence dans EMS et sur la santé de l'animal.

Analyse bactériologique:

Isolement de Staphylococcus spp.
Lecture après 48h: milieu Chapman (Mannitol Salt Agar, MSA2, BioMérieux) et milieu chromogène pour *S. aureus* (SAID, BioMérieux) avec enrichissement au préalable dans bouillon MRSA;

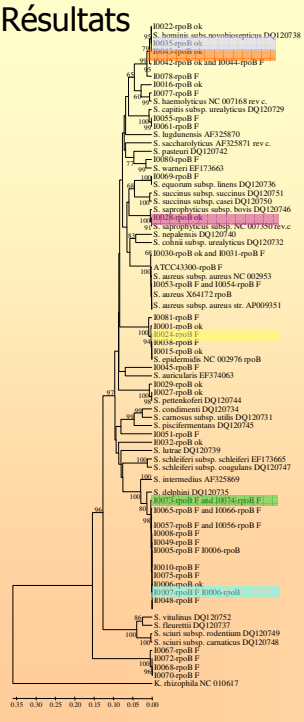
Identification de Staphylococcus spp.
Coloration de Gram, test catalase, fermentation du mannitol pour *S. aureus* (sur milieu Chapman);
PCR gène *rpoB* (primers 10µM: *Staph rpoB 1418f* et *Staph rpoB 3554r*; 5' 94°C, 35x 45'' 94°C / 1' 52°C / 1'30'' 72°C, 10' 72°C, ∞ 4°C) et séquençage partiel du gène *rpoB* (primers 1µM: *Staph ropB 1418f* et *Staph rpoB 1975rs*; 1' 96°C, 25x 10'' 96°C / 5'' 50°C / 4' 60°C, ∞ 4°C).

Profil de résistance antibiotique:

Diffusion sur plaque Mueller-Hinton agar sang (24 antibiotiques testés). Lecture selon NCCLS avec mesure du diamètre de diffusion et classification des souches comme "résistant", "intermédiaire", "sensible";

Détection gène *mecA* par PCR (primers 10µM: *mecA L* et *mecA R*; 15' 94°C, 30x 1' 94°C / 1'30'' 55°C / 1'30'' 72°C, 10' 72°C, ∞ 4°C).

Résultats



Staphylocoques isolés (n=34) chez les chats et chiens dans EMS des Cantons Berne et Tessin (Suisse).

Arbre UPGMA sur le fragment de 372 pb du gène *rpoB* (Nucleotide Maximum Composite Likelihood; les valeur de bootstrap pour 1 000 réplications sont reportés sur les noeuds).

Portage de *Staphylococcus* spp.: - Chiens 12/21 (57%)
- Chats 9/22 (41%)

dont *Staphylococcus aureus*: - Chiens 0/21 (0%)
- Chats 2/22 (9%)

Isolats résistants à 4 différents principes actifs ou plus: 7/34 (20.6%).

Animaux	Résistance aux antibiotiques	Présence du gène *mecA*
Chat 1	**P; AM; OX; TE; E; SXT; AMC; CAZ; NN; FA; FOX**	**OUI**
Chat 2	**P; AM; E; FA**	NON
Chien 1	**P; AM; OX; CAZ; FA; FOX**	**OUI**
Chien 2	**P; AM; OX; FA**	NON
Chien 3	**P; AM; E; CC; C; K**	NON
Chien 4	**P; AM; TE; E; CC; SXT; K**	NON
Total n = 6	équivalent à 14% du total des animaux échantillonnés (n=43)	

Antibiotiques:

P	penicilline
AM	ampicilline
OX	oxacilline
TE	tetracycline
E	erythromycine
SXT	Bactrim
FA	acide fusidique
CC	clindamycine
C	chloramphenicol
FOX	cefoxitine
K	kanamycine
CAZ	ceftazidime
AMC	augmentine
NN	tobramycine

Cette étude pilote nous a fourni des données quant à la situation des animaux de compagnie dans les EMS vis-à-vis du portage de staphylocoques résistants aux antibiotiques. Ces informations essentielles seront prises en compte lors de la mise en place d'une prochaine étude visant à investiguer le rôle des animaux en tant que réservoir de résistances aux antibiotiques.

10.1.2. POSTER 2 - Carriage of multidrug resistant staphylococci in healthy cats and dogs in Swiss nursing homes

Presented at the: ASM (American Society of Microbiology) Conference – Antimicrobial resistance in zoonotic bacteria and foodborne pathogens in animals, humans and the environment, Toronto, Canada, 8th-11th of June 2010

By: P. Decristophoris, G. Regula, E. Schelling, J. Zinsstag, O. Petrini

Carriage of multidrug resistant staphylococci in healthy cats and dogs in Swiss nursing homes

P. Decristophoris [1,2], G. Regula [3], E. Schelling [2], J. Zinsstag [2], O. Petrini [1]

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Background

Healthy cats and dogs may carry multidrug resistant staphylococci, including methicillin resistant *S. aureus* (MRSA) [1].

Pets are increasingly used for psychological support in the therapy of chronically diseased or elderly patients [2], thus knowledge about the presence of multidrug resistant bacteria in these animals is becoming crucial.

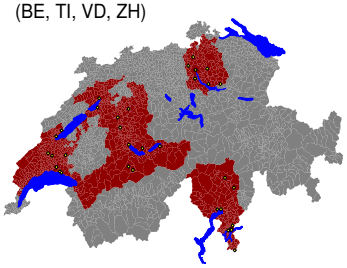
Aim

To assess the carriage of multidrug resistant staphylococci by healthy cats and dogs living in or regularly visiting nursing homes.

Methods

Pets

- 39 Swiss nursing homes (♦), randomly selected in 4 different Swiss Cantons (BE, TI, VD, ZH)



- Sampling of all cats and dogs living in or weekly visiting these homes (n=98)

	Females (n)	Males (n)	
Dogs	30	15	45 (45.9%)
Cats	34	19	53 (54.1%)
	64 (65.3%)	34 (34.7%)	98 (100%)

Demographics (n = 98)

- Age in years: 7.39 (SD ± 4.37), min 0.33, max 19.00
- Sterilized: 80.6%

Owners	%
nursing home	43.9
resident	14.3
pet-therapy	10.2
visitor	11.2
care staff	15.3
other	5.1

- 77.6 % of the pets had access to bedroom of residents
- 12.2 % visited several nursing homes
- No pets had an history of MRSA
- Antibiotic treatment in the last 3 months

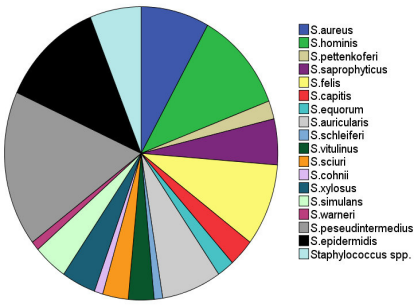
Antibiotics	%
yes	5.1
none	90.8
do not remember	3.1
n.d.	1.0

Sample collection and laboratory analyses

- Nasal and ear swabs (Amies agar gel 108C, Copan)
- Swabs plated out on mannitol salt agar (Biomérieux) within 24h after collection and enriched in MRSA broth followed by culture on Chrom ID *S. aureus* (Biomérieux)
- Isolates identified by MALDI-TOF MS
- Phenotypic antibiotic resistance assessed by disk diffusion method on Mueller-Hinton blood agar according to CLSI guidelines
- Multidrug resistance defined as resistance to at least 3 drugs of different antibiotic classes
- *mecA* gene detected by PCR

Results

Isolated *Staphylococcus spp.* (n=99)



- Multidrug resistant strains = 26.3% (26/99)
- Strains carrying *mecA* gene = 7.1% (7/99)
- Pets carrying multidrug resistant strains = 16.3% (16/98); cats n=7, dogs n= 9
- Pets carrying strains with *mecA* gene = 5.1% (5/98); cats n=3, dogs n=2
- Methicillin resistant *S. aureus* (MRSA) or *S. pseudintermedius* (MRSP) were not detected

Phenotypic antibiotic resistance (%)

R = resistant; I = intermediate; S = susceptible

Antibiotic	R (%)	I (%)	S (%)
penicillin	43.4		56.6
ampicillin	39.4		60.6
oxacillin	22.2		77.8
amoxicillin-clavulanic acid	1		99
gentamicin	1		99
kanamycin	7.1	1	91.9
tobramycin	1		99
cefazolin	1		99
ceftazidim	3	9.1	87.9
cefoxitin	8.1		91.9
tetracycline	11.1	1	87.9
doxycyclin	2	3	95
erythromycin	25.3	5	69.7
clindamycin	10.1	5.1	84.8
trimethoprin-sulfamethoxazole	5.1	1	93.9
ciprofloxacin	2	3	95
imipenem	0		100
fusidic acid	32.3		67.7
rifampicin	0		100
chloramphenicol	5.1		94.9
linezolid	0		100
quinopristin-dalfopristin	0	3	97
vancomycin	0		100
mupirocin	0		100

The emergence of multidrug resistant *Staphylococcus* spp. in pets is of concern in terms of animal and human health.

Investigations are needed to determine the risk of transmission of resistant strains between humans an animals.

References [1] O'Mahony et al. 2007. Methicillin resistant Staphylococcus aureus (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology*, 109, 285-296.
[2] Colombo et al. 2006. Pet-therapy and institutionalized elderly: a study on 144 cognitively unimpaired subjects. *Archives of Gerontology and Geriatrics*, 42, 207-216.

