Distribution and antimicrobial resistance profiles of bacterial species in stray cats, hospital-visiting cats, and veterinary staff in South Korea

Woo Kyung Jung
Seoul National University College of Veterinary Medicine
Sook Shin
Seoul National University College of Veterinary Medicine
Young Kyung Park
Seoul National University College of Veterinary Medicine
Suk-Kyung Lim
Animal and Plant Quarantine Agency
Dong-Chan Moon
Animal and Plant Quarantine Agency
Kun Taek Park
Inje University
Yong Ho Park (yhp@snu.ac.kr)
Seoul National University College of Veterinary Medicine https://orcid.org/0000-0002-4168-6884

Research article

Keywords: Cat, Antimicrobial resistance, Staphylococcus spp., Enterobacteriaceae, Enterococcus spp.

Posted Date: November 5th, 2019

DOI: https://doi.org/10.21203/rs.2.15626/v2

Version of Record: A version of this preprint was published at BMC Veterinary Research on April 9th, 2020. See the published version at https://doi.org/10.1186/s12917-020-02326-2.

Abstract

Background Antimicrobial resistance is becoming increasingly important in both human and veterinary medicine. According to the One Health concept, an important step is to monitor the resistance patterns of pathogenic bacteria. In this study, the antimicrobial susceptibility patterns and trends of bacteria isolated from stray cats, hospital-visiting cats, and veterinary staff in South Korea between 2017 and 2018 were investigated. Results The minimum inhibitory concentrations of different antibiotics for Staphylococcus spp., Enterobacteriaceae , and Enterococcus spp. were determined to establish representatives of different antibiotic classes relevant for treatment or surveillance. For Coagulase-positive and Coagulase-negative Staphylococci, resistance to fluoroquinolones was below 13%, but resistance to ampicillin and penicillin was high (20–88%). A total of 9.5%, 12.1%, and 40.3% of staphylococcal isolates from stray cats, hospital-visiting cats, and veterinary staff, respectively, were confirmed to be mecA positive. For Enterobacteriaceae , resistance to carbapenems, fluoroquinolones, and 3rd generation cephalosporins was low (0–11.1%). The Enterococcus spp. isolates showed no resistance to vancomycin. The antimicrobial resistance rates of the Staphylococcus spp. and Enterobacteriaceae isolates from stray cats were usually lower than those of isolates from hospital-visiting cats and veterinary staff, but the Enterococcus spp. isolates revealed the opposite. Thus, the antimicrobial resistance varied across bacterial species according to the source from which they were isolated. Conclusions Resistance to critically important compounds were low. These results emphasize the fact that more attention should be paid to the use of antimicrobials and the occurrence of antimicrobial resistance in cats.

Background

Recently, the term ‘One Health’ was introduced, which states there is actually only ‘one’ health shared by humans, animals, and the ecosystem, and that what affects one affects all these three components [1, 2]. Currently, the One Health concept is mainly advocated with regards to antimicrobial resistance [3]. Therefore, a One Health-based approach to fight antimicrobial resistance is promoted on a global level [4]. To support the application of a One Health-based approach to combat antimicrobial resistance, research on the human-animal-environmental interface is important [3].

Antimicrobials, including agents of importance to human medicine, are commonly used in companion animal-related veterinary practice [5, 6]. Especially, veterinarians and staff in veterinary hospitals are frequently exposed to companion animals; thus, they may also play a role in the spread of microbial diseases, acting as carriers. Transmission of antimicrobial resistance among bacteria from animals and veterinarians has been reported [7, 8]. Multidrug-resistant (MDR) *Staphylococcus* (*pseud*)*intermedius* isolates are increasingly being reported to cause problems in small-animal practices [9-11]. According to Rusher et al., wounds and the ear canal are the most common sites for infections with methicillin-resistant *S.* (*pseud*)*intermedius* [12]. Knowledge about drug resistance trends over time is important to ensure the long-term efficacy of antibacterial products, and data regarding antimicrobial susceptibility could help veterinarians select the most appropriate antibiotic for treatment purposes. Despite the importance of this aspect, standardized, ongoing surveys regarding antimicrobial resistance are barely available.

Companion animals such as dogs and cats not only share a common environment with humans, but are also administered drugs similar to those prescribed to humans. It has been postulated that companion animals may be an integral part of the transfer of drug resistance, owing to their close and direct contact with humans [13]. All kinds of companion animals (owned and stray animals) are involved in the transmission of drug resistance, even if the particular implication of each animal population has not yet been established clearly [14]. However, most of these concerns have been directed towards parasites of stray cats [15, 16]. Further, in Korea, a large number of studies have focused on dogs, rather than cats [17-19]. The rate of pet ownership is increasing globally, as animals enrich the lives of humans. In Korea, it has been estimated that over 29% (a total of 5.74 million) of households own a companion animal, with the estimated number of dogs and cats being 6.32 and 2.43 million, respectively [20]. Especially, the total number of companion cats is increasing rapidly, showing a growth of about 41% in 2017, compared to the statistic mentioned in a study from 2015 [20]. Stray cats that move freely within urban environments have also been shown to exist, although their population in Seoul has decreased from 200,000 in 2015 to 139,000 in 2017 due to the Trap-Neuter-Return program [21]. Therefore, the systematic control and prevention of drug resistance, through the implementation of a national antimicrobial resistance surveillance method, are greatly needed and should be applied in case of both cats and dogs. To our knowledge, this is the first nation-wide cross-sectional study on antimicrobial resistance in isolates from cats and veterinary staff in Korea. This study aimed to determine the antimicrobial susceptibility in the most common bacteria isolated from stray cats, hospital-visiting cats, and veterinary staff.

