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One Health **Bekämpfung und Kontrolle**

Prevalence and identification of critical points for transmission of multidrug-resistant bacteria in small animal clinics - towards evidence-based guidelines for infection prevention and control

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Key words
Multidrug-resistant bacteria, colonization, contamination, resistance genes, risk factors, infection prevention and control standards, hygiene concepts, molecular typing

Aims of the study

1. Investigate the prevalence and risk factors for carriage of multidrug resistant organisms (MDRO) and the role of veterinary clinics as a source of MDRO acquisition in companion animals, veterinary personnel and owners.
2. Assess current standards in IPC in first and second opinion veterinary practices and develop evidence-based hygiene concepts.
3. Gain knowledge on the prevalence, antibiotic resistance and spread of *Macrococcus* spp. in small animals and contact persons.

Material and methods

Rectal and oro-nasal swabs were obtained from cats and dogs presented to 5 veterinary care facilities in Switzerland. Fecal samples and nasal swabs were collected from owners of these animals and clinical staff. Dogs, cats and owners carrying MDRO were resampled for up to 8 months. Rectal swabs were tested for the presence of Gram-negative MDRO using non-selective enrichment in LB followed by selection on CHROMID® ESBL, plates, CHROMID OXA-48, CHROMID CARBA and CHROMID® Colistin R. Nasal swabs were tested for the presence of methicillin-resistant (MR) staphylococci and macrococci using a two-step selective enrichment followed by selection on MRSA selective agar. Samples from predetermined high-touch surfaces were taken in three large clinics, two medium-sized clinics and two primary opinion practices using swabs and processed as above. Isolates were identified to the species level using MALDI ToF (Bruker). Antibiotic susceptibility was tested by the measurement of the minimal inhibitory concentrations of different antibiotics using broth dilution the method and Sensititre plates. Selected strains were tested via rep-PCR or ERIC-PCR and sequenced using Illumina and Oxford Nanopore technologies for clonality and the presence of specific resistance genes. Novel resistance genes of Macrococcus spp and their mobile genetic elements were identified by whole genome analysis obtained with Illumina and Oxford Nanopore technologies.

A structured one-day audit of IPC practices was performed in three large clinics, two medium-sized clinics and two primary opinion practices. A written report was provided including a list of deficiencies that need to be addressed in these facilities. Hand hygiene compliance of the staff was assessed in the

seven clinics/practices by direct observation and according to WHO standards using the CleanHands application.

Results and significance

Specific aim 1: Rectal and nasal/oropharyngeal swabs were collected from 88 cats and 183 dogs presented to 5 veterinary care facilities and 187/271 animals were resampled at discharge (Table 1). The prevalence of MDRO carriage in pets at admission was 15.5% (95%CI 11.4-20.4) with ESBL-producing *E. coli* (5.5%) and MR Coagulase negative Staphylococci (MRCoNS 8.5%) predominating. Overall discharge prevalence was 32.6% (95%CI 26-39.8) but varied significantly between different practices and hospitals (range 17.2-42.7%). Predominant hospital-acquired isolates in the institution with the highest discharge prevalence were: ESBL-producing *E. coli* (16.7%), and ESBL-producing *K. pneumoniae* (13.7%). *E. coli* commonly (82.1%) displayed carbapenemase (CP) encoding genes ($bla_{OXA-181}$, bla_{OXA-48}, bla_{NDM-5}) and showed clonality (ST410, $bla_{OXA-181}$), suggesting transmission from a common source rather than *de novo* selection. Resistant bacteria were isolated from 9/46 owners (22%, 6/10 ESBL-producing *E. coli*; 3/10 MRCoNS; 2/10 MRSA). Persistence of ESBL-producing or CP *E. coli* or *K. pneumoniae* was shown in 7/34 MDRO positive animals for up to 138 days. Transfer of MDRO between pets and owners was not documented. The results of this study showed that veterinary hospitals play a significant role in the selection and transmission of MDRO amongst veterinary patients. However, the risk of colonization of owners via their pets appears low.