10.1.3. POSTER 3 - Effective antibiotic treatment in a dog with methicillin resistant *Staphylococcus pseudintermedius* infection: A case report

Presented at the: ASM (American Society of Microbiology) Conference – Antimicrobial resistance in zoonotic bacteria and foodborne pathogens in animals, humans and the environment, Toronto, Canada, 8th-11th of June 2010

By: P. Decristophoris, F. Mauri, A. Carnelli, T. Vanzetti, J. Zinsstag, O. Petrini

Effective antibiotic treatment in a dog with methicillin resistant *Staphylococcus pseudintermedius* infection: A case report

P. Decristophoris[1,2], F. Mauri[1], A. Carnelli[1], T. Vanzetti[3], J. Zinsstag[2], O. Petrini[1]

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Background

Coagulase-positive staphylococci may be opportunistic pathogens of humans and animals. Of them, *Staphylococcus pseudintermedius* is the most commonly isolated species in cats and dogs, with methicillin resistance (*mecA* gene) already documented [1]. This species may become a pathogen of skin and soft tissue infections in dogs [2].

Case report

- A 5 years old, mixed breed, female dog with an ongoing mastitis was brought to a veterinary practice. The dog had an ovariectomy 3 months before presenting to the practice.
- The first antibiotic treatment consisted of amoxicillin-clavulanic acid and spiramycin-metronidazole; due to lack of efficacy after 7 days, treatment with these antibiotics was stopped and enrofloxacin and ceftriaxone were administered to the dog (Figure 1).
- At that stage the dog was anemic with liver pain and febrile (40.8°C); pyoderma with exudates and blood appeared on the back and around the occipital bone (Figure 2).

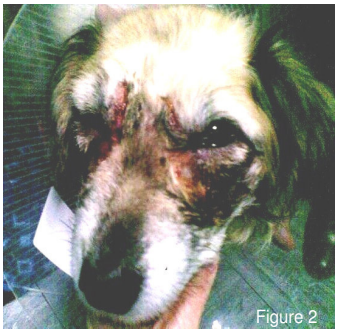
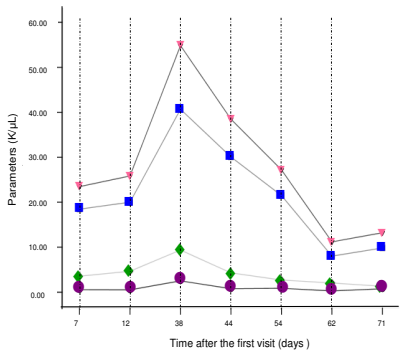


Figure 2

Figure 1. Antibiotic treatments and trends in blood parameters



▼ = leukocytes (reference range: 5.50 - 16.90 K/µL)
■ = neutrophiles (reference range: 2.00 - 12.00 K/µL)
♦ = monocytes (reference range: 0.30 - 2.00 K/µL)
● = eosinophiles (reference range: 0.10 - 1.49 K/µL)

Antibiotic treatments:
- day 7 to 12: enrofloxacin and ceftriaxone
- day 12 to 38: ceftriaxone and methylprednisolone
- day 38 to 44: ceftriaxone, clindamycin, itraconazole and methylprednisolone
- day 44 to 54: doxycycline and methylprednisolone
- day 54 to 62: minocycline

Laboratory analyses

- At day 54, a swab (Amies agar gel 108C, Copan) was taken directly from the eyebrow arch wound, plated out on blood agar-nalidixin within 24h after collection and incubated during 24h at 35°C; heavy growth of Gram positive haemolytic, catalase positive cocci was observed.
- MALDI-TOF MS analysis identified the strain as *S. pseudintermedius* (confidence level: 99.9%), identification was confirmed by partial *hsp60* gene sequencing.
- The strain harboured the *mecA* gene but not the Panton Valentine Leukocidin gene (detection by PCR).
- Phenotypic antibiotic resistance patterns were tested for 25 different drugs (Table 1). The strain was resistant to 18 out of 25 antibiotics tested.

Table 1. Resistance patterns of the strain isolated

Antibiotic	Phenotypic behaviour of the strain
Kanamycin, Gentamicin, Tobramycin	Resistant, Resistant, Resistant
Doxycycline, **Minocycline**, Tetracycline	Resistant, **Susceptible**, Resistant
Cefazolin, **Cefoxitin**, Ceftazidime, Ceftriaxone	Resistant, **Susceptible**, Resistant, Resistant
Ampicillin, Oxacillin, Amoxicillin and clavulanic acid	Resistant , Resistant, Resistant
Chloramphenicol	Resistant
Ciprofloxacin	Resistant
Imipenem	Resistant
Clindamycin	Resistant
Erythromycin	Resistant
Fusidic acid	Resistant
Linezolid	**Susceptible**
Mupirocin	**Susceptible**
Quinupristin-dalfopristin	**Susceptible**
Rifampicin	**Susceptible**
Trimethoprim-sulfamethoxazole	Resistant
Vancomycin	**Susceptible**

- Microbiological analysis allowed identification within 48h of the appropriate antibiotic treatment for the methicillin resistant *S. pseudintermedius* infection.
- An accurate microbiological diagnosis is of primary importance for the effective treatment of severe cutaneous infections in pets, particularly when previous therapy has not been successful.

References: [1] Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant Staphylococcus pseudintermedius in a veterinary teaching hospital. J Clin Microbiol. 2007;45: 1118-1125.
[2] Weese JS, Poma R, James F, Buenviaje G, Foster R, Slavic D. Staphylococcus pseudintermedius necrotizing fasciitis in a dog. Can Vet J. 2009;50: 655-656.

10.1.4. POSTER 4 - Carriage of multidrug resistant staphylococci in cats, dogs and nursing home residents of the Canton Ticino

Presented at the: Annual Congress of the SSM (Swiss Society of Microbiology), Zürich, Switzerland, 24th-25th of June 2010

By: P. Decristophoris, V. Francini, A. De Benedetti, E. Schelling, J. Zinsstag, O. Petrini

Carriage of multidrug resistant staphylococci in cats, dogs and nursing home residents of the Canton Ticino

P. Decristophoris[1,2], V. Francini[2], A. De Benedetti[3], E. Schelling[2], J. Zinsstag[2], O. Petrini[1]

[1]Istituto cantonale di microbiologia, 6500 Bellinzona, Switzerland, [2]Swiss Tropical and Publich Health Institute, 4002 Basel, Switzerland, [3]Ufficio del medico cantonale, Bellinzona, Switzerland

BACKGROUND

Staphylococci have developed resistance to several antibiotics and are a major cause of nosocomial infections. Antibiotic resistance in staphylococci has also been described in pets and transmission of these bacteria between pets and humans has been documented. Knowledge of multidrug resistant staphylococcal carriage in nursing homes where pets are used for psychological support in the therapy of chronically diseased or elderly patients is of primary importance.

AIM

Evaluation of the epidemiology of multidrug resistant staphylococci in cats, dogs and humans living in nursing homes in the Ticino Canton, Switzerland.

METHODS

- 10 nursing homes (♦), randomly selected (Figure 1)
- 58 enrolled residents; 6 residents randomly selected in each nursing home
- all animals (n= 26) living in or weekly visiting the nursing homes were included in the study

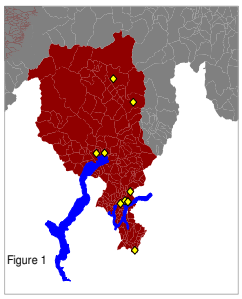


Figure 1

- nasal swabs collected from humans (Amies agar gel 108C, Copan)
- nasal and ear swabs collected from pets (Amies agar gel 108C and 110C, Copan)
- swabs plated out on mannitol salt agar (BioMérieux) within 24h after collection and enriched in MRSA broth followed by culture on Chrom ID *S. aureus* (Biomérieux)
- isolates identified by MALDI-TOF MS
- phenotypic antibiotic resistance assessed by disk diffusion method on Mueller-Hinton blood agar according to CLSI guidelines
- multidrug resistance defined as resistance to at least 3 drugs of different antibiotic classes
- *mecA* gene detected by PCR

RESULTS

Demographics

Humans (n=58)

- Male 15.5 % (9/58); Female 84.5 % (49/58)
- Age 86.3± 5.5 years, range 68 – 100
- History of MRSA 17.2% (10/58)
- Antibiotic treatment in the last 3 months 29.3% (17/58)
- 43.1% (25/58) actively in contact with pets once a week

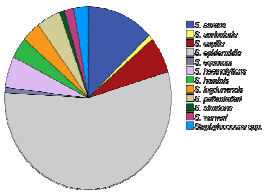
Pets (cats n=11; dogs n=15)

	Female (n)	Male (n)	
Dogs	10	5	15 (57.7%)
Cats	9	2	11 (42.3%)
	19 (73.1%)	7 (26.9%)	26(100%)

- Dogs: age 6.97±4.36 years , range 0.5 – 15
- Cats: age: 6.81±3.46 years, range 2 – 11
- No history of MRSA in both species
- Antibiotic treatment in the last 3 months 11.5 % (3/26)

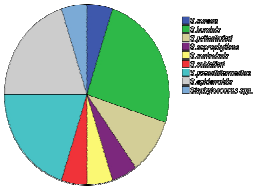
Isolated strains

humans (n = 108)



- 25% (27/108) coagulase negative staphylococcal strains carrying *mecA* gene in 22 residents;
- 34.3% (37/108) multidrug resistant staphylococcal strains;
- methicillin resistant *S. aureus* (MRSA) or *S. pseudintermedius* (MRSP) were not isolated;
- multidrug resistant *S. epidermidis* (n=2) carrying *mecA* gene and resistant against mupirocin in 2 residents.