Results

Bacterial species

A total of 1,829 *Staphylococcus* spp., *Enterobacteriaceae*, and *Enterococcus* spp. isolates were recovered (281 from stray cats, 978 from hospital-visiting cats, and 570 from veterinary staff). Table 2 shows the bacterial species isolated based on their origin (stray cats, hospital visiting cats, and veterinary staff). The most frequently isolated pathogens were *E. coli* in case of stray cats and hospital-visiting cats (n=31, 11% and n=161, 16.5%, respectively) and *S. epidermidis* in case of the veterinary staff (n=89, 15.6%). CNS were isolated at a significantly higher ($P < 0.05$) rate from stray cats (64/78, 82.1%) than from hospital-visiting cats (230/350, 65.7%) and veterinary staff (160/294, 54.4%). Among the staphylococcal isolates, *S. felis* was isolated at a significantly higher frequency ($P < 0.05$) from stray cats and hospital-visiting cats (29/74, 39.2% and 60/264, 22.7%, respectively) than from veterinary staff (6/176, 3.4%). On the contrary, the isolation frequency of *S. epidermidis* from veterinary staff (89/176, 50.6%) was significantly higher ($P < 0.05$) than that from stray cats (6/74, 8.1%) and hospital-visiting cats (39/264, 14.8%).

Antimicrobial susceptibility of *Staphylococcus* spp.

In case of the isolates of CNS from stray cats, the rates of resistance to ampicillin, penicillin, and oxacillin varied between 20 and 34%, and the rates of resistance to amox/clav, gentamicin, and tetracycline were 6–9%. Resistance to clindamycin, enrofloxacin, marbofloxacin, and chloramphenicol was absent or low (0–3%) (Table 4). For cephalexin, no CLSI breakpoints are available. The number of isolates of Coagulase-positive Staphylococci (CPS) from stray cats was too small; hence, the resistance rates of these isolates have not been discussed.

More than half of the isolates of CPS from hospital-visiting cats showed resistance to ampicillin, penicillin, and amox/clav (59–82%) (Table 3). The rates of resistance to oxacillin, enrofloxacin, marbofloxacin, and tetracycline varied between 12 and 29%, and the rates of resistance to gentamicin, clindamycin, and chloramphenicol were lower (6–9%). For the isolates of CNS from hospital-visiting cats, the rates of resistance to ampicillin, penicillin, and oxacillin were between 35 and 60%, and the rates of resistance to amox/clav, gentamicin, and tetracycline were 11–24% (Table 4). The rates of resistance to clindamycin, enrofloxacin, marbofloxacin, and chloramphenicol were low (4–7%) (Table 4). In case of cephalexin, for which no breakpoints were available, the MIC distributions suggest the presence of acquired resistance for some isolates. The rates of resistance to ampicillin and penicillin for isolates from hospital-visiting cats were significantly higher ($P < 0.05$) than those for the isolates from stray cats.

In case of the CNS isolates from the veterinary staff, the rates of resistance to ampicillin, penicillin, oxacillin, and tetracycline varied between 59 and 80%, and the rates of resistance to amox/clav and gentamicin were 38%. The rates of resistance to clindamycin, enrofloxacin, marbofloxacin, and chloramphenicol was low (3–11%) (Table 4). In case of cephalexin, for which no breakpoints were available, the MIC distributions suggest the presence of acquired resistance for some isolates, like the case for the isolates of CNS from the hospital-visiting cats. The rates of resistance to ampicillin, penicillin, oxacillin, amox/clav, gentamicin, enrofloxacin, marbofloxacin, and tetracycline for isolates from the veterinary staff were significantly higher ($P < 0.05$) than those for the isolates from stray cats or hospital-visiting cats.

Antimicrobial susceptibility of *Enterobacteriaceae*

For *Enterobacteriaceae* isolates from stray cats, resistance to ceftiofur, ceftriaxone, gentamicin, imipenem, meropenem, enrofloxacin, marbofloxacin, and tetracycline was absent or low (0–5%). The rate of resistance to cefoxitin was shown to be 13%. The rates of resistance to ampicillin, amox/clav, and cephalothin varied between 72 and 100% (Table 5).

For *Enterobacteriaceae* isolates from hospital-visiting cats, resistance to gentamicin, imipenem, meropenem, enrofloxacin, and marbofloxacin was absent or low (0–6%). The rates of resistance to cefoxitin, ceftiofur, ceftriaxone, and tetracycline ranged from 10 to 22%. The rates of resistance to ampicillin, amox/clav, and cephalothin varied between 76 and 99% (Table 5). Tetracycline resistance in isolates from hospital-visiting cats was significantly higher ($P < 0.05$) than that in isolates from stray cats.

For *Enterobacteriaceae* isolates from veterinary staff, resistance to ceftiofur, ceftriaxone, gentamicin, imipenem, meropenem, enrofloxacin, and marbofloxacin was absent or low (0–8%). The rates of resistance to cefoxitin and tetracycline were between 36 and 44%, and were significantly higher ($P < 0.05$) than those of isolates from stray cats or hospital-visiting cats. The rates of resistance to ampicillin, amox/clav, and cephalothin varied between 88–98% (Table 5).

Antimicrobial susceptibility of *Enterococcus* spp.

For *Enterococcus* spp. isolates from stray cats, rates of resistance to erythromycin, tetracycline, and minocycline were between 51 and 68%; however, resistance to ampicillin, vancomycin and high levels of resistance to streptomycin was absent or low (0–9%) (Table 6). The rates of resistance to penicillin, chloramphenicol, and ciprofloxacin, and high levels of resistance to gentamicin were between 16 and 21%.

For *Enterococcus* spp. from hospital-visiting cats, rates of resistance to erythromycin, tetracycline, and minocycline were between 39 and 62%; however, resistance to vancomycin and high levels of resistance to streptomycin were absent or low (0–9%) (Table 6). The rates of resistance to ampicillin, penicillin, chloramphenicol, and ciprofloxacin, and high levels of resistance to gentamicin were between 11 and 20%.

