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Short communication/Kurzbericht

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Diversity of antimicrobial-resistant pheno- and genotypes of *Staphylococcus aureus* from clinically healthy cats kept in city households

Vielfalt von Resistenz – Phäno- und Genotypen bei Staphylococcus aureus-Isolaten von klinisch gesunden Katzen aus städtischen Haushalten

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Summary

This study provides detailed information on *Staphylococcus* (*S.*) *aureus* strains isolated from clinically healthy cats and on the drug resistance of these bacteria to different antibiotic classes. A total of 101 isolates of *S. aureus* collected from the groin, conjunctival sacs, nares and anuses of healthy cats between January 2013 and November 2014 were screened for the presence of antibiotic-resistance genes and phenotypic resistance using the disc diffusion method. More than 80% of *S. aureus* isolates contained antimicrobial-resistance genes such as: *bla*Z (100%), *erm*A (92.08%), *erm*B (95.05), *aac(6')le-aph(2")la* (84.16%), *tet*(K) (98.02%) and *tet*(M) (84.16%). The presence of *mec*A gene was detected in 29.7% while only 3.96% were resistant to cefoxitin. About 57% of all isolates in cats showed phenotypic resistance to penicillin. Tetracycline, erythromycin and clindamycin resistance were detected in 7.9%, 15.8% and 9.9% of isolates respectively. Multidrug resistance, according only to the results of the disc diffusion method, was observed in 5 isolates (5.0%%) whereas a significantly larger number of multidrug-resistant strains were obtained using the molecular method (99%). We observed that cats, that had received chemotherapeutics over the course of the previous 12 months, were significantly more frequent carriers ($p = 0.015$) of the multidrug-resistant phenotype of *S. aureus* isolates. The report shows that healthy cats may serve as reservoirs of antimicrobial-resistant *S. aureus* strains that harbour resistance genes also found in isolates from humans.

Keywords: MRSA, pet, multidrug resistance, public health, zoonosis

Zusammenfassung

Das Ziel dieser Studie war es, *Staphylococcus* (*S.*) *aureus*-Stämme, die von klinisch gesunden Katzen isoliert worden waren, zu charakterisieren und die Resistenzlage dieser Bakterien zu untersuchen. Die Studie umfasste 101 zwischen Januar 2013 und November 2014 aus Abstrichen aus Leiste, Bindehautsäcken, Nasenlöchern und Anus der Katzen isolierte *S. aureus*-Stämme. Die Untersuchung auf Antibiotikaresistenzen dieser Bakterienstämme erfolgte phänotypisch mithilfe eines Agardiffusionstests sowie genotypisch durch den Nachweis der Resistenzgene. Mehr als 80 % der *S. aureus*-Isolate enthielten solche Gene für die Resistenz wie: *bla*Z (100 %), *erm*A (92,08 %), *erm*B (95,05 %), *aac(6')le-aph(2")la* (84,16 %), *tet*(K) (98,02 %) und *tet*(M) (84,16 %). Das Auftreten des *mec*A-Gens wurde bei 29,7 % der Isolate entdeckt, während nur 3,96 % die Cefoxitin-Resistenz aufwiesen. Circa 57 % der von den Katzen isolierten Stämme zeigten phänotypisch eine Resistenz gegen Penicillin. Die Resistenz gegen Tetracyclin, Erythromycin und Clindamycin wurde bei 7,9 %, 15,8 % und 9,9 % der Isolate beobachtet. Multiresistenz wurde anhand der Ergebnisse der Agardiffusionstests bei 5 Stämmen festgestellt (5,0 %), wogegen eine deutlich größere Zahl der Stämme mit den molekularbiologischen Methoden genotypisch als Multiresistent identifiziert wurde (99 %). Es konnte festgestellt werden, dass Katzen, die innerhalb von 12 Monaten vor der Pro-

benentnahme Chemotherapeutika verabreicht bekommen hatten, signifikant häufiger Träger (p = 0.015) des multiresistenten Phänotypes von *S. aureus* waren. Die Studie zeigt, dass klinisch gesunde Katzen ein Reservoir für Antibiotika-resistente *S. aureus*-Stämme sein können, die Resistenzgene besitzen, welche auch in von Menschen isolierten
S. aureus-Stämmen gefunden werden.