Pets (n = 20)



- 20% (4/20) coagulase negative staphylococcal strains carrying *mecA* gene in 2 dogs and 1 cat;
- 25% (5/20) multidrug resistant coagulase negative staphylococcal strains;
- methicillin resistant *S. aureus* (MRSA) or *S. pseudintermedius* (MRSP) were not isolated;
- no strains resistant against mupirocin.

Multidrug resistant *Staphylococcus* spp. were isolated from residents and pets in nursing homes in Ticino. The emergence of multidrug resistance in these bacteria is of concern in terms of human and animal health in nursing homes.

This study is part of a wider nested case-control study carried out in the nursing homes of four different Swiss Cantons (BE, TI, VD, ZH), aiming at assessing whether people in close contact with pets are at higher risk of carrying multidrug resistant staphylococci compared to people without close contact with pets.

10.1.5. POSTER 5 - Antibiotic resistance: a challenge to human-pet relationship

Presented at the: EcoHealth 2010 – Global Ecohealth Challenges; Multiple Perspectives, London, United Kingdom, 18th-20th of August 2010

By: P. Decristophoris, O. Petrini, E. Schelling, J. Zinsstag

Antimicrobial resistance: a challenge to human-pet relationship

P. Decristophoris [1,2], O. Petrini [1], E. Schelling [2], J. Zinsstag [2]

Swiss TPH

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Background

The emergence and spread of antibiotic resistant microbes is a challenge to public health. By developing resistance to several antibiotics, staphylococci have emerged as a major cause of nosocomial infections. Antibiotic resistance of staphylococci has also been described in pets and transmission of these bacteria between pets and humans has been documented [1]. Pets are increasingly used for psychological support in the therapy of chronically diseased or elderly patients [2]. Because people in nursing homes often suffer from infections or are immunosuppressed, they may be at increased risk of disease if infected by resistant staphylococci.

? Is there a potential for pets to act as reservoirs of multidrug resistant staphylococci ?

Figure 1. Causal web relating carriage of multidrug-resistant staphylococci in pets and in people to their causes and consequences

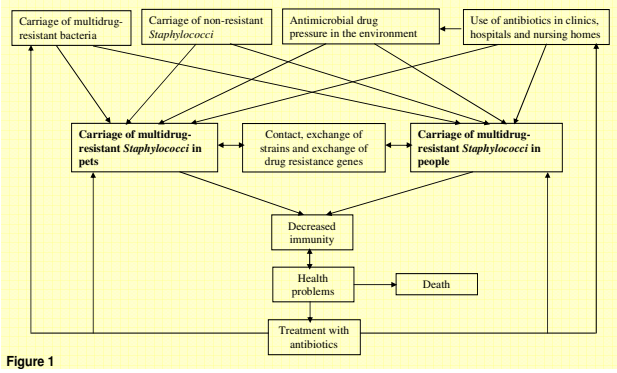


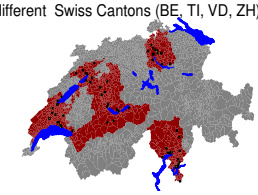
Figure 1

Figure 2. Close contact between pets and nursing home residents


Figure 2

Methods

- 77 Swiss nursing homes (♦), randomly selected in 4 different Swiss Cantons (BE, TI, VD, ZH)



- Sampling of all cats and dogs living in or weekly visiting these homes (n=98)
- 445 enrolled residents; 6 residents randomly selected in each nursing home (229 residents in nursing homes with pets; 216 in nursing homes without pets)
- Nasal and ear swabs (Amies agar gel 108C, Copan)
- Swabs plated out on mannitol salt agar (Biomérieux) within 24h after collection and enriched in MRSA broth followed by culture on Chrom ID *S. aureus* (Biomérieux)
- Isolates identified by MALDI-TOF MS
- Phenotypic antibiotic resistance assessed by disk diffusion method on Mueller-Hinton blood agar according to CLSI guidelines
- Multidrug resistance defined as resistance to at least 3 drugs of different antibiotic classes
- MLST scheme for typing of *S. epidermidis* applied to a set of strains isolated from 6 residents and the dog visiting a nursing home

Results

PETS

- Multidrug resistant staphylococcal strains = 26.3% (26/99)
- Pets carrying multidrug resistant strains = 16.3% (16/98; 7 cats, 9 dogs)
- Methicillin resistant *S. aureus* (MRSA) or *S. pseudintermedius* (MRSP) were not detected

RESIDENTS

- Multidrug resistant staphylococcal strains = 29.6% (241/815)
- Nursing home residents carrying multidrug resistant strains = 41.8% (186/445)
- Residents carrying methicillin resistant *S. aureus* (MRSA) =2.25 % (10 /445)
- Methicillin *S. pseudintermedius* (MRSP) was not detected

… A CASE…

5 out of 6 investigated residents of a nursing home (total residents n= 63) and a dog visiting the same institution were found to carry multidrug resistant *S.epermidis* harbouring the *mecA* gene coding for methicillin resistance in staphylococci (Table 1)

Table 1. MLST profiles and antibiotic resistance of the *S. epidermidis* isolates

	Typing	Resistance gene	Phenotypic antibiotic resistances
Dog	ST 200	*mecA*	P, AM, OX, SXT,FA, FOX
Resident 1	ST 2	*mecA*	P, AM, OX, CIP, CAZ, FA, FOX
Resident 2	ST5	*mecA*	P, AM, OX, CZ, GM, SXT, CIP, AMC, CAZ, IPM, NN, FA, FOX, K
Resident 3	ST5	*mecA*	P, AM, OX, CIP, CAZ, FOX
Resident 4	ST5	*mecA*	P, AM, OX, E, CC, CIP, CAZ, FA, FOX
Resident 5	ST5	*mecA*	P, AM, OX, CIP, FOX

Multidrug resistance was observed in staphylococci isolated from humans and animals in nursing homes. No direct link between individual isolates in the same home was established. We are now analysing data of nursing homes residents to assess whether there is an association between carriage of multidrug resistant staphylococci and living in a nursing home with pets.

References
[1] O'Mahony et al. 2007. Methicillin resistant Staphylococcus aureus (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology*, 109, 285-296.
[2] Colombo et al. 2006. Pet-therapy and institutionalized elderly: a study on 144 cognitively unimpaired subjects. *Archives of Gerontology and Geriatrics*, 42, 207-216.

10.2. Report to the nursing homes

This report was written and translated in the three main Swiss languages (German, French and Italian) and was mailed to all 77 nursing homes that participated into the study in the four different Swiss Cantons (Bern, Ticino, Vaud and Zürich).

10.2.1. German version

- ERGEBNISBERICHT -

Die Epidemiologie der Antibiotika-resistenten Staphylokokken bei Katzen, Hunden und Bewohnern in Alters- und Pflegeheimen in der Schweiz

Paola Decristophoris, Anna De Benedetti, Giovanni Marvin, Jan Guillaume, Christiane Petignat, Monica Attinger, Orlando Petrini

Aus dem Französischen von Lena Fiebig

Hintergrund der Studie

Antibiotika-resistente Mikroorganismen stellen weltweit eine Gefahr für die öffentliche Gesundheit dar. Bakterien der Gattung *Staphylococcus*, welche bei Mensch und Tier zur natürlichen Mikroflora der Haut und der Schleimhäute gehören, können über verschiedene Mechanismen Resistenzen gegenüber ein oder mehrere Antibiotika entwickeln.

Diese Bakterien können ein Gen (*mec*A) tragen, über welches eine Resistenz gegen Methicillin und alle andere Betalaktame vermittelt wird. Durch eine solche Resistenz wird die Behandlung von Infektionen durch diese Mikroorganismen erschwert. Aktuellen Studien zufolge, welche in Alters- und Pflegeheimen von vier Schweizer Kantonen durchgeführt wurden, sind etwa 6-10% der Bewohner symptomlose Träger von MRSA (Methicillin-resistenter *Staphylococcus aureus*) (1, 2).

Auch koagulasenegative Staphylokokken, wie *S. epidermidis*, können zu nosokomialen (in medizinischen Einrichtungen erworbenen) Infektionen führen, die subakut oder ohne deutliche Krankheitsanzeichen verlaufen können. Diese Staphylokokken spielen auch eine wichtige Rolle als opportunistische Erreger. Im Laufe der vergangenen Jahre wurde ein Anstieg an durch diese Erreger bedingte Infektionen verzeichnet, und zwar vor allem bei Personen mit Prothesen oder bei welchen ein Intravenös - oder ein Harnkatheter gelegt wurde (3).