For *Enterococcus* spp. from veterinary staff, rates of resistance to erythromycin, tetracycline, and minocycline were between 25 and 50%; however, resistance to ampicillin, penicillin, vancomycin, chloramphenicol, ciprofloxacin and high levels of resistance to both streptomycin and gentamicin were absent or low (0–10%) (Table 6).

mecA gene detection

In total, 110 (21.4%) *Staphylococcus* spp. isolates harbored the *mecA* gene: 7 (9.5%) from stray cats, 32 (12.1%) from hospital-visiting cats, and 71 (40.3%) from veterinary staff. The rates of *mecA* among the staphylococcal isolates from the veterinary staff were higher than those among the staphylococcal isolates from the stray cats or hospital-visiting cats ($P < 0.05$). *S. epidermidis* was the most prevalent bacterium among the isolates from both the hospital-visiting cats and veterinary staff (15/46.9% and 53/74.7%, respectively) (data not shown). The oxacillin-resistance rates among the isolates of CNS were higher than those among the isolates of CPS from the hospital-visiting cats and veterinary staff ($P < 0.05$). Generally, the susceptibility ranges of the *mecA*-containing staphylococci isolated from the stray cats, hospital-visiting cats, and veterinary staff were similar. The rates of resistance to ampicillin and penicillin were between 84.4 and 98.6%, and the rates of resistance to amox/clav, gentamicin, and tetracycline were between 28.6 and 88.7%. Resistance to clindamycin, enrofloxacin, marbofloxacin, and chloramphenicol was absent or low (0–15.5 %) (Table 7).

ESBL and carbapenemase detection

Among the 278 *Enterobacteriaceae* isolates, 1 *K. pneumoniae* isolate from a stray cat and 1 *K. pneumoniae*, 1 *Enterobacter asburiae*, 1 *Enterobacter cloacae*, and 11 *E. coli* isolates from hospital-visiting cats fulfilled the selection criteria for being termed as ESBL-producing bacteria. Among them, five *E. coli* isolates from hospital-visiting cats were confirmed to possess both bla_{TEM} and $bla_{CTX\text{-}M}$, while one *K. pneumoniae* isolate from a stray cat and one *K. pneumoniae* isolate from a hospital-visiting cat possessed only bla_{TEM}. None of the isolates possessed bla_{SHV}. A total of eight isolates that satisfied the ESBL-producing bacteria selection criteria were negative for all resistance genes tested.

None of the *Enterobacteriaceae* isolates that were not susceptible to imipenem or meropenem showed enhanced growth in the modified Hodge test.

Phenotypic resistance patterns derived from human clinical breakpoints

Overall, 15% (9/60) of the isolates of CPS, 25.3% (115/454) of the isolates of CNS, 48.6% (135/278) of the *Enterobacteriaceae* isolates, and 27.9% (63/226) of the *Enterococcus* spp. isolates were susceptible to all antimicrobial agents tested. Additionally, 30% (18/60) of the isolates of CPS, 33% (150/454) of the isolates of CNS, 25.5% (71/278) of the *Enterobacteriaceae* isolates, and 29.2% (66/226) of the *Enterococcus* spp. isolates showed MDR. The MDR rates of the isolates of CNS obtained from the veterinary staff (92/160, 57.5%) were significantly higher ($P < 0.05$) than those of the isolates of CNS obtained from the stray cats (7/64, 10.9%) or hospital-visiting cats (51/230, 22.2%) (Table 8). The MDR rates of the isolates of CNS obtained from the hospital-visiting cats were also significantly higher ($P < 0.05$) than those of the isolates of CNS obtained from the stray cats. The MDR rates of *Enterobacteriaceae* isolates obtained from veterinary staff (22/50, 44%) were significantly higher ($P < 0.05$) those of *Enterobacteriaceae* isolates obtained from the stray cats (4/39, 10.3%) or hospital-visiting cats (45/189, 23.8%) (Table 9). On the contrary, the MDR rates of *Enterococcus* spp. isolates obtained from the veterinary staff (1/20, 5.0%) were significantly lower ($P < 0.05$) than those of *Enterococcus* spp. isolates obtained from the stray cats (18/57, 31.6%) or hospital-visiting cats (47/149, 31.5%) (Table 10).

The most common resistance profile among the CNS isolates was BLA-OXA, which was detected in 57 isolates of CNS, while the most common MDR profile was AMG-BLA-BLI-OXA-TET, which was observed in 4 isolates of CNS obtained from the hospital-visiting cats and 24 isolates of CNS obtained from the veterinary staff. Among the *Enterobacteriaceae* isolates, the most common resistance profile was BLA-BLI-GC, which was detected in 1 isolates of *Enterobacteriaceae* obtained from the stray cats, 11 isolates from hospital-visiting cats, and 9 isolates from the veterinary staff. In the *Enterococcus* spp. isolates, the most common resistance profile was MAC-TET, which was detected in 29 isolates of *Enterococcus* spp., while the most common MDR profile was MAC-PNC-TET, which was observed in 5 isolates of *Enterococcus* spp. obtained from the stray cats, 8 isolates from the hospital-visiting cats, and 9 isolates from the veterinary staff.

Discussion

In this study, we report the findings of the first nation-wide cross-sectional study of antimicrobial resistance in *Staphylococcus* spp., *Enterobacteriaceae*, and *Enterococcus* spp. isolates from stray cats, hospital-visiting cats, and veterinary staff in South Korea. Among our culture collection, the most prevalent bacterial species in the stray cats and hospital-visiting cats was *E. coli*, followed by *S. felis* and *E. faecalis*. Interestingly, the detection rate of CNS was significantly higher among isolates from stray cats ($P < 0.05$) than that among the isolates from hospital-visiting cats and veterinary staff. This may be caused by the poor, unhygienic surroundings of the stray cats. Among the samples from the veterinary staff, *S. epidermidis* was the major species isolated, followed by *E. coli* and *Serratia marcescens*. The most commonly identified staphylococcal species among isolates from both the stray cats and hospital-visiting cats was *S. felis*; this is in accordance with a previous German study, which showed that half of the *Staphylococcus* spp. isolates obtained were *S. felis*, but inconsistent with the results of studies in USA and South Africa, which have shown that *S.* (*pseud*)*intermedius* and *S. epidermidis* were the representative staphylococci present in cats [27-29]. *S. felis* has been regarded as a normal commensal organism present on the skin, the conjunctival sac and eyelid margins, and in the saliva of normal healthy cats, as well as an etiological agent that causes skin infections such as pyoderma and otitis [30, 31]. On the other hand, the most frequently isolated staphylococcal species among samples from the veterinary staff was *S. epidermidis*, which is consistent with the results of other studies [32, 33]. This is presumably due to the different host specificities of the staphylococcal species [8].