Schlüsselwörter: MRSA, Haustier, Multiresistenz, öffentliches Gesundheitswesen, Zoonose

Introduction, Material and Methods

Staphylococcus (*S.*) *aureus* is a common commensal microorganism and opportunistic pathogen in humans and animals. These bacteria can colonise individuals without symptoms, for either short or extended periods of time. They can also cause a wide range of infections when the immune system becomes compromised. *S. aureus* of humans and animals are responsible for a wide range of infections, such as those of the respiratory and urinary tracts, skin (wound) infections and bacteraemia (Oliveira et al., 2002). Diagnostic and treatment options for bacterial infections of companion animals frequently include techniques and drugs important in human medicine. The ability of bacteria such as staphylococci to develop resistance to antimicrobial therapies used across a range of species has necessitated an increase in studies of antimicrobial resistance in companion animals. An increasing number of studies report companion animals infected or colonized with clinically and epidemiologically significant pathogenic bacterial strains such as multidrug resistant *S. aureus*, known to be transmissible between species. Thus, responsible use of antimicrobials by veterinarians is essential to contain antimicrobial resistance in pathogens relevant to public health (Grave et al., 2014) .

Many reports focus on antimicrobial patterns and transfer of resistance genes from zoonotic pathogens and commensals in food animals to humans, whereas the role of companion animals in the persistence and dissemination of antimicrobial resistance to humans is less defined (Guardabassi et al., 2004). It is critical for medical and veterinary institutions to partner and collaborate in researching this topic. The objective of this investigation was to find out if house cats are reservoirs of antibiotic-resistant *S. aureus*. This study provides detailed information on *S. aureus* strains isolated from clinically healthy cats and on the drug resistance of these bacteria to different antibiotic classes.

Bacterial strains

A total of 101 archival isolates of *S. aureus* (bacterial glycerol stocks in 15% sterile glycerol, stored at −80°C), collected between January 2013 and November 2014 from clinically healthy cats in the Wrocław city area, were investigated. Only cats without outdoor access were sampled. *S. aureus* strains were isolated from four anatomical locations: conjunctival sacs (n = 30), nares (n = 37), groin (n = 22), and anus (n = 12). Owners of the pets at the time of sample collection were asked to fill out a brief questionnaire investigating factors which could potentially influence the study results, such as age (range from 1 months to 12 years), sex (female n = 30; male n = 38), breed (pure breed n = 36; crossbreed n = 32), the hospitalisation of a household member during the last year (n = 5), the antimicrobial therapy of the individual during the last year (n = 36), and the other animals in the household and their antimicrobial treatment during the last year (n = 35).

Identification of *S. aureus* isolates

Identification of the *S. aureus* strains was performed as previously described (Bierowiec et al., 2016).

Amplification of the short sequence repeat region of the *spa* gene was conducted using specific PCR primers (Harmsen et al., 2003). The thermal cycling conditions were set up according to Shopsin et al. (1999) and the completed reaction mixtures were sent to sequencing services (MagroGene, Netherlands). The sequences were analysed using the Ridom SpaServer (http://spa.ridom.de).

Antimicrobial susceptibility testing

Antimicrobial-resistant phenotyping of isolates was performed by the agar disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute document (CLSI, 2015). Antimicrobial tests were as follows (µg/disc): penicillin G (10), cefoxitin (30), erythromycin (15), clindamycin (2), gentamicin (10), tetracycline (30), norfloxacin (10), chloramphenicol (30), mupirocin (200), fusidic acid (10), vancomycin (30), tigecycline (15) and linezolid (30) (Mast Diagnostics, UK). The double-disc diffusion test (D-test) was performed on all isolates to detect inducible clindamycin resistance. The interpretation of the test was as follows: a flattening of the inhibition zone around the clindamycin disc, near the erythromycin disc, indicated that erythromycin had induced clindamycin resistance ($iMLS_B$). The phenotype $cMLS_B$ was characterised by erythromycin and clindamycin resistance. The phenotype (MS_B) was characterised by clindamycin susceptibility and erythromycin resistance, with a negative D-test (Spode Coutinho et al., 2010).