Bei Haustieren (vor allem bei Katzen und Hunden) werden am häufigsten Staphylokokken gefunden, welche zur Art *S. pseudintermedius* gehören. Auch diese Staphylokokken können Antibiotikaresistenzen entwickeln, unter anderem gegenüber Methicillin (MRSP: Methicillin-resistenter *Staphylococcus pseudintermedius*). Es sind Fälle beschrieben, in welchen diese Erreger zwischen Haustieren und Mitarbeitern von Tierkliniken übertragen wurden. Haustiere wurden hierbei als mögliches Reservoir Antibiotika-resistenter Bakterien vermutet (4).

Katzen und Hunde spielen eine immer wichtigere Rolle bei therapeutischen Massnahmen für ältere und chronisch erkrankte Menschen. In Alters- und Pflegeheimen durchgeführte Studien zeigen, dass

sich der therapeutische Einsatz von Tieren günstig auf das psychische und physische Wohl der Bewohner auswirkt (5). Allerdings sind Bewohner von Alters- und Pflegeheimen häufig immungeschwächt und können daher einem besonders hohen Risiko einer Infektion mit multiresistenten Staphylokokken ausgesetzt sein. Bislang ist wenig darüber bekannt, in welchem Ausmass Haustiere mit Staphylokokken kolonisiert sind und welche Rolle eine Bakterienübertragung zwischen Mensch und Tier in Alters- und Pflegeheimen spielt.

Zielstellung

Die Ziele der Studie umfassten

- die Bestimmung der Prävalenz, mit welcher multiresistente Staphylokokken bei Katzen, Hunden und Bewohnern in Alters- und Pflegeheimen in den Kantonen Bern, Tessin, Waadt und Zürich vorkommen;

- die Beschreibung der epidemiologischen Zusammenhänge zwischen Staphylokokken, welche bei Tieren isoliert wurden und jenen, welche bei Personen isoliert wurden, die Kontakt zu den Tieren hatten; sowie

- eine Einschätzung der möglichen Bedeutung von Haustieren als Reservoir für Antibiotika-resistente Bakterien.

Studienablauf und Auswahl der Studienteilnehmer

Die Studie wurde von den Ethikkommissionen der Kantone Bern, Tessin, Waadt und Zürich, durch die Veterinärämter dieser Kantone, sowie durch das Bundesamt für Veterinärwesen (BVET) bewilligt.

Die Alters- und Pflegeheime der Kantone Bern, Tessin, Waadt und Zürich wurden in zwei Gruppen eingeteilt: eine Gruppe bestand aus Einrichtungen, bei welchen Katzen und Hunde als Haustiere zugelassen waren, und die andere Gruppe bestand aus Einrichtungen ohne Haustiere. Aus beiden Gruppen wurden Alters- und Pflegeheime nach dem Zufallsprinzip ausgewählt und zu einer freiwilligen Teilnahme an dieser Studie eingeladen. Als **Einrichtungen mit Tieren** wurden jene Alters- und Pflegeheime eingestuft, in welchen

- mindestens eine Katze oder ein Hund von der Einrichtung oder von einem der Bewohner gehalten wurde oder

- mindestens einmal wöchentlich eine Katze oder ein Hund zu Therapiezwecken einbezogen wurde.

Innerhalb jeder teilnehmenden Einrichtung wurden dann zufällig ausgewählte Bewohner gefragt, ob sie bereit wären, an der Studie teilzunehmen. Anhand einer Pilotstudie ergab sich, dass für die Durchführung der Studie je fünf bis sieben Teilnehmer pro Alters- und Pflegeheim nötig wären. Nach Einverständnis der Besitzer wurden ausserdem alle Katzen und Hunde der teilnehmenden Einrichtungen in die Probenentnahme einbezogen. Alle Studienteilnehmer hatten das 18. Lebensjahr vollendet und gaben ihr schriftliches Einverständnis zur Studienteilnahme. Ausgeschlossen von der Studie wurden alle Personen, welche bereits an anderen klinischen Studien teilnahmen, oder zum Zeitpunkt der Nasentupferentnahme akut erkrankt waren.

Probennahme und Laboranalysen

Jedem Tier, welches in die Studie einbezogenen war, wurde je ein Nasen- und ein Ohrabstrich, den teilnehmenden Personen jeweils ein Nasenabstrich entnommen. Für alle Teilnehmer (Tiere und Personen) wurde ausserdem ein Datenformular ausgefüllt, um auch demographische Angaben, sowie Informationen zum Gesundheitszustand und über Kontakte der Bewohner zu den Tieren zu erfassen.

Die Proben wurden innerhalb von 24 bis 48 Stunden nach Probenentnahme im Labor auf Staphylokokken untersucht. Bei positivem Befund wurden die Bakterien selektiv in Fest- und Flüssigmedien angereichert, und eine Speziesbestimmung mittels molekularer Methoden wurde durchgeführt.

Die isolierten Staphylokokken wurden dann auf Resistenzen gegenüber 24 verschiedenen Antibiotika getestet. Anhand der Testergebnisse wurden Bakterienstämme nach standardisierten Verfahren (6, 7) als "resistent" (bei fehlenden Medikamentensensibilität) oder als "sensibel" gegenüber den jeweiligen Antibiotika eingestuft. Als "multiresistent" wurden in dieser Studie diejenigen Bakterienstämme bezeichnet, welche gegenüber mindestens drei Wirkstoffe verschiedener Antibiotikaklassen resistent waren. Mittels molekularbiologischer Methoden wurde untersucht, ob die Bakterien das *mec*A-Gen trugen. Dieses Gen kommt bei Staphylokokken vor, welche ähnliche Antibiotikaresistenzen wie MRSA aufweisen und ist für die Resistenz gegenüber Methicillin und andere Betalaktame verantwortlich. Es ist leicht von einem auf einen anderen Bakterienstamm übertragbar (8).

Statistische Auswertung

Die für die Studie benötigte Teilnehmerzahl wurde über eine Pilotstudie ermittelt. Aus dieser ergaben sich Schätzwerte, dass bei 10% der Bewohner und bei 5% der Tiere mindestens ein multiresistenter Staphylokokkenstamm vorkommt. Alle erhobenen (Labor- und Befragungs-) Daten wurden deskriptiv und vergleichend statistisch ausgewertet.

Ergebnisse und Diskussion

Bei insgesamt 445 Bewohnern und 98 Haustieren (53 Katzen und 45 Hunden) aus 77 Alters- und Pflegeheimen wurden Proben entnommen (Tabelle 1). Die durchschnittliche Teilnahmequote der

Alters- und Pflegeheime aus vier Kantonen betrug 53,2%. Die demographischen Angaben zu den Studienteilnehmern sind in den Tabellen 2A und 2B dargestellt.

Tabelle 1. Anzahl der Studienteilnehmer (Bewohner und Tiere) nach Kanton und nach der Gruppe des Alters- und Pflegeheims (mit oder ohne Tiere).

	Alters- und Pflegeheime mit Tiere (n = 39)	**Alters- und Pflegeheime ohne Tiere (n = 38)**
Anzahl Bewohner (insgesamt)	**229**	**216**
Im Kanton Bern	59	58
Im Kanton Tessin	58	58
Im Kanton Waadt	60	60
Im Kanton Zürich	52	40
Anzahl Tiere (insgesamt)	**98**	-
Im Kanton Bern	32	-
Im Kanton Tessin	26	-
Im Kanton Waadt	20	-
Im Kanton Zürich	20	-

Tabelle 2: A. Demographische Angaben zu den Bewohnern der Alters- und Pflegeheime mit und ohne Tiere. **B.** Angaben zu den Tieren.

A.	**Alters- und Pflegeheime mit Tiere**		**Alters- und Pflegeheime ohne Tiere**	
Alter (Median)	86.6 ± 10.8		85.2 ± 11.4	
	%	**n**	**%**	**n**
Anteil Frauen	75	(172/229)	69	(148/216)
Früherer Befund von MRSA	5	(12/229)	3	(7/216)
Katheter innerhalb des vergangenen Jahres gelegt	16	(36/229)	14	(30/216)
Harnwegsinfektionen (vergangenen Jahr)	22	(50/226)	26	(55/212)
Chirurgischer Eingriff (vergangenen Jahr)	14	(32/227)	6	(14/216)
Aufenthalt in anderen Alters- / Pflegeheim (verg. Jahr)	23	(53/228)	22	(47/215)
Behandlung mit Antibiotika (innerhalb letzten 3 Monate)	23	(52/228)	21	(45/214)
Wöchentlicher Kontakt zu einer Katze oder einem Hund	60	(137/229)	9	(20/216)
B.	**Katzen (n=53)**		**Hunde (n=45)**	
	%	**n**	**%**	**n**
Besitzer des Tieres ist				
das Alters- und Pflegeheim	81	43	0	0
ein Bewohner	17	9	11	5
ein Therapeut	0	0	24	11
ein Besucher	0	0	24	11
ein Mitglied des Pflegepersonals	0	0	31	14
Andere	2	1	9	4

Bewohner

Bei den 445 untersuchten Personen wurden insgesamt 815 Staphylokokkenstämme gefunden. In Alters- und Pflegeheimen mit Tieren wurden bei 36,7% (84/229) der Bewohner multiresistente Staphylokokken festgestellt und bei 24% (55/229) Methicillin-resistente Staphylokokken (mit *mec*A-Gen). In Alters- und Pflegeheimen ohne Tiere wurden bei 45,8% (99/226) der untersuchten Bewohner multiresistente Staphylokokken festgestellt, und bei 29,6% (64/216) Methicillin-resistente Staphylokokken.