Specific aim 2: Assessment of infection prevention and control (IPC) standards in small animal clinics and practices in Switzerland

Of a maximum total IPC score of 68, the institutions reached a median (range) score of 33 (19–55). MDROs were detected in median (range) 8.2% (0–33.3%) of the sampling sites. Clinics with low IPC standards showed extensive environmental contamination, i.e. of intensive care units, consultation rooms and utensils. CPE were detected in two clinics; one of them showed extensive contamination with CP *K. pneumoniae* (ST11, bla_{OXA-48}) and MR *Staphylococcus pseudintermedius* (ST551). Despite low IPC scores, environmental contamination with MDROs was low in primary opinion practices. Three employees were colonized with *E. coli* ST131 ($bla_{CTX-M-15}$, $bla_{CTX-M-27}$, $bla_{CTX-M-14}$). Two employees carried CP *E. coli* closely related to environmental (ST410, $bla_{OXA-181}$) and patient-derived isolates (ST167, bla_{NDM-5}). MRSA (ST225) and MRSP (ST551) of the same sequence types and with similar resistance profiles were found in employees and the environment in two clinics.

Hand hygiene compliance (% [CI]) was low in all seven clinics/practices, with an overall compliance of 32% [30–34], ranging from 26–47% across institutions. Hand hygiene compliance was highest in the consultation area (41% [38–45]) and after contact to body fluids (45% [40–50]), and lowest in the pre-OR area (20% [15–24]) and before clean/aseptic procedure (12% [9–15]). Veterinarians showed a higher HH compliance (37% [34–40]) than veterinary nurses (25% [22–28]).

The study indicates that IPC standards are variable and that hand hygiene compliance is generally low in companion animal clinics and practices in Switzerland. The results suggest that insufficient IPC standards contribute to the evolution of MDROs which can be transferred between the environment and working personnel. The implementation of IPC concepts should thus urgently be promoted and efficient hand hygiene trainings established that include all veterinary personnel.

Specific aim 3: *Macrococcus spp.* as reservoir of antbioic resistance and presence in human infection

Four novel macrolide resistance determinants were identified in *Macrococcus* spp. The novel *msr*(F) *msr*(H) and *mef*(D) were identified on chromosomal macrolide resistance islands McRI$_{msr}$, and the novel *msr*(G), and *mef*(F) on plasmids. One *M. canis* strain harbored a plasmid, which was almost identical to the *mecB*-containing plasmid reported in *S. aureus* suggesting *M. canis* as potential source of *mecB* in staphylococci. Presence of methicillin-resistant *Macrococcus* spp. was rare in animals and clinical environment and not detected in humans. However, *M. canis* was associated in one case of human infections.

Publications, posters and presentations

Infection prevention and control concepts for small animal clinics and practices (finalisation End of February 2020). These guidelines will be made available to veterinarians in Switzerland through the project partners GST and SVK-ASMPA.

Peer reviewed publications

A Endimiani, M Brilhante, O Bernasconi, V Perreten, JS Schmidt, V Dazio, A Nigg, S Gobeli Brawand, SP Kuster, S Schuller B Willi (2019). Employees of Swiss veterinary clinics colonized with epidemic clones of carbapenemase-producing *Escherichia coli.* J. Antimicrob. Chemother. 10.1093/jac/dkz470

A Nigg, M Brilhante, V Dazio, M Clément, A Collaud, S Gobeli, B Willi, A Endimiani, S Schuller, V Perreten (2019). Shedding of OXA-181 carbapenemase-producing *Escherichia coli* from companion animals after hospitalisation in Switzerland: an outbreak in 2018.). Eurosurveillance, 24(39): pii=1900071.