Additionally 30 randomly selected isolates of *S. aureus* were tested by minimal inhibitory concentration (MIC) to analyse the correlation between the harbouring of antimicrobial genes and phenotypic features. The MIC tests were performed using E-test strips (MIC Test Strip, Liofilchem, Italy): oxacillin (0.16–256), penicillin (0.016–256), erythromycin (0.016–256), clindamycin (0.016–256), gentamicin (0.064–1024), tetracycline (0.016–256), mupirocin (0.064–1024), fusidic acid (0.016256). The antimicrobial-resistant phenotyping of isolates was performed and interpreted according to the Clinical and Laboratory Standards Institute document M100-S25 (CLSI, 2015). Additionally due to lack of the formally validated CLSI zone diameter other references were used to interpretive criteria for fusidic acid, mupirocin and tigecycline (Brink et al., 2012; Coutant et al., 1996; Malaviolle et al., 2008).

TABLE 1: *Primers and optimised PCR conditions used for testing of antimicrobial resistance genes in S. aureus isolates obtained from clinically healthy cats kept in city households*

Gene	Primer pair	Sequence 5'-3'	Amplicon size (bp)	Anneling temperature (Ta °C)	Amplification cycle	Reference for primers
tet(L)	tetL-F	ATAAATTGTTTCGGGTCGGTAAT	1077	54	1	Emaneini et al. (2013)
	tetL-R	AACCAGCCAACTAATGACAATGAT				
tet(K)	tetK-F	TCGATAGGAACAGCAGTA	169	55	2	Rizzotti et al. (2005)
	tetK-R	CAGCAGATCCTACTCCTT				
tet(M)	tetM-F	GTGGACAAAGGTACAACGAG	406	53	2	
	tetM-R	CGGTAAAGTTCGTCACACAC				
tet(O)	tetO-F	AACTTAGGCATTCTGGCTCAC	515	54	2	
	tetO-R	TCCCACTGCTCCATATCGTCA				
*erm*A	ermA-F	TCTAAAAAGCATGTAAAAGAA	645	52	3	
	ermA-R	CTTCGATAGTTTATTAATATTAGT				
*erm*B	ermB-F	GAAAAGGTACTCAACCAAATA	639	52	3	
	ermB-R	AGTAACGGTACTTAAATTGTTTAC				
*erm*C	ermC-F	TCAAAACATAATATAGATAAA	642	52	3	
	ermC-R	GCTAATATTGTTTAAATCGTCAAT				
*van*A	vanA-F	GGGAAAACGACAATTGC	732	54	3	
	vanA-R	GTACAATGCGGCCGTTA				
*van*B	vanB-F	ATGGGAAGCCGATAGTC	635	635	3	
	vanB-R	GATTTCGTTCCTCGACC				
aac(6')Ie-aph(2")Ia	aph-F	GAGCAATAAGGGCATACCAAAAATC	480	54	2	
	aph-R	CCGTGCATTTGTCTTAAAAAACTGG				
blaZ	blaZ-F	ACTTCAACACCTGCTGCTTTC	173	55	2	
	blaZ-R	TGACCSCTTTTATCAGCAACC				
*mec*A	mecA-F	TCCAGATTACAACTTCACCAGG	162	54	2	Oliveira and de Lencastre (2002)
	mecA-R	CCACTTCATATCTTGTAACG				
*mec*C	mecC-F	TCACCAGGTTCAAC[Y]CAAAA	356	55	4	Garcia-Alvarez et al. (2011)
	mecC-R	CCTGAATC[W]GCTAATAATATTTC				
*mup*A	mupA-F	TATATTATGCGATGGAAGGTTGG	457	53	2	Seah et al. (2012)
	mupA-R	AATAAAATCAGCTGGAAAGTGTTG				
*fus*B	fusB-F	CCGTCAAAGTTATTCAATCG	492	50	2	Chen et al. (2010)
	fusB-R	ACAATGAATGCTATCTCGACA				

Amplification cycles used in the study were as follow:

Cycle 1: 94°C for 4 min; 35 cycles of 94°C for 45s, Ta for 45s, 72°C for 90s; 72°C for 7 min.

Cycle 2: 94°C for 4 min; 35 cycles of 94°C for 30s, Ta for 30s, 72°C for 30s; 72°C for 7 min.