Darüber hinaus wurden 9 MRSA-Stämme aus Nasentupfern von 8 Personen isoliert. Von diesen Personen wohnten zwei Personen in Alters- und Pflegeheimen mit Tieren und sechs Personen in Einrichtungen ohne Tiere. In keiner der Proben wurde ein MRSP (Methicillin-resistenter *Staphylococcus pseudintermedius*) gefunden, welcher hauptsächlich bei Haustieren vorkommen kann.

Katzen und Hunde

Aus den Nasen- und Ohrtupferproben der 98 untersuchten Tiere wurden insgesamt 109 Staphylokokkenstämme isoliert. Von den Tieren trugen 16,3% (16/98) multiresistente Staphylokokken und 6,1% (6/98) Methicillin-resistente Staphylokokken.

Weder MRSA noch MRSP wurden bei den untersuchten Tieren gefunden.

Kontakt der Bewohner zu den Katzen und Hunden

Insgesamt 63,1% (281/445) der Studienteilnehmer gaben an, dass ein Kontakt zu Haustieren sehr wichtig für ihre Lebensqualität sei. Jedoch hatten nicht alle Personen, welche in Alters- und Pflegeheimen mit Tieren wohnten, tatsächlich direkten Kontakt zu einem Tier: nur 55,9% (128/229)

der befragten Bewohner von Alters- und Pflegeheimen mit Tieren gaben an, mindestens einmal wöchentlich ein oder mehrere Tiere der Einrichtung berührt zu haben.

In einem Fall wurde derselbe Stamm eines multiresistenten *S. epidermidis* in der Nasentupferprobe eines Bewohners und in der Nasentupferprobe von einer Katze gefunden. Gemäss den erhobenen Kontaktdaten hatte dieser Bewohner die Katze etwa einmal wöchentlich berührt. Anhand dieses Ergebnisses kann jedoch nicht gesagt werden, ob eine direkte Übertragung des Bakterienstammes zwischen Mensch und Tier stattgefunden hat, oder ob es eine gemeinsame Expositionsquelle in der Umgebung gab, von welcher sowohl die Katze als auch der Bewohner die Bakterien unabhängig voneinander erworben hatten.

Es stellte sich heraus, dass für die Bewohner von Alters- und Pflegeheimen kein erhöhtes Risiko bestand, multiresistente Staphylokokken zu tragen (relatives Risiko = 0,80; CI: 0,64-1,00), wenn Katzen und Hunde in der Einrichtung gehalten wurden (bzw. Zugang hatten) und berührt wurden. Wurde jedoch innerhalb der drei Monate vor Befragung eine Antibiotikabehandlung durchgeführt, war das Risiko dreimal so hoch, dass multiresistente Staphylokokken vorkamen.

Schlussfolgerungen

Die Ergebnisse dieser Studie zeigen, dass Bewohner in Alters- und Pflegeheimen durch den Kontakt zu Katzen und Hunden keinem erhöhten Risiko ausgesetzt sind, multiresistente Staphylokokken zu tragen. Bedenkt man die günstigen Auswirkungen, welche die Anwesenheit von Tieren insbesondere auf das psychische Wohlbefinden der Bewohner hat, kann man zu einer Zulassung von Katzen und Hunden in Alters- und Pflegeheime ermutigen. Es sollte aber darauf geachtet werden, dass im Umgang mit den Tieren Hygieneregeln strikt eingehalten werden und der Gesundheitszustand der Haustiere regelmässig untersucht wird, um eine Übertragung von Infektionskrankheiten zu vermeiden. Dazu sollten Tiere mit akuten infektiösen Erkrankungen nicht

in direkten Kontakt mit den Bewohnern kommen. Daher ist es wichtig, auch eine Möglichkeit vorzusehen, dass Tiere innerhalb der Einrichtung zeitweilig getrennt von Personen gehalten werden können.

Literaturangaben

1. Petignat C, Attinger M. Enquête de prevalence des residents porteurs de MRSA dans le Canton de Vaud. Forum Hygiène. 2004.

2. Gaia V, De Benedetti A, Valsangiacomo C, Poloni C. Prevalenza di *Staphylococcus aureus* meticillino-resistente (MRSA) negli istituti a lunga degenza in canton Ticino: studio multicentrico 2008. Trib Med Tic. 2009;74:103-6.

3. Piette G, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.

4. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

5. Colombo G, Buono MD, Smania K, Raviola R, De Leo D. Pet therapy and institutionalized elderly: A study on 144 cognitively unimpaired subjects. Arch Gerontol Geriat. 2006;42:207-16.

6. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Third Edition M 31-A3. 2008;28:1-99.

7. CLSI. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement M 100-S19. 2010;29.

8. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46:8-20.

10.2.2. Italian version

- RAPPORTO DELLO STUDIO -

Epidemiologia degli stafilococchi multiresistenti agli antibiotici nei gatti, nei cani e nei residenti di istituti di lunga degenza in Svizzera

Paola Decristophoris, Anna De Benedetti, Giovanni Marvin, Jan Guillaume, Christiane Petignat, Monica Attinger, Orlando Petrini

Traduzione tedesca a cura di Lena Fiebig

Contesto dello studio

I microrganismi multiresistenti agli antibiotici sono una preoccupazione per la salute pubblica a livello mondiale. Anche batteri del genere *Staphylococcus*, appartenenti alla comunità batterica naturale della cute e delle mucose di uomini e animali, hanno sviluppato vari meccanismi che conferiscono resistenze a diversi tipi di antibiotici. In particolare, questi germi, possono avere un gene (*mecA*) che conferisce loro la capacità di essere resistenti alla meticillina e a tutti i beta-lattamici. Possono quindi insorgere difficoltà nel trattamento di eventuali infezioni derivanti da questi microrganismi. Secondo studi recenti condotti negli istituti di lunga degenza di alcuni Cantoni svizzeri, circa il 6% di residenti è portatore sano di MRSA (*Staphylococcus aureus* meticillino resistente) (1, 2). Pure gli stafilococchi coagulasi negativa (ad es. *S. epidermidis*) causano infezioni nosocomiali, con manifestazioni cliniche subacute e dei decorsi cronici, senza segni fulminanti d'infezioni, e rivestono un ruolo importante quali patogeni opportunisti. Negli ultimi anni vi è stato un aumento d'infezioni riconducibili a questi microorganismi, in particolare nei pazienti con cateteri e protesi (3).

Negli animali domestici, in particolare nei cani e nei gatti, gli stafilococchi più frequentemente isolati appartengono alla specie *Staphylococcus pseudintermedius*. Anche questi stafilococchi possono sviluppare resistenze a vari tipi di antibiotici, tra i quali pure la meticillina (MRSP: *Staphylococcus pseudintermedius* meticillino resistente). Sono stati documentati anche dei casi di trasmissione di questi microorganismi tra animali domestici e personale di cliniche veterinarie. Questi animali sono quindi stati descritti come potenziale serbatoio per batteri resistenti agli antibiotici (4).

Gli animali, in particolare cani e gatti, sono impiegati sempre più spesso quale parte integrante della terapia alle persone anziane o malati cronici. Studi condotti in istituti di lunga degenza hanno mostrato come l'impiego di animali nella terapia porti a dei benefici sia a livello psichico sia fisico nei residenti (5). Tuttavia, gli ospiti presenti negli istituti di lunga degenza sono, frequentemente,

delle persone immunodepresse esposte quindi a un potenziale rischio d'infezioni da stafilococchi multiresistenti agli antibiotici. Abbiamo a disposizione poche informazioni riguardanti la colonizzazione degli animali domestici da parte degli stafilococchi e l'eventuale trasmissione di questi batteri all'ambiente di un istituto di lunga degenza.

Scopi dello studio

- Conoscere la prevalenza di colonizzazione da stafilococchi multiresistenti agli antibiotici in gatti, cani e nelle persone residenti in istituti di lunga degenza nei Cantoni Ticino, Berna, Vaud e Zurigo;

- Descrivere le relazioni epidemiologiche tra gli stafilococchi isolati negli animali e quelli isolati nelle persone in contatto con questi ultimi;

- Chiarire il ruolo degli animali domestici quali serbatoi di resistenze batteriche.

Disegno dello studio e scelta dei soggetti

Tutti gli istituti di lunga degenza dei Cantoni Berna, Ticino, Vaud e Zurigo sono stati classificati in due categorie, in base alla presenza o meno di animali da compagnia quali cani e gatti. All'interno di ogni categoria è stata eseguita una scelta casuale degli istituti cui richiedere, su base volontaria, la partecipazione allo studio. Gli istituti classificati come **istituti con animali da compagnia** presentavano almeno una delle seguenti caratteristiche:

- presenza di un gatto o di un cane appartenente all'istituto o ad un residente, oppure

- svolgimento di un'attività di pet-therapy con gatto o cane almeno una volta a settimana.

I residenti ai quali è stata richiesta la partecipazione sono stati scelti in modo casuale all'interno di ogni singolo istituto. Il numero di partecipanti necessario allo studio è stato calcolato sulla base di

uno studio pilota svolto in precedenza. All'interno di ogni istituto hanno quindi partecipato tra le cinque e le sette persone. Tutti gli animali presenti al momento dello svolgimento dello studio sono stati inclusi nel campionamento, previo consenso del proprietario. Tutti i partecipanti erano maggiorenni e hanno fornito il loro consenso scritto allo studio. Sono stati esclusi dallo studio i soggetti già partecipanti ad altri studi clinici e quelli affetti da una patologia acuta al momento dello striscio di una narice. Lo studio è stato autorizzato dai Comitati Etici dei Cantoni Berna, Ticino, Vaud e Zurigo e dai rispettivi Uffici cantonali e federale di veterinaria.