Chanchaithong P, Perreten V, Schwendener S. (2019). *Macrococcus canis* contains recombinogenic methicillin resistance elements and the *mecB* plasmid found in *Staphylococcus aureus*. 2019. J Antimicrob Chemother 74(9): 2531-2536.JS Schmidt, SP Kuster, A Nigg, V Dazio, M Brilhante, H Rohrbach, Helene; Bernasconi, Odette J.; Büdel, Thomas; Campos-Maduenof, Edgar I.; Gobeli Brawand, Stefanie; S Schuller, A Endimiani, V Perreten, B Willi. Poor infection prevention and control standards are associated with environmental contamination with carbapenemase-producing Enterobacterales and other multidrug-resistant bacteria in Swiss companion animal clinics. *Submitted BMC Antimicrobial Resistance and Infection Control*

V Dazio, A Nigg, SJ Schmidt, M Brilhante, M Clément, A Collaud, SP Kuster, S Gobeli Brawand, B Willi, A Endimiani, V Perreten, S Schuller. High rate of acquisition and long term carriage of 3rd generation cephalosporin and carbapenem resistant *Enterobacteriaceae* in dogs and cats treated in small animal care facilities without evidence of transmission to their owners In preparation

JS Schmidt, S Hartnack, S Schuller, A Ebert, SP Kuster, B. Willi. Evaluation of hand hygiene compliance in small animal clinics and practices in Switzerland. In preparation

Presentations

Schuller S. Prevalence, acquisition and persistence of rectal and naso-/oropharyngeal carriage of multidrug-resistant organisms in dogs and cats presented to veterinary practices and their owners. Annual congress of the European College of Veterinary Internal Medicine, Milano (Italy), 19th-21st September, 2019

Willi B. Evaluation of infection prevention and control standards and carriage of multidrug-resistant organisms in working staff in small animal clinics and practices in Switzerland. Annual congress of the European College of Veterinary Internal Medicine, Milano (Italy), 19th-21st September, 2019

Willi B. Evaluation of hand hygiene compliance in small animal clinics and practices in Switzerland using the CleanHands application. JS Schmidt, S Kuster, S. Hartnack, A Ebert, S Schuller, B Willi. Annual congress of the European College of Veterinary Internal Medicine, Milano (Italy), 19th-21st September, 2019

Brilhante M. Whole genome sequencing of high-risk clones of OXA-48-producing *Klebsiella pneumoniae* from the veterinary setting in Switzerland Swiss Society for Microbiology, 3rd-4th September *2019*

Brilhante M. Clinical contamination with high-risk clones of carbapenemase-producing *Klebsiella pneumoniae* causing infection in pets in Switzerland. GCB Students' Symposium, University of Bern, Bern, Switzerland, 30 January 2020.

Perreten V. Carbapenemase producing *Enterobacteriaceae* in animals. One Health meets Sequencing – KKL Lucerne, May 21st 2019

Perreten V. Use of next generation sequencing (NGS) for antibiotic resistance surveillance 3rd International Seminar on Animal and Plant Health (SISA 2019) and the 20th Congress of the Latin American Phytopathological Association, Varadero, Cuba, 6 – 10 May 2019.

Perreten V. Zwanzig Jahre Forschung über Antibiotika-Resistenzen *„Twenty years of technology advancement for the identification of antibiotic resistance".* Veranstaltung der Alumni Vetsuisse-Fakultät Bern, Bern, 16. Mai 2019.

Posters

Brilhante M. Promiscuous carbapenemase-containing plasmid spreads in three different *Enterobacteriaceae* species in a veterinary hospital. Microbiology Society Annual Conference 2020, Edinburgh International Conference Centre (EICC), Edinburgh, Scotland, United Kingdom, 30 March – 3 April 2020.

Fernandez J. Novel plasmid-encoded macrolide resistance genes in *Macrococcus canis*. Annual Swiss Society for Microbiology Meeting, Zurich, Switzerland, 3 – 4 September 2019.

Perreten V. *Macrococcus canis* isolated from a human wound. Annual Swiss Society for Microbiology Meeting, Zurich, Switzerland, 3 – 4 September 2019.

Perreten V. *Staphylococcus aureus* and *Macrococcus canis* share the same multidrug and *mecB*-containing plasmid. 8th Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE 2019), Vinci International Convention Centre, Tours Val de Loire – France, 1 – 3 July 2019.

Perreten V. Whole-genome sequencing of OXA-48-producing *Klebsiella pneumoniae* causing nosocomial infections in pets in Switzerland. 8th Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE 2019), Vinci International Convention Centre, Tours Val de Loire – France, 1 – 3 July 2019.

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