Cycle 3: 94°C for 4 min; 35 cycles of 94°C for 45s, Ta for 45s, 72°C for 45s; 72°C for 7 min.

Cycle 4: 94°C for 5 min; 30 cycles of 94°C for 1 min, Ta for 1 min, 72°C for 2 min; 72°C for 7 min.

Antimicrobial-resistant genotypes of isolates were obtained by PCR. The presence of genes involved in resistance to penicillinase (*bla*Z), aminoglycosides (*aac(6')Ie-aph(2")Ia*), ß-lactamase (*mec*A and *mec*C), glycopeptides (*van*A and *van*B), macrolide-lincosamide-streptogramins (*erm*A, *erm*B and *erm*C), tetracyclines (*tet*[K], *tet*[L], *tet*[M] and *tet*[O]), mupirocine (*mup*A) and fusidic acid (*fus*B) was determined by PCR amplification with the use of specific primers and conditions reported in Table 1. As a positive control the following strains were used: *S. aureus* ATCC 43300 for *mec*A, ,*Enterococcus fecium* ATCC 700221 for *van*A and *Enterococcus faecalis* ATCC 512299 for *van*B. Considering the lack of a positive control for residual genes we sequenced one of the representative replicons of an expected size and we have established it as a positive control. The nucleotide sequence of the *erm*B and *erm*C genes has been assigned the GenBank accession number AKS43594 and KP893742. As a negative control *S. aureus* ATCC 29213 was used.

As there is no universally accepted definition of "multiresistance", the interpretation of the results was performed according to the suggestions of Schwarz et al. (2010). Resistance of the phenotypes of *S. aureus* isolates to 3 or more classes of antimicrobial agents was interpreted as multiresistance. The phenotypic susceptibility testing was supplemented with molecular analysis for the resistance genes present, thus multiresistance was also assessed at the molecular level. Bacterial isolates that exhibited the presence of 3 or more resistance genes to different antimicrobial groups were referred to as multiresistant.

Statistical methods

Statistical analysis was carried out using the R statistical package (v2.11.1). The characteristics of the cats and questionnaire answers were compared with scores of antibiotic-resistant phenotypes and genotypes of *S. aureus* isolates. Data was entered into a computerised database and analysed using the Shapiro-Wilk test, the Wilcoxon test, the Kruskal–Wallis test, 2 × 2 contingency

tables and bootstrapped chi-square tests. $P < 0.05$ was considered indicative of a statistically significant association. Residue tables were constructed for statistically significant results and used to detect existing relationships between characteristics. A residue table shows the frequency distribution of the values of the dependent variable, given the occurrence of the independent variable's values.

Results and Discussion

A total of 101 previously confirmed *S. aureus* strains were included in this study. All of them were tested for antimicrobial resistance and typed using the *spa* method. 39 different *spa* types were determined during the study. Most of the isolates were assigned to t091, t008, t4474, t002, t11639 and t8420. There were no differences between the frequency of the *spa* type isolation and the sample collection place.

The antimicrobial resistance profiles of *S. aureus* isolates obtained via PCR were verified by testing each isolate with a suitable antibiotic. A correlation between PCR results and the agar disc diffusion method and E-tests for selected strains, was observed only for some isolates harbouring erythromycin, tetracycline, methicillin and gentamicin resistance genes. Conversely, the majority of strains which harboured genetic determinants of resistance were fully sensitive to the investigated chemotherapeutics. Antimicrobial gene patterns and E-test results for the 30 randomly selected *S. aureus* isolates are presented in Table 2. None of the isolates were resistant to chloramphenicol, linezolid, tigecycline, vancomycin, fusidic acid and mupirocin. The residual results of disc diffusion method are presented in Table 3. Any genetic determinants of resistance to the following antibiotics were not detected: vancomicin (*van*A and *van*B genes) and mupirocin (*mup*A gene). Comparisons of residual results from PCR are presented in Table 4.

There were 3 types of MLS_B resistance observed: 21.43% $iMLS_B$, 64.29% $cMLS_B$ and 14.28% MS_B. More MLS_B induction (78.57%) was caused by *erm*A/B genes (66.67% = $iMLS_B$, 88.89% = $cMLS_B$ and 100% = MS_B). *Erm*A/B/C genes caused 33.33% of $iMLS_B$ and 11.11% of $cMLS_B$.