Raccolta dei campioni e analisi di laboratorio

Per ogni animale sono stati raccolti uno striscio dal naso e uno dall'orecchio, mentre ai residenti è stato eseguito un solo striscio nasale. Per ogni soggetto incluso nello studio, è stato inoltre compilato un questionario per la raccolta di dati demografici e d'informazioni sullo stato di salute e sul contatto residente-animale.

Gli strisci raccolti sono stati analizzati in laboratorio per la ricerca di stafilococchi entro le 24-48 ore dalla raccolta. Questi microorganismi sono stati isolati da piastre e arricchimenti selettivi e sono stati identificati a livello di specie per mezzo di tecniche molecolari.

Sono state pure testate le reazioni degli stafilococchi nei confronti di 24 diversi antibiotici. I ceppi sono poi stati classificati come "resistenti", con sensibilità ridotta o "sensibili" ai diversi antibiotici secondo specifiche linee guida (6, 7). In questo studio abbiamo definito quale "multiresistente" ogni ceppo che presentava resistenze ad almeno tre principi attivi appartenenti a diverse classi di antibiotici. Abbiamo inoltre investigato con metodi molecolari la presenza del gene *mecA*. Questo gene è presente negli stafilococchi che hanno caratteristiche di resistenza antibiotica paragonabili a quelle dell'MRSA, conferisce la resistenza alla meticillina e agli altri beta-lattamici ed è facilmente trasmesso da un ceppo di stafilococco all'altro (8).

Analisi statistiche

Il numero di soggetti da investigare in questo studio é calcolato sulla base dei risultati di uno studio pilota che stima la presenza di almeno un ceppo di stafilococco multiresistente agli antibiotici nel 10% dei residenti e nel 5% degli animali.

Risultati e discussione

Sono stati raccolti campioni da 445 residenti e da 98 animali domestici (53 gatti e 45 cani) in 77 diversi istituti di lunga degenza (Tabella 1). Il tasso di partecipazione allo studio degli istituti dei quattro Cantoni investigati é stato del 53.2%. Il dettaglio delle caratteristiche demografiche dei soggetti investigati è presentato nelle Tabella 2A e 2B.

Tabella 1. Numero di soggetti partecipanti allo studio suddivisi per Cantone e per tipologia di istituto a lunga degenza (con o senza animali).

	Istituti con animali (n = 39)	**Istituti senza animali (n = 38)**
Totale persone	**229**	**216**
Canton Berna	59	58
Canton Ticino	58	58
Canton Vaud	60	60
Canton Zurigo	52	40
Totale animali	**98**	**-**
Canton Berna	32	-
Canton Ticino	26	-
Canton Vaud	20	-
Canton Zurigo	20	-

Tabella 2: A. Caratteristiche demografiche dei residenti investigati negli istituti a lunga degenza con e senza animali. **B.** Caratteristiche demografiche degli animali investigati.

A.	**Istituti con animali**		**Istituti senza animali**	
Età (mediana)	86.6 ± 10.8		85.2 ± 11.4	
	%	**n**	**%**	**n**
Sesso femminile	75	(172/229)	69	(148/216)
Precedente storia di MRSA	5	(12/229)	3	(7/216)
Portatori di catetere (ultimo anno)	16	(36/229)	14	(30/216)
Infezioni urinarie (ultimo anno)	22	(50/226)	26	(55/212)
Intervento chirurgico (ultimo anno)	14	(32/227)	6	(14/216)
Permanenza in un altro istituto (ultimo anno)	23	(53/228)	22	(47/215)
Trattamento antibiotico (ultimi 3 mesi)	23	(52/228)	21	(45/214)
Contatto con cane/gatto ogni settimana	60	(137/229)	9	(20/216)

B.	**Gatti (n=53)**		**Cani (n=45)**	
	%	**n**	**%**	**n**
Proprietari				
Istituti a lunga degenza	81	43	0	0
Residenti	17	9	11	5
Personale di pet-therapy	0	0	24	11
Visitatori	0	0	24	11
Personale curante	0	0	31	14
Altro	2	1	9	4

Residenti

Sono stati isolati 815 ceppi di stafilococco dalle narici dei 445 residenti. Negli istituti di lunga degenza con animali la proporzione di residenti portatori di stafilococchi multiresistenti agli antibiotici è del 36.7% (84/229) e quella dei residenti portatori di stafilococchi resistenti alla meticillina (presenza del gene *mecA*) è del 24% (55/229). Negli istituti di lunga degenza senza animali il 45.8% (99/216) dei residenti è portatore di stafilococchi multiresistenti agli antibiotici, e il 29.6% (64/216) è portatore di stafilococchi resistenti alla meticillina.

Dalle narici di 8 residenti (2 residenti in istituti con e 6 residenti in istituti senza animali) sono stati isolati 9 ceppi di MRSA, con un residente portatore di due ceppi diversi. Non è stato isolato nessun MRSP (stafilococco resistente alla meticillina riscontrato di solito negli animali domestici).

Cani e gatti

Sono stati isolati 109 ceppi dalle narici e dalle orecchie dei 98 animali. Il 16.3% (16/98) degli animali è portatore di stafilococchi multiresistenti agli antibiotici, e il 6.1% (6/98) è portatore di stafilococchi resistenti alla meticillina.

Non è stato isolato nessun MRSA dai cani e dai gatti investigati in questo studio, né nessun MRSP.

Contatti dei residenti con cani e gatti

Il 63.1% (281/445) dei residenti inclusi nello studio ha dichiarato di considerare il contatto con un animale domestico di primaria importanza per la propria qualità di vita. Non tutti residenti in istituti dove cani e gatti sono presenti hanno però un contatto con questi animali, infatti, solo il 55.9% (128/229) ha dichiarato d'avere un contatto fisico diretto con gli animali dell'istituto almeno una volta a settimana.

In un solo caso si é riscontrato lo stesso ceppo di stafilococco meticillino resistente, appartenente alla specie *S. epidermidis*, nelle narici di un residente e in quelle di un gatto con cui il residente dichiarava di avere contatto una volta a settimana. Non è tuttavia chiaro se i due soggetti si siano scambiati il ceppo tramite contatto fisico diretto o se i due abbiano acquisito questo ceppo da una fonte comune di colonizzazione (ad es. ambiente circostante).

In base al nostro studio, la presenza di cani e gatti negli istituti di lunga degenza e il contatto dei residenti con questi animali non è un fattore che aumenta nei residenti il rischio di essere portatori

di stafilococchi multiresistenti agli antibiotici (rischio relativo = 0.80; 95% CI: 0.64-1.00). È invece chiaro che un trattamento antibiotico nei tre mesi precedenti lo studio è un fattore che aumenta di tre volte il rischio di essere portatore di stafilococchi multiresistenti agli antibiotici.

Conclusioni

I risultati di questo studio mostrano che il contatto con cani e gatti all'interno di istituti di lunga degenza non aumenta il rischio nei residenti di essere portatori di stafilococchi multiresistenti agli antibiotici. La presenza di cani e gatti nel rispetto delle regole d'igiene di base dovrebbe quindi essere promossa considerando i benefici, in particolare a livello psichico, per le persone che hanno un contatto con questi animali. Per il benessere di residenti e animali sarebbe tuttavia auspicabile evitare la presenza di animali affetti da patologie acute o prevederne l'isolamento temporaneo all'interno degli istituti.

Referenze

1. Petignat C, Attinger M. Enquête de prevalence des residents porteurs de MRSA dans le Canton de Vaud. Forum Hygiène. 2004.

2. Gaia V, De Benedetti A, Valsangiacomo C, Poloni C. Prevalenza di *Staphylococcus aureus* meticillino-resistente (MRSA) negli istituti a lunga degenza in canton Ticino: studio multicentrico 2008. Trib Med Tic. 2009;74:103-6.

3. Piette G, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.

4. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

5. Colombo G, Buono MD, Smania K, Raviola R, De Leo D. Pet therapy and institutionalized elderly: A study on 144 cognitively unimpaired subjects. Arch Gerontol Geriat. 2006;42:207-16.

6. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Third Edition M 31-A3. 2008;28:1-99.

7. CLSI. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement M 100-S19. 2010;29.

8. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46:8-20.