Comparison of the results obtained in this study with those from international studies are difficult to interpret due to differences between the study design, drugs tested, breakpoint determination, and temporal or geographic variation. According to the ComPath project of Europe, *Pasteurella* spp. has been reported to be the major dermatological bacterial pathogen in cats, followed by *S.* (*pseud*)*intermedius* and *S. aureus* [34]. For *S.* (*pseud*)*intermedius*, the rates of resistance to penicillin, gentamicin, and chloramphenicol were 16–23%, while the rates of resistance to oxacillin, amox/clav, enrofloxacin, and marbofloxacin were 8–11%. In comparison, in case of *S. aureus*, high rates of resistance to penicillin (62%), as well as low rates of resistance to oxacillin, gentamicin, enrofloxacin, marbofloxacin, and chloramphenicol (0–7%) were found. In our study, the isolates of CPS which are including both of *S.* (*pseud*)*intermedius* and *S. aureus* obtained from the stray cats and hospital-visiting cats showed higher rates of resistance to penicillin, oxacillin, and amox/clav than those observed in case of the isolates analyzed in the ComPath study. However, we observed slightly lower levels of resistance to enrofloxacin and marbofloxacin among isolates of stray cats than those observed in the ComPath study.

The isolates of CNS showed higher rates of resistance to oxacillin than the isolates of CPS from the stray cats, hospital-visiting cats and veterinary staff. This has been previously reported in case of studies on both human and veterinary medicine [8, 35]. The isolates of CPS showed higher resistance than CNS to most of the tested antimicrobials except oxacillin; additionally, the isolates of CPS from stray cats showed significantly higher resistance to ampicillin, penicillin, amox/clav, and tetracycline, and those from hospital-visiting cats showed significantly higher resistance to ampicillin, penicillin, and amox/clav ($P < 0.05$).

In this study, 7 staphylococcal isolates from stray cats and 32 from hospital-visiting cats were identified to harbor the *mecA* gene; this corresponds to 9.5% of the isolates from the stray cats and 12.1% of the isolates from the hospital-visiting cats, respectively. These statistics are similar with those of the ComPath study, which showed that 10.3% of the isolates from cats harbored the *mecA* gene [34]. Among the 176 staphylococcal isolates from the veterinary staff, 71 (40.3%) possessed the *mecA* gene; this finding is similar to that of our previous study, which showed that the prevalence of the *mecA* gene in *S.* (*pseud*)*intermedius* isolates from veterinary staff was 35% [36].

In an Australian study, 341 clinical *E. coli* isolates were collected from cats [37]. In this study, lower rates of resistance to cefoxitin, ceftiofur, ceftriaxone, gentamicin, imipenem, enrofloxacin, marbofloxacin, and tetracycline (0–10%) and higher rates of resistance to ampicillin, amox/clav, and cephalothin (15–100%) were observed. This is similar to the results of our study, except for the fact that in our study, the rates of resistance to ampicillin, cephalothin, cefoxitin and tetracycline were found to be higher.

Among the *Enterococcus* spp. isolates, the most prevalent species was *E. faecalis*, followed by *E. faecium*; this is in accordance with a German study, which showed that *E. faecalis* represented the vast majority of the *Enterococcus* spp. isolates (26 of 29 isolates) [29]. No *Enterococcus* spp. isolates were found to be resistant to vancomycin, a critically important drug whose use is strongly discouraged because it can be considered to be 'reserved for the treatment of serious MRSA infections in humans' [38].

Enterobacteriaceae and *Enterococcus* spp. were isolated from the samples of veterinary staff only at a low frequency; this may be caused by sampling bias, for e.g., bias due to the fact that only the skin and nasal swab samples were included.

Although limited data are available for comparison, *E. coli* isolates from hospitalized dogs have been found to be more resistant than those from stray dogs [17]. Similarly, the frequency of the resistance to every antimicrobial tested in this study was higher in case of the isolates of CNS from hospital-visiting cats than in case of those from stray cats; the isolates of CNS from hospital-visiting cats showed significantly higher levels of resistance to penicillin and tetracycline than those from stray cats ($P < 0.05$). The number of isolates of CPS was too small to perform a comparison. The resistance rates of *Enterobacteriaceae* and *Enterococcus* spp. isolates from stray cats and hospital-visiting cats were similar. The stray cats tested in this study could not be simply assumed to be healthy cats, because the health status of the stray cats was unavailable; however, it is true that the hospital-visiting cats had a greater chance of receiving medication. This could be the reason why the isolates of CNS from hospital-visiting cats showed higher levels of drug resistance. However, why there were no differences between the drug resistance rates of the *Enterobacteriaceae* and *Enterococcus* spp. isolates from stray cats and hospital-visiting cats was not understood; this finding has been considered to be a good indicator of the selection pressure caused by the use of antimicrobials [39].