Multidrug resistance according only to the results of the disc diffusion method was observed in 5 isolates (4.95%). A significantly larger number of multidrug-resistant strains were obtained using the molecular method. There were 99% multiresistant isolates using genotypic characterization methods.

Multivariate analysis revealed that there were no statistically significant differences in age, sex or breed between carriers of multidrug-resistant *S. aureus* strains. In addition, no influence of hospitalisation of the owner, or treatment of co-habiting pets with antimicrobials could be detected. There was an observed correlation between the owners' occupation and the frequency of some genetic determinants of resistance. We observed that in households where owners work in veterinary healthcare, isolated *S. aureus* strains harboured genes such as were frequently statistically significant: *mec*A ($p=0.0477$), *aac(6')Ie-aph(2")Ia* ($p=0.031$), *erm*A ($p=0.041$) and *tet*(O) ($p = 0.0119$). A statistically significant relationship was found between the previous hospitalisation of an owner and the phenotypic resistance to tetracycline in isolates of *S. aureus* strains from his/her cat ($p = 0.05$). Additionally the resistance to tetracycline was frequently observed parallel with a resistance to cefoxitin ($p = 0.006$). We observed that cats that had been treated with chemotherapeutics during the previous year were frequent carriers ($p = 0.003$) of multidrug-resistant phenotypes of *S. aureus* isolates, although such an association was not found on the molecular level ($p = 0.411$). Moreover, treatment of the cat during the previous year had an impact on later phenotypic resistance to clindamycin ($p = 0.015$), whereas treatment of other animals

TABLE 2: *Diversity of spa types and antibiotic resistance patterns of randomly selected isolates of S. aureus obtained from clinically healthy cats kept in city households*

Anatomical location of isolation	*spa* type	Genotypic resistance/Phenotypic resistance
Nares	t002	*bla*Z, *mec*A, *erm*A, *erm*B, *tet*(K), *tet*(M)/PN,
	t005	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet(K)*, *tet*(M)/PN
	t008	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M)/PN
	t008	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *erm*C, *tet*(K), *tet*(M), *tet*(O)/PN
	t037	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN, OX, GN, ER, TET
	t189	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)
	t189	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(L), *tet*(M), *tet*(O)
	t521	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)
	t8420	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M)
	t11455	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
	t12411	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *erm*C, *tet*(K), *tet*(M), *tet*(O)/PN, OX
Conjunctival sacs	t002	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K)/PN
	t005	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *erm*C, *tet*(K), *tet*(L), *tet*(M), *tet*(O)/PN
	t008	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M)/PN
	t091	*bla*Z, *erm*A, *erm*B, *tet*(K), *tet(O)*
	t700	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
	t852	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *erm*C, *tet*(K), *tet*(M), *tet*(O)
	t7482	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)
	t8420	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*B, *tet*(K), *tet*(M), *tet*(O)
	t11455	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
Skin	t425	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet(M)*, *tet*(O)/PN, TET
	t8420	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*B, *tet*(K), *tet*(L), *tet*(M), *tet*(O)
	t8420	*bla*Z, *Meca*, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)
	t1451	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/ER
	t11024	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
	t11639	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
Anus	t008	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
	t008	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN
	t700	*bla*Z, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(O)/PN
	t13105	*bla*Z, *mec*A, *aac(6')Ie-aph(2")Ia*, *erm*A, *erm*B, *tet*(K), *tet*(M), *tet*(O)/PN

Phenotypic resistant according to E-test result: PN – penicillin, OX – oxacillin, GN – gentamicin, ER – erythromycin, TET – tetracycline

TABLE 3: *Percentage of antibiotic resistance according to disc diffusion test in all S. aureus strains isolated from clinically healthy cats kept in city households*

Peinicilin	Cefoxitin	Gentamycin	Erythromycin	Clindamycin	Tetracycline	Norfloksacin
57.43%	3.96%	0.99%	15.84%	9.9%	7.92%	0.99%

TABLE 4: *Percentage of antibiotic resistance genes in all S. aureus strains isolated from clinically healthy cats kept in city households*

*bla*Z	*mec*A	*aac(6')le-aph(2")la*	*erm*A	*erm*B	*erm*C	*tet*K	*tet*L	*tet*M	*tet*O	*fus*B	*mup*A
100%	29.7%	84.16%	92.08%	95.05%	9.9%	98.02%	15.84%	84.16%	71.29%	4.95%	0,99%

in the household was connected with resistance to erythromycin (p = 0.0415), clindamycin (p = 0.046) and the presence of *fus*B genes (p = 0.025). Gene *mec*A was frequently present in isolates from cats younger than 2 years (p = 0.027).