10.2.3. French version

- RAPPORT D'ETUDE -

Epidémiologie des staphylocoques multirésistants aux antibiotiques chez les chats, les chiens et les personnes dans les EMS en Suisse

Paola Decristophoris, Anna De Benedetti, Giovanni Marvin, Jan Guillaume, Christiane Petignat, Monica Attinger, Orlando Petrini

Traduction allemande par Lena Fiebig

Contexte de l'étude

Les microorganismes multirésistants aux antibiotiques sont un souci pour la santé publique au niveau mondial. Les bactéries du genre *Staphylococcus*, qui font partie de la communauté bactérienne naturelle de la peau et des muqueuses chez l'homme et chez les animaux, ont développé divers mécanismes qui les rendent résistants à plusieurs types d'antibiotiques. Ces germes peuvent en particulier être porteurs d'un gène (*mecA*) qui leur confère la capacité d'être résistants à la méticilline et à tous les autres bêta-lactames. Des difficultés peuvent donc se présenter lors du traitement d'infections provoquées par ces microorganismes. Selon des études récentes conduites dans les EMS de quatre cantons suisses, environ 6-10% des résidants sont porteurs sains du MRSA (*Staphylococcus aureus* résistant à la méticilline) (1, 2). Les staphylocoques coagulase-négative (par ex. *S. epidermidis*) peuvent également causer des infections nosocomiales avec manifestations subaiguës et décours sans signe d'infection fulminante, et jouent un rôle important en tant qu'agents pathogènes opportunistes. Au cours des dernières années, on a relevé une hausse des infections causées par ces microorganismes, surtout chez les patients avec prothèse ou porteurs de cathéter intraveineux ou de sonde urinaire (3).

Chez les animaux domestiques, en particulier les chats et les chiens, les staphylocoques les plus fréquemment isolés appartiennent à l'espèce *Staphylococcus pseudintermedius* et peuvent développer également des résistances à divers types d'antibiotiques, dont la méticilline (MRSP: *Staphylococcus pseudintermedius* résistant à la méticilline). Des cas de transmission de ces microorganismes entre animaux domestiques et personnel de clinique vétérinaire ont été documentés. Ces animaux ont donc été décrits comme réservoirs potentiels de bactéries résistantes aux antibiotiques (4).

Les chats et les chiens font de plus en plus partie intégrante des thérapies appliquées aux personnes âgées ou aux malades chroniques. Des études conduites dans des EMS ont montré que le recours à des animaux apporte des bénéfices psychiques et physiques aux résidants (5). Ces derniers sont

cependant souvent immunodéprimés et, partant, exposés à un risque potentiel d'infection à des staphylocoques multirésistants aux antibiotiques. On dispose actuellement de peu d'éléments quant à la colonisation des animaux de compagnie par les staphylocoques et la possible transmission de ces bactéries dans les EMS.

Buts de l'étude

- Connaître le taux de prévalence de la colonisation par les staphylocoques multirésistants aux antibiotiques chez les chats, les chiens et les résidants d'EMS dans les cantons de Berne, du Tessin, de Vaud et de Zurich.

- Décrire les relations épidémiologiques entre les staphylocoques isolés chez les animaux d'une part, et chez les personnes en contact avec ces derniers d'autre part.

- Evaluer le rôle potentiel des animaux domestiques comme réservoirs de bactéries résistantes aux antibiotiques.

Dessin de l'étude et choix des sujets

L'étude a été autorisée par les commissions d'éthique des cantons de Berne, du Tessin, de Vaud et de Zurich ainsi que par les offices cantonaux concernés et par l'Office vétérinaire fédéral.

Les EMS des cantons de Berne, du Tessin, de Vaud et de Zurich ont été classés en deux catégories, en fonction de la présence ou non de chats ou de chiens comme animaux de compagnie. Nous avons ensuite sélectionné de manière aléatoire les institutions de chaque catégorie appelées à participer à l'étude sur une base volontaire. Les EMS étaient catalogués comme **institutions avec animaux** de compagnie s'ils présentaient au moins une des caractéristiques suivantes:

- présence d'un chat ou d'un chien appartenant à l'EMS ou à un résidant,

- activité de thérapie animale avec un chat ou un chien au moins une fois par semaine.

Nous avons également choisi de façon aléatoire, au sein de chaque institution, les résidants dont nous avons sollicité la participation. Calculé sur la base d'une étude pilote préalable, le nombre de participants nécessaire au déroulement de l'étude a été fixé entre cinq et sept résidants par EMS. Tous les chats et les chiens présents dans l'institution ont été inclus dans l'échantillonnage, avec le consentement de leur propriétaire. Les participant étaient âgés de plus de 18 ans et ont donné leur accord écrit. Ont été exclus de l'étude les sujets qui participaient déjà à d'autres études cliniques ou qui souffraient d'une ou de plusieurs maladies aiguës au moment du prélèvement nasal.

Prélèvement d'échantillons et analyses de laboratoire

Un frottis de nez et d'oreille a été effectué chez tous les animaux et un frottis de nez chez tous les résidents inclus dans l'étude. Nous avons en outre rempli un questionnaire pour chaque sujet inclus dans l'étude afin de disposer de données démographiques et d'informations sur l'état de santé ainsi que sur le contact résidant-animal.

Les prélèvements microbiologiques ont été analysés en laboratoire dans les 24 à 48 heures suivant leur prélèvement afin d'y déceler la présence éventuelle de staphylocoques. Ceux-ci ont été isolés au moyen de plaques et de bouillons d'enrichissement sélectifs et les espèces identifiées par technique moléculaire.

Nous avons aussi testé les réactions des staphylocoques à 24 antibiotiques, puis classé les souches isolées comme "résistantes" avec sensibilité réduite ou "sensibles" aux différents antibiotiques selon des lignes directrices spécifiques (6, 7). Dans cette étude, les souches ont été définies comme "multirésistantes" en présence de résistances à au moins trois principes actifs appartenant à diverses classes d'antibiotiques. Nous avons par ailleurs recouru à des méthodes moléculaires pour détecter le gène *mecA*, que l'on trouve dans les staphylocoques présentant des résistances aux antibiotiques

comparables à celles observées pour le MRSA. Responsable de la résistance à la méticilline et aux autres beta-lactames, celui-ci est facilement transmissible d'une souche de staphylocoque à l'autre (8).

Analyses statistiques

Le nombre de participants a été déterminé sur la base des résultats obtenus lors d'une étude pilote préalable, qui estimait la présence d'au moins une souche de staphylocoques multirésistants aux antibiotiques chez 10% des résidants et 5% des animaux.

Résultats et discussion

Au total, nous avons prélevé des échantillons sur 445 résidants et 98 animaux domestiques (53 chats et 45 chiens) dans 77 EMS (Tableau 1). Le taux de participation des EMS à l'étude menée dans les quatre cantons susmentionnés a été de 53,2%. Le détail des caractéristiques démographiques des sujets est présenté aux Tableaux 2A et 2B.

Tableau 1 Nombre de sujets ayant participé à l'étude, par canton et par typologie d'EMS (avec ou sans animaux).

	EMS avec animaux (n = 39)	**EMS sans animaux (n = 38)**
Total résidants	**229**	**216**
Canton de Berne	59	58
Canton du Tessin	58	58
Canton de Vaud	60	60
Canton de Zurich	52	40
Total animaux	**98**	**-**
Canton de Berne	32	-
Canton du Tessin	26	-
Canton de Vaud	20	-
Canton de Zurich	20	-

Tableau 2: A. Caractéristiques démographiques des résidants dans les EMS avec et sans animaux. **B.** Caractéristiques démographiques des animaux.

A.	**EMS avec animaux**		**EMS sans animaux**	
Age (médiane)	86.6 ± 10.8		85.2 ± 11.4	
	%	**n**	**%**	**n**
Sexe féminin	75	(172/229)	69	(148/216)
Historique du MRSA	5	(12/229)	3	(7/216)
Porteurs de cathéter/sonde (dernière année)	16	(36/229)	14	(30/216)
Infections urinaires (dernière année)	22	(50/226)	26	(55/212)
Intervention chirurgicale (dernière année)	14	(32/227)	6	(14/216)
Séjour dans un autre EMS (dernière année)	23	(53/228)	22	(47/215)
Traitement antibiotique (3 derniers mois)	23	(52/228)	21	(45/214)
Contact avec chat/chien chaque semaine	60	(137/229)	9	(20/216)

B.	**Chats (n=53)**		**Chiens (n=45)**	
	%	**n**	**%**	**n**
Propriétaires				
EMS	81	43	0	0
Résidants	17	9	11	5
Animateur de thérapie animale	0	0	24	11
Visiteurs	0	0	24	11
Personnel de soins	0	0	31	14
Autres	2	1	9	4

Résidants

Nous avons isolé 815 souches de staphylocoques chez les 445 résidants inclus dans l'étude. Dans les EMS avec animaux, la proportion de résidants porteurs de staphylocoques multirésistants aux antibiotiques était de 36,7% (84/229) et celle des résidants porteurs de staphylocoques résistants à la méticilline (présence du gène *mecA*) de 24% (55/229). Dans les EMS sans animaux, 45,8% (99/216) des résidants étaient porteurs de staphylocoques multirésistants aux antibiotiques et 29,6% (64/216) de staphylocoques résistants à la méticilline.

Par ailleurs, 9 souches de MRSA ont été isolées dans les frottis nasaux de 8 résidants (respectivement 2 résidants dans des EMS avec et 6 dans des EMS sans animaux). Un résident était porteur de deux souches différentes de MRSA. Nous n'avons en revanche isolé aucun MRSP (staphylocoque résistant à la méticilline habituellement présent chez les animaux domestiques).

Chats et chiens

Les écouvillons nasaux et auriculaires prélevés sur les 98 animaux ont révélé la présence de 109 souches de staphylocoques. Au total, 16,3% (16/98) des animaux étaient porteurs de staphylocoques multirésistants aux antibiotiques et 6,1% (6/98) de staphylocoques résistants à la méticilline.

Nous n'avons pas isolé de MRSA ni de MRSP.