Interestingly, the MDR rates of CNS and *Enterobacteriaceae* isolates from the veterinary staff were significantly higher than those of the isolates from both the stray cats and hospital-visiting cats ($P < 0.05$). These results support the hypothesis that veterinary staff may serve as possible reservoirs for the dissemination of multidrug resistance in veterinary hospitals. This highlights the importance of vigilance by veterinary staff. Veterinary staff may serve as carriers for the pathogens; in this study, it was observed that the veterinary staff from whom the bacterial isolates were obtained showed no clinical signs of diseases. The MDR rates of CNS and *Enterobacteriaceae* isolates from the stray cats were significantly lower than that of the isolates from the hospital-visiting cats ($P < 0.05$). This may reflect the fact that stray cats only had a rare opportunity to receive medication. However, it has been known that stray dogs show a higher rate of parasitic infection than housed dogs; this may be due to the scavenging habits of stray dogs, which make them more vulnerable to natural infection than housed dogs [14, 16].

The MDR rate of *Enterobacteriaceae* isolates from hospital-visiting cats was 23.8%, which was much higher than that seen in case of the Australian study (11.7% among *E. coli* isolates from cats) [37].

The ESBL detection rates among the isolates were as follows: 2.6% (1/39) of the isolates from stray cats, 3.2% (6/189) of the isolates from hospital-visiting cats, and 0% of the isolates from veterinary staffs. Among them, five *E. coli* isolates from hospital-visiting cats were confirmed to harbor both

bla$_{TEM}$ and *bla*$_{CTX-M}$, while one *K. pneumoniae* isolate from a stray cat and one *K. pneumoniae* isolate from a hospital-visiting cat were observed to harbor only *bla*$_{TEM}$. None of the isolates possessed *bla*$_{SHV}$. Since 2000, CTX-M β-lactamases have been identified as the most widespread type of ESBLs, replacing classical TEM- and SHV-type ESBLs [40]. The ComPath study has reported a low prevalence of ESBL-producing bacteria in dogs (2.8%) and the absence of ESBL-producing bacteria in cats [34]. However, a previous Korean study has shown a higher prevalence of ESBL-producing isolates in dogs and cats (29.2% in dogs and 13.5% in cats) [41]. The differences in their prevalence may be caused by the use of an ESBL-selective agar (CHROMagar ESBL). AmpC β-lactamases were not investigated in this study; they need to be focused on in future studies.

Conclusion

Staphylococcus spp., and *Enterobacteriaceae* isolates from stray cats and hospital-visiting cats in Korea showed higher resistance to most of the tested antimicrobials, compared to the isolates from cats in European countries and Australia. Additionally, some isolates showed resistance against antimicrobials that are regarded as critically important for use in humans, such as third-generation cephalosporins and quinolones. It is unclear if the higher resistance to certain kinds of antimicrobials was attributed to a result of misuse of antimicrobials in animal hospitals because the treatment history of cats was not collected in the current study. Nevertheless, considering the risk of cross-transmission of resistant bacteria between cats and humans, emergence and dissemination of antimicrobial resistance in cat will undoubtedly continue to be a challenge in veterinary medicine, from both patient health and public health standpoint. More organized surveillance is required to better understand the mechanism of antimicrobial resistance transmission between veterinary and human medicine, in accordance with the One Health perspective.

Methods

Sample collection

A total of 2,278 samples were obtained in convenience from 78 stray cats (n=333), 350 hospital-visiting cats (n=1,357), and 294 veterinary hospital staff including veterinarians, veterinary technicians, and receptionists (n=588) across 20 veterinary hospitals in Seoul, Gangwon-do, Gyeonggi-do, Chungcheong-do, Gyeongsang-do, Jeolla-do, and Jeju-do between 2017 and 2018 (Table 1). The stray cats were from the trap-neuter-return program. The captured cats were sent to the local veterinary hospital and the sampling was performed immediately after their arrival. Samples from the 333 stray cats and 1,357 hospital-visiting cats were obtained from the anus (n=425), horizontal ear canal (n=416), nasal mucosa (n=417), skin (n=384), and urine (n=48). All samples were collected by skilled veterinarians without anesthesia. The captured stray cats were released after the neutering. The hospital-visiting cats were proceeded to the appropriated treatment after sampling. Human samples were taken from the hand (palm and the skin between fingers; n=294) and nasal cavity (n=294). All samples from the cats and humans were obtained using BD BBL Culture Swabs (Becton-Dickinson, Sparks, MD, USA), placed on ice, and transported to our lab within 6 h of their collection.

Bacterial isolation and identification

The swabs were directly plated on 5% defibrinated sheep blood agar (Hangang, Gunpo, South Korea) and incubated overnight at 37ºC for 24 h under aerobic and anaerobic conditions. All the colonies that showed different morphologies and hemolysis patterns were collected and subjected to Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (microflex LT/SH spectrometer Bruker, Bruker Daltonics, Bremen, Germany) for their identification. One bacterial species from each individual was selected for further analysis. *Staphylococcus* spp., *Enterobacteriaceae*, and *Enterococcus* spp. which were most frequently isolated bacteria were included for this study. Both *Staphylococcus intermedius* and *S. pseudintermedius* were designated as "*S.* (*pseud*)*intermedius*" because of the difficulty associated with their differentiation.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations were determined using the standardized agar dilution methodology, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines [22, 23]. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were included as quality control strains in the tests, as recommended by the CLSI guidelines. The antimicrobial susceptibility data of the isolates were obtained for the following antibiotics: gentamicin from the aminoglycoside except streptomycin (AMG); streptomycin (STM); ampicillin, and penicillin from the β-lactam (BLA) groups; oxacillin (OXA); amoxicillin/clavulanate (amox/clav, ratio 2:1) from the β-lactam/β-lactamase inhibitor combination (BLI); enrofloxacin, marbofloxacin, and ciprofloxacin from the fluoroquinolones (FQN); clindamycin from the lincosamide (LIN); chloramphenicol from the phenicol (PNC); cephalexin, cephalothin, and cefoxitin from the 1st and 2nd generation cephalosporins (GC); ceftiofur, and ceftriaxone from the 3rd generation cephalosporins (3GC); imipenem, and meropenem from the carbapenems (CPM); minocycline and tetracycline from the tetracyclines (TET); and vancomycin from the glycopeptide (GLP). The concentration ranges indicated in Tables 3–6. The isolates were defined as MDR if they showed resistance to at least one drug from three or more antimicrobial classes using human clinical breakpoints, as previously described [24].