This investigation provides detailed information on *S. aureus* in healthy cats and on drug resistance of these bacteria to different antibiotic classes. The study showed that *Staphylococcus* strains which colonise healthy cats were frequently multi-drug resistant. There was also a large difference in the amount of multidrug resistance on the phenotypic and the genotypic level. This suggests that results based only on phenotypic methods could be undervalued.

We also isolated 39 different *spa* types around one city area. 8 of them – t002, t005, t008, t015, t037, t091, t159 and t233 – were previously reported in Poland (http://www.spatialepidemiology.net/srl-maps and http://spa.ridom.de/frequencies.shtml). The other *spa* types had previously been isolated from a variety of locations around the world (http://spa.ridom.de/frequencies.shtml). Such a diversity of *spa* types had been previously observed in *S. aureus* of pet origin (Vincze et al., 2013). No correlation was found between *spa* type and the multi-drug resistance pattern. Additionally no specific *spa* type was associated with a resistance pattern, and major differences were observed in the distribution of antimicrobial resistance genes and phenotypic susceptibility among the same *spa* type.

The results of this study indicate that healthy cats might be a source of *S. aureus* strains that harbour antimicrobial resistant genes of clinical relevance to human health. Furthermore, the *S. aureus* isolates vary in combinations of antimicrobial-resistant genes, as well as among the same *spa* type. All investigated isolates contained *bla*Z. This gene is one of the most frequently isolated in *S. aureus* strains (Davis et al., 2014), but only half of the isolates examined in this study showed phenotypic resistance to penicillin. It has been previously observed by El Feghaly et al. (2012) that conventional methods for *S. aureus* penicillin susceptibility testing may not reliably detect penicillin resistance in all isolates.

Similar results were found in the frequency of methicillin resistance. There were only four strains where the disc diffusion method had shown resistance to methicillin, whereas the *mec*A gene was identified in 30 isolates using PCR. The difference in results obtained using these methods may reflect a situation where the *mec*A gene is present in genomic DNA but not expressed, or expressed at a low level due to growth conditions (Shahraz et al., 2012). There is a difficulty in comparing our results with other reports because of the methods used. In the USA according to the results of only phenotypic methods, 20% (Abraham et al., 2007) to 32% (Lin et al., 2011) of *S. aureus* strains isolated from clinically healthy and treated cats respectively were MRSA. A study by Kottler et al. (2010) demonstrated that 40% of *S. aureus* strains isolated from cats were MRSA – but only the presence of the *mec*A gene was checked without the confirmation of antibiotic resistance genes expression.

More than 90% of strains harboured macrolide-resistant genes – *erm*A and *erm*B, whereas *erm*C was detected in only 9.9% of isolates in this study. This data is in contrast to the results of Piątkowska et al. (2012) where the *erm*C gene was dominant and *erm*B was detected only once. Also other reports regarding macrolide-resistance genes in *S. aureus* isolates show a dominance of *erm*C or *erm*A genes (Davis et al., 2014) in *Staphylococcus* isolates. The coexistence of two different ribosomal methylases was also more frequent in our study than previously described (Davis et al., 2014; Piatkowska et al., 2012). Distribution of the *erm*B gene in our isolates is more similar to the distribution of this gene in enterococci isolated from healthy cats and dogs (Jackson et al., 2010) therefore this issue should be better investigated in future studies.