Contacts des résidants avec des chats et des chiens

Sur la totalité des sujets ayant participé à l'étude, 63,1% (281/445) ont déclaré que le contact avec un animal domestique était très important pour leur qualité de vie. Les résidants d'EMS avec animaux n'avaient cependant pas tous un contact réel avec ces animaux: seuls 55,9% (128/229) d'entre eux ont indiqué avoir un contact physique direct avec les animaux de l'EMS au moins une fois par semaine.

Dans un cas, nous avons retrouvé la même souche de staphylocoque résistant à la méticilline appartenant à l'espèce *S. epidermidis* dans le frottis nasal d'un résidant et dans l'écouvillon nasal d'un chat avec qui le résidant a dit avoir un contact hebdomadaire régulier. Il n'est toutefois pas possible de déterminer si les deux sujets ont échangé cette souche par contact physique direct ou s'ils ont été contaminés par une source commune de colonisation (par ex. environnement voisin).

Selon notre étude, la présence de chats et de chiens dans les EMS et le contact des résidants avec ces animaux n'augmentent pas le risque pour les résidants d'être porteurs de staphylocoques multirésistants aux antibiotiques (risque relatif = 0,80; 95% CI: 0,64-1,00). En revanche, il ressort de façon manifeste qu'un traitement antibiotique dans les trois mois précédant l'étude accroît de trois fois le risque d'être porteur de staphylocoques multirésistants aux antibiotiques.

Conclusions

Les résultats de cette étude montrent que le contact avec des chats et des chiens dans les EMS n'augmente pas le risque chez les résidants d'être porteurs de staphylocoques multirésistants aux antibiotiques. Vu les bénéfices apportés par ces animaux aux personnes avec qui ils entrent en contact, en particulier au niveau psychique, la présence de chats et de chiens dans les EMS peut donc être encouragée. Cette pratique nécessite tout de même le strict respect des règles d'hygiène et un contrôle régulier de la santé des animaux de compagnie, ceci afin d'éviter la transmission de pathologies infectieuses. Pour le bien-être des résidants et des animaux, il conviendrait cependant d'éviter la présence d'animaux atteints de pathologies aiguës ou de prévoir leur isolement temporaire au sein des EMS.

Références

1. Petignat C, Attinger M. Enquête de prevalence des residents porteurs de MRSA dans le Canton de Vaud. Forum Hygiène. 2004.

2. Gaia V, De Benedetti A, Valsangiacomo C, Poloni C. Prevalenza di *Staphylococcus aureus* meticillino-resistente (MRSA) negli istituti a lunga degenza in canton Ticino: studio multicentrico 2008. Trib Med Tic. 2009;74:103-6.

3. Piette G, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.

4. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321-32.

5. Colombo G, Buono MD, Smania K, Raviola R, De Leo D. Pet therapy and institutionalized elderly: A study on 144 cognitively unimpaired subjects. Arch Gerontol Geriat. 2006;42:207-16.

6. CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard - Third Edition M 31-A3. 2008;28:1-99.

7. CLSI. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement M 100-S19. 2010;29.

8. Hanssen AM, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006;46:8-20.

11. Curriculum vitae

PERSONAL INFORMATION

Name	**PhD Paola GANDOLFI - DECRISTOPHORIS**
Address	In L'Era 4, 6705 Cresciano, Switzerland
Telephone	+41 (0)79 256 09 19
E-mail	paola.decristophoris@gmail.com
Nationality	Swiss
Date of birth	30th September 1981

WORK EXPERIENCE

• Dates (from – to)	**SINCE MARCH 2008**
• Name and address of employer	Hintermann & Weber AG, Ökologische Beratung, Planung und Forschung, Austrasse 2, CH-4153 Reinach
• Type of business or sector	BDM Program – Biodiversity monitoring Switzerland
• Occupation or position held	Scientific collaborator
• Main activities and responsibilities	Monitoring of macroinvertebrate larvae in Switzerland, Z9 indicators. Field and laboratory analyses
• Dates (from – to)	**JUNE - AUGUST 2006 AND JUNE - AUGUST 2007**
• Name and address of employer	Alpine Centre of Biology, Piora, CH-6777 Quinto
• Occupation or position held	Assistant (University of Geneva and High School of Lugano and Bellinzona)
• Main activities and responsibilities	Field and laboratory work
• Dates (from – to)	**NOVEMBER 2005 - JANUARY 2006**
• Name and address of employer	Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2009 Neuchâtel
• Type of business or sector	PhD project on the reproductive behaviour and ecology of Alpine ibex (*Capra ibex*)
• Occupation or position held	Scientific collaborator during the field work in Les Diablerets, VD
• Main activities and responsibilities	Behavioural observations and faecal sample collection
• Dates (from – to)	**MAY - AUGUST 2005**
• Name and address of employer	BirdLife Switzerland, Wiedingstr. 78, CH-8036 Zurich
• Type of business or sector	Species action plan for the Corn Crake in Switzerland
• Occupation or position held	Practical training
• Main activities and responsibilities	Night investigation of meadows for calling male Corn Crakes

• Dates (from – to)	**AUGUST 2004**
• Name and address of employer	University of Pavia, I-27100 Pavia
• Type of business or sector	Master in management and conservation of environment and fauna
• Occupation or position held	Field assistant in the Gran Paradiso National Park, Italy
• Dates (from – to)	**AUGUST 2003**
• Name and address of employer	Cantonal Institute of Microbiology, V. Mirasole 22a, CH-6500 Bellinzona
• Occupation or position held	Practical training
• Dates (from – to)	**2003 - 2011**
• Name and address of employer	Scuola Cantonale Agraria, Mezzana Scuola Cantonale di Commercio, Bellinzona Liceo Cantonale, Bellinzona Scuola Media, Biasca
• Occupation or position held	Temporary teaching positions

EDUCATION AND TRAINING

• Dates (from – to)	**2009-2011**
• Name and type of organisation	Swiss School of Public Health
• Attended courses	- Biostatistics - Multilevel modelling: Analysis of Clustered Data - Epidemiological Concepts and Epidemiological Methods - Disease Ecology and Human-Animal Interfaces
• Title of qualification awarded	Certificate SSPH+
• Dates (from – to)	**2008-2011**
• Name and type of organisation	University of Basel and Swiss Tropical and Public Health Institute, Basel in collaboration with the Cantonal Institute of Microbiology, Bellinzona
• Principal subjects	Epidemiology of multidrug resistant staphylococci in cats, dogs and people in Switzerland
• Title of qualification awarded	PhD
• Dates (from – to)	**2006 – 2007**
• Name and type of organisation	University of Geneva and Cantonal Institute of Microbiology, Bellinzona
• Principal subjects	Eco-physiological behaviour of Lake Cadagno phototrophic sulphur bacteria: distribution in the lake water column and CO_2 assimilation in isolated cultures
• Title of qualification awarded	Advanced master in microbiology and parasitology
• Dates (from – to)	**2004 – 2005**
• Name and type of organisation	Behavioural Ecology Department, University of Zurich in collaboration with the Alpine Wildlife Research Centre, Gran Paradiso National Park, Aosta, Italy
• Principal subjects	Testing the immunocompetence handicap hypothesis in male Alpine ibex (*Capra ibex ibex*)
• Title of qualification awarded	Master thesis
• Dates (from – to)	**2000 – 2005**
• Name and type of organisation	University of Neuchâtel
• Principal subjects	Biology, specializations: Parasitology, Animal Behaviour and Ecological system modelling
• Title of qualification awarded	Master of Science in Parasite Biology
• Dates (from – to)	**1996 – 2000**
• Name and type of organisation	ICEC, Istituto Cantonale di Economia e Commercio, Bellinzona
• Title of qualification awarded	Maturity

PERSONAL SKILLS AND COMPETENCES

MOTHER TONGUE	**ITALIAN**
OTHER LANGUAGES	
	ENGLISH
• Writing skills	good - excellent
• Verbal skills	good
	FRENCH
• Writing skills	excellent
• Verbal skills	excellent
	GERMAN
• *Writing skills*	basic- good
• Verbal skills	good
COMPUTER SKILLS	Word, Excel, PowerPoint, Access, MSProject, EndNote, Bionumerics, SPSS, STATA
SOCIAL SKILLS AND COMPETENCES	Teamwork skills and living in group attitude acquired and trained during the field works with Alpine ibex in the Gran Paradiso National Park and in Les Diablerets. Multidisciplinary teamwork attitude trained during the PhD thesis at the Swiss Tropical and Pubic Health Institute and at the Cantonal Institute of Microbiology in Bellinzona. Communication skills improved during the periods spent as assistant at the Alpine Centre of Biology in Piora as well as during temporary teaching.
ORGANISATIONAL SKILLS AND COMPETENCES	Organisational skills of an epidemiological study: acquired with the organisation of the PhD project consisting in a multicentre study requiring ethical and veterinary clearances. Organisational and communication skills trained during the PhD thesis while co-supervising three master thesis: - in veterinary medicine at the Ecole Nationale Vétérinaire d'Alfort (France) and Bundesamt für Veterinärwesen (BVET- Switzerland) - in pharmacological sciences at the University of Geneva (Switzerland) - in epidemiology at the Swiss Tropical and Public Health Institute, Basel (Switzerland)

ADDITIONAL INFORMATION

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Cresciano 12/12/2011

Paola Gandolfi - Decristophoris