Data analyses

The MIC_{50} and MIC_{90} values of the tested antimicrobials were determined for *Staphylococcus* spp., *Enterobacteriaceae*, and *Enterococcus* spp., which were the major bacterial species isolated. Based on the MICs, the isolates were categorized as susceptible, intermediate, or resistant to the antibiotics for which CLSI veterinary breakpoints [23] or breakpoints based on human data [22] are available. The CLSI susceptibility and resistance breakpoints utilized are indicated in Tables 3–6.

The isolation and antimicrobial resistance rates of each bacterial species from different origins (stray cats, veterinary hospital visiting cats, and veterinary hospital staff) were analyzed and compared by Fisher's exact test or Chi square test. All statistical analyses were performed using MedCalc statistical software ver. 11. 2. 1 (MEDCALC, Acacialaan, Belgium). In all tests, *P* values less than 0.05 were considered significant.

Detection of the *mecA* gene

All *S.* (*pseud*)*intermedius* and coagulase-negative Staphylococci (CNS) isolates for which the MICs of oxacillin were ≥ 0.5 μg/ml and all *S. aureus* isolates for which the MICs of oxacillin were ≥ 4 μg/ml [23] were screened by PCR for the presence of the *mecA* gene according to a method adapted from Oliverira D. C. et al. [25]. Concurrently, the *mecA*-positive strain *S. aureus* ATCC 43300 was used as a quality control organism.

Detection of extended spectrum β-lactamases (ESBLs) and carbapenemase

The mechanisms underlying the resistance of *Enterobacteriaceae* towards β-lactams were further characterized. The presence of ESBLs was tested by a combined double-disc test using clavulanic acid and cephalosporin indicators (cefotaxime and cefrazidime), according to the CLSI guidelines [22]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as a quality control strains for a combined double-disc test. Genotypic characterization of the ESBL-positive strains was achieved by performing PCR to detect the major groups of genes encoding β-lactamases, including $bla_{CTX\text{-}M}$, bla_{SHV}, and bla_{TEM} [26].

All *Enterobacteriaceae* strains that were not susceptible to imipenem or meropenem were tested by performing the modified Hodge test according to the CLSI guidelines [22]. This test represents a simple method for detecting carbapenemase-producing *Enterobacteriaceae*.

Abbreviations

MIC: Minimum inhibitory concentration; MDR: Multidrug-resistant; CLSI: Clinical and Laboratory Standards Institute; CNS: Coagulase-negative Staphylococci; CPS: Coagulase-positive Staphylococci; AMG: aminoglycosides except streptomycin; STM: streptomycin; BLA: β-lactam groups; OXA: oxacillin; BLI: β-lactam/β-lactamase inhibitor combinations; FQN: fluoroquinolones; LIN: lincosamides; PNC: phenicols; GC: 1st and 2nd generation cephalosporins; 3GC: 3rd generation cephalosporins; CPM: carbapenems; TET: tetracyclines; GLP: glycopeptides; amox/clav: amoxicillin/clavulanate; ESBL: extended spectrum β-lactamases

Declarations

Ethics approval and consent to participate

The experiment was approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-170809-4) and Seoul National University Institutional Review Board (1608/001-004). Individual written informed consent for the use of samples was obtained from all cat owners and veterinary staff.

Consent for publication

Not applicable.

Availability of data and materials

Data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture and Forestry (Z-1543081-2017-18-03) and the Cooperative Research Program of Center for Companion Animal Research (Project No. PJ013985022019) of Rural Development Administration,

South Korea.

Authors' contributions

WKJ analyzed MIC data and drafted the manuscript; SS and YKP was responsible for laboratory testing of bacterial isolates; SKL and DCM contributed to the study design; KTP analyzed statistic and drafted the manuscript; YHP contributed to the study design and drafted the manuscript.

Acknowledgements

The authors wish to thank Seong Mi Nho and Se Ra Shin for their outstanding technical assistance.