In our study, prevalence of the gentamicin-resistant gene was 84.16%, which is comparable to other reports regarding aminoglycoside resistance in clinical samples (Yıldız et al., 2014). Similarly tetracycline resistance genes were common. In our research *tet*(K) was the most widespread, followed by *tet*(M). These results are similar to other reports concerning the distribution of *tet* genes in *S. aureus* of human and animal origin (Schwarz et al., 1998). Usually there was more than one *tet* gene. 7 isolates had all of them. The combination of *tet*(K) and *tet*(M) is the most frequently described in staphylococci (Youn et al., 2014). The proportion of tetracycline-resistant isolates was 7.92%, whereas 98% of isolates harboured one or more genetic determinants of resistance. It is possible that the prevalence of resistance to tetracycline in *S. aureus* has been underestimated owing to the false identification of susceptibility with phenotypic methods (Trzcinski et al., 2000).

There are several antibiotics currently available that are suitable for use in managing MRSA in humans. These include inter alia vancomycin, linezolid, tigecycline, chloramphenicol, mupirocin and fusidic acid (Gould et al., 2009), to which antibiotic resistance in this study was investigated. All strains were sensitive to these chemotherapeutics. In a similar study, where phenotypic and genotypic resistance to vancomycin in companion animals was investigated, no resistant isolates were found as well, (Youn et al., 2014) albeit Ranjbary et al. (2014) reported the dominance of the *van*B gene in *S. aureus* from pets.

Mupirocin and fusidic acid are recommended for the treatment of superficial staphylococcal infections and for decolonisation in dogs and cats (Loeffler et al., 2008). Our study showed full sensitivity to the chemotherapeutics although a low level of mupirocin resistance in isolates from cats was noted by Loeffler et al. (2008). Resistance to fusidic acid in previous studies in Poland and Europe was described as low (1–3%) (Castanheira et al., 2010), while resistance to fusidic acid among staphylococci isolated from dogs with pyoderma ranged from 0.8% to 3.7% (Kruse et al., 1996).

The results of this study indicate a large difference in the amount of multidrug-resistant isolates on the phenotypic and genotypic levels. Results of both phenotypic methods used to antimicrobial resistance testing are comparable and recommended for staphylococci (Gosden et al., 2008; Farahani et al., 2013). A correlation between phenotype and molecular indicators of multidrug resistance was not found but we investigated only selected antimicrobial-resistant genes and chemotherapeutics. Thus the relationship should be further examined. The inconsistency between these results of phenotype and genotype drug susceptibility tests could be explained as differences in resistance gene expression between isolates (Resch et al., 2008) and the PCR results show the inherent potential of the isolate to develop high levels of resistance to antibiotics in the future (Mohanasoundaram and Lalitha, 2008). In our study we noted that almost all *S. aureus* strains harboured 3 or more antimicrobial resistance genes. Multidrug-resistant genes are wide spread in staphylococci, not only of human origin, but they are also common in bacteria from animals (Wendlant et al., 2015). Such a high percentage of multi-drug resistance staphylococci of pet origin was previously described by Wedley et al. (2014). 87.5% and 21.8% of methicilin-resistance coagulase negative and coagulase positive staphylococci respectively, were phenotypicaly multidrug-resistant associated with harbouring *blaZ*, *mec*A, *erm*C or *cfr* genes. We observed that cats, that had received chemotherapeutics over the course of the previous 12 months, were significantly more frequent carriers of the multidrug-resistant phenotype of *S. aureus* isolates. This observation is similar to Loeffler et al. (2011) results, where previous treatment had had an influence on MRSA carriage.

Foregoing reports have shown that companion animals may serve as a reservoir of antimicrobial-resistant genes that can be transferred from pets to people and within the environment (Harrison et al., 2014). This ability of cross-staphylococcal transfer is an additional means to acquire new resistance and virulence genes encoded by mobile genetic elements (McCarthy et al., 2014). It is especially important when the same antimicrobials are used to treat infections in both humans and animals (Jackson et al., 2010). This study has shown that previous a one-year treatment of cat under investigation does influence the phenotypic resistance pattern of *S. aureus* isolates. More studies on staphylococci from healthy companion animals should be performed to fully evaluate the extent of antimicrobial resistance and the genetic diversity of those bacteria. The findings of this study support the need for the "One Health" Initiative, to expand interdisciplinary collaboration and communication in all aspects of health care for humans, animals and the environment (www.onehealthinitiative.com).

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Conflict of interest

The corresponding author (representative for all authors) has to confirm that the authors have no protected, financial, occupational or other personal interests in a product, service and/or a company which could influence the content or opinions presented in the manuscript.

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