References

1. Perez A, Pierce WJS. One Environmental Health: an emerging perspective in toxicology. F1000Res. 2018;7:918.
2. Gibbs EP. The evolution of One Health: a decade of progress and challenges for the future. Vet Rec. 2014;174(4):85-91.
3. Sikkema R, Koopmans M. One Health training and research activities in Western Europe. Infect Ecol Epidemiol. 2016;6:33703.
4. WHO. Global action plan on antimicrobial resistance. 2015. https://www.who.int/antimicrobial-resistance/global-action-plan/en/. Accessed 14 May 2019
5. Buckland EL, O'Neill D, Summers J, Mateus A, Church D, Redmond L, Brodbelt D. Characterisation of antimicrobial usage in cats and dogs attending UK primary care companion animal veterinary practices. Vet Rec. 2019;179(19):489.
6. Pedersen K, Pedersen K, Jensen H, Finster K, Jensen VF, Heuer OE. Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. J Antimicrob Chemother. 2007;60(4):775-781.
7. Youn JH, Hwang SY, Kim SH, Koo HC, Shin S, Lim SK, Park YH. *mecA* gene transferrability and antibiogram of zoonotic *Staphylococcus intermedius* from animals, staff and the environment in animal hospitals in Korea. J Microbiol Biotechnol. 2010;20(2):425-432.
8. Moon BY, Youn JH, Shin S, Hwang SY, Park YH. Genetic and phenotypic characterization of methicillin-resistant staphylococci isolated from veterinary hospitals in South Korea. J Vet Diagn Invest. 2012;24(3):489-498.
9. van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyorala S, Ruzauskas M, Sanders P, Threlfall EJ, Torren-Edo J *et al*. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother. 2011;66(12):2705-2714.
10. Lehner G, Linek M, Bond R, Lloyd DH, Prenger-Berninghoff E, Thom N, Straube I, Verheyen K, Loeffler A. Case-control risk factor study of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infection in dogs and cats in Germany. Vet Microbiol. 2014;168(1):154-160.
11. Weese JS, van Duijkeren E. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol. 2010;140(3-4):418-429.
12. Ruscher C, Lubke-Becker A, Wleklinski CG, 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(1-2):197-201.
13. So JH, Kim J, Bae IK, Jeong SH, Kim SH, Lim SK, Park YH, Lee K. Dissemination of multidrug-resistant *Escherichia coli* in Korean veterinary hospitals. Diagn Microbiol Infect Dis. 2012;73(2):195-199.
14. Sowemimo OA. The prevalence and intensity of gastrointestinal parasites of dogs in Ile-Ife, Nigeria. J Helminthol. 2009;83(1):27-31.
15. Dashti A, Santin M, Cano L, de Lucio A, Bailo B, de Mingo MH, Koster PC, Fernandez-Basterra JA, Aramburu-Aguirre J, Lopez-Molina N *et al*. Occurrence and genetic diversity of Enterocytozoon bieneusi (Microsporidia) in owned and sheltered dogs and cats in Northern Spain. Parasitol Res. 2019, in press.
16. Fu Y, Huang Y, Abuzeid AMI, Hang J, Yan X, Wang M, Liu Y, Sun Y, Ran R, Zhang P *et al*. Prevalence and potential zoonotic risk of hookworms from stray dogs and cats in Guangdong, China. Vet Parasitol Reg Stud Reports. 2019;17:100316.
17. Nam HM, Lee HS, Byun JW, Yoon SS, Jung SC, Joo YS, Lim SK. Prevalence of antimicrobial resistance in fecal *Escherichia coli* isolates from stray pet dogs and hospitalized pet dogs in Korea. Microb Drug Resist. 2010;16(1):75-79.
18. Kang JH, Chung TH, Hwang CY. Clonal distribution of methicillin-resistant *Staphylococcus pseudintermedius* isolates from skin infection of dogs in Korea. Vet Microbiol. 2017;210:32-37.
19. Chung YS, Hu YS, Shin S, Lim SK, Yang SJ, Park YH, Park KT. Mechanisms of quinolone resistance in *Escherichia coli* isolated from companion animals, pet-owners, and non-pet-owners. J Vet Sci. 2017;18(4):449-456.
20. Ji I, Kim H, Kim W, Seo G (2017) Development Strategies for the Companion Animal Industry. In: Korea Rural Economic Institute. 11-13.
21. Cho YJ, Lee YA, Hwang BR, Han JS, Hwang JS. Stray cat inhabitation status monitoring in Seoul. In: Seoul city; 2017. P. 59-60.
22. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Approved Standard. 27th ed. Wayne, Pennsylvania, CLSI; 2017.
23. CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptivility Tests for Bacteria Isolated from Animals. 3rd ed. Wayne, Pennsylvania, CLSI; 2015.
24. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired

resistance. Clin Microbiol Infect. 2012;18(3):268-281.

25. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2002;46(7):2155-2161.
26. Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of *bla*SHV, *bla*TEM and *bla*CTX-M genes in *Enterobacteriaceae*. APMIS. 2007;115(12):1400-1408.
27. Lane MJ, Roy AF, Kearney MT, Pucheu-Haston CM. Characterization, distribution, antimicrobial resistance and resistance risk factors in staphylococci isolated from cats from 2001 to 2014. Vet Med Sci. 2018;4(4):315-325.
28. Qekwana DN, Sebola D, Oguttu JW, Odoi A. Antimicrobial resistance patterns of *Staphylococcus* species isolated from cats presented at a veterinary academic hospital in South Africa. BMC Vet Res. 2017;13(1):286.
29. Teichmann-Knorrn S, Reese S, Wolf G, Hartmann K, Dorsch R. Prevalence of feline urinary tract pathogens and antimicrobial resistance over five years. Vet Rec. 2018;183(1):21.
30. Espinola MB, Lilenbaum W. Prevalence of bacteria in the conjunctival sac and on the eyelid margin of clinically normal cats. J Small Anim Pract. 1996;37(8):364-366.
31. 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(1-2):182-188.
32. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27(4):870-926.
33. Xu Z, Shah HN, Misra R, Chen J, Zhang W, Liu Y, Cutler RR, Mkrtchyan HV. The prevalence, antibiotic resistance and *mecA* characterization of coagulase negative staphylococci recovered from non-healthcare settings in London, UK. Antimicrob Resist Infect Control. 2018;7:73.
34. Ludwig C, de Jong A, Moyaert H, El Garch F, Janes R, Klein U, Morrissey I, Thiry J, Youala M. Antimicrobial susceptibility monitoring of dermatological bacterial pathogens isolated from diseased dogs and cats across Europe (ComPath results). J Appl Microbiol. 2016;121(5):1254-1267.
35. Garza-Gonzalez E, Morfin-Otero R, Llaca-Diaz JM, Rodriguez-Noriega E. Staphylococcal cassette chromosome *mec* (SCC *mec*) in methicillin-resistant coagulase-negative staphylococci. A review and the experience in a tertiary-care setting. Epidemiol Infect. 2010;138(5):645-654.
36. Youn JH, Yoon JW, Koo HC, Lim SK, Park YH. Prevalence and antimicrogram of *Staphylococcus intermedius* group isolates from veterinary staff, companion animals, and the environment in veterinary hospitals in Korea. Vet Diagn Invest. 2011;23(2):268-274.
37. Saputra S, Jordan D, Mitchell T, Wong HS, Abraham RJ, Kidsley A, Turnidge J, Trott DJ, Abraham S. Antimicrobial resistance in clinical *Escherichia coli* isolated from companion animals in Australia. Vet Microbiol. 2017;211:43-50.
38. Hillier A, Lloyd DH, Weese JS, Blondeau JM, Boothe D, Breitschwerdt E, Guardabassi L, Papich MG, Rankin S, Turnidge JD *et al*. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). Vet Dermatol. 2014;25(3):163-e143.
39. Caprioli A, Busani L, Martel JL, Helmuth R. Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int J Antimicrob Agents. 2000;14(4):295-301.
40. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect. 2012;18(7):646-655.
41. Hong JS, Song W, Park HM, Oh JY, Chae JC, Shin S, Jeong SH. Clonal Spread of Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* Between Companion Animals and Humans in South Korea. Front Microbiol. 2019;10:1371.

Tables

Table 1. Geographical distribution of each samples from Korean veterinary hospitals (stray cats, hospital-visiting cats, and veterinary staff).

	Stray cat		Hospital-visiting cat		Veterinary staff	
	Individual no.	Sample no.	Individual no.	Sample no.	Individual no.	Sample no.
Seoul	35	140	220	880	221	442
Gangwon-do	22	88	39	149	32	64
Gyeonggi-do			78	266	4	8
Chungcheong-do			10	50	10	20
Gyeongsang-do	20	100			20	40
Jeolla-do			3	12	6	12
Jeju-do	1	5			1	2
Total	78	333	350	1357	294	588

Table 2. Bacterial isolates from 78 stray cats, 350 hospital-visiting cats, and 294 veterinary staff

Species identified		Stray cat (78)	Hospital-visiting cat (350)	Veterinary staff (294)
Staphylococcus spp.		74	264	176
CPS	*S. aureus*	5	29	10
	S. (pseud)intermedius	3	5	5
	S. hyicus	2		1
CNS	*S. capitis*		7	4
	S. caprae	1	2	
	S. chromogenes	1	1	1
	S. cohnii	2	5	3
	S. condimenti		2	
	S. epidermidis	6	39	89
	S. equorum	1	8	1
	S. felis	29	60	6
	S. gallinarum			1
	S. haemolyticus		5	14
	S. hominis	3	17	4
	S. lugdunensis		1	5
	S. nepalensis	1	1	1
	S. pettenkoferi		6	1
	S. saprophyticus		14	9
	S. schleiferi	3	3	4
	S. sciuri	6	6	1
	S. simulans	9	26	6
	S. warneri	1	6	7
	S. xylosus	1	21	3
Enterobacteriaceae		39	189	50
Citrobacter braakii		1	1	
C. spp.		1	4	
Enterobacter aerogenes			4	12
Ent. asburiae			2	
Ent. cloacae		1	2	
Ent. hormaechei		1		
Ent. kobei				1
Ent. ludwigii			2	
Ent. spp.		1		
Escherichia coli		31	161	18
Klebsiella pneumoniae		2	4	2
K. variicola			4	
Serratia liquefaciens			1	
Serratia marcescens			1	17
Serratia spp.		1		
Enterococcus spp.		57	149	20
Enterococcus avium		3	5	
E. casseliflavus		2	2	2
E. durans				1
E. faecalis		25	74	9
E. faecium		13	38	5
E. gallinarum		4	7	
E. hirae		10	22	3
E. saccharolyticus			1	
Others		74	215	45
Total		281	978	570

Table 3. MICs of 11 antimicrobial agents against 57 Coagulase-positive Staphylococci[1] isolated in this study

Antimicrobials	MIC_{50}	MIC_{90}	MIC values (μg/ml)																		
	(μg/ml)	(μg/ml)	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	% R	% I	% S
Ampicillin[2]	1/2/1[3]	8/16/4		0/2/0		1/1/0	2/4/2	0/2/2	1/2/2	1/4/2	2/9/6	1/3/1	1/1/0	0/4/1	1/1/0	0/1/0		70/74/69	0/0/0	30/27/31
Penicillin[4]	1/2/2	16/16/16	1/0/0		0/1/0	1/2/0	1/3/2	0/2/1	0/3/1	2/3/3	1/6/4	0/6/2	2/1/0	1/4/2	0/2/1	1/1/0		70/82/88	0/0/0	30/18/13
Oxacillin[4]	0.25/0.5/0.5	32/16/4				2/1/0	1/2/2	2/4/2	2/17/6	1/3/1	0/3/2	0/0/2		0/1/0	2/1/1	0/2/0		20/21/29	0/0/0	80/80/65
Amox/clav	1/1/0.5	2/4/4			2/2/0		0/3/1	1/6/6	1/3/1	4/14/6	1/2/0	0/0/2	1/0/0		0/1/0			60/59/50	10/9/6	30/32/44
Cephalexin[5]	4/4/4	64/16/8							1/0/0	1/2/0	3/2/4	1/17/9	2/8/1	0/2/0		2/2/1	0/1/0			
Gentamicin[4]	0.25/0.5/0.5	0.5/8/32				3/4/2		3/2/1	3/17/8	0/4/2	0/3/0		0/2/1	0/1/0	1/1/2			10/6/13	0/6/6	90/88/81
Clindamycin[2]	0.12/0.12/0.12	1/0.5/≥ 64				2/10/2	4/20/9	1/0/0	0/1/0	2/0/0	0/0/2			1/0/1		0/3/2		10/9/19	20/0/13	70/91/69
Enrofloxacin	0.12/0.25/0.25	0.25/4/4		1/0/1		2/6/0	5/10/5	1/11/6	0/3/1	0/0/1	1/0/0	0/2/1		0/1/0	0/0/1	0/1/0		0/12/13	10/0/6	90/88/81
Marbofloxacin	0.25/0.5/0.25	0.5/4/4			1/0/1		1/0/0	6/15/8	1/15/4		1/0/1	0/1/1		0/1/1	0/2/0			0/12/13	10/0/6	90/88/81
Chloramphenicol[4]	8/8/8	8/16/64							1/0/0		2/1/0	0/8/5	7/21/6	0/1/4	0/2/0	0/1/3		0/9/19	0/3/13	100/88/69
Tetracycline[2]	0.5/0.5/0.5	64/64/64				0/2/0		3/11/3	211/6	1/3/2			0/1/0	1/1/0	0/0/1	3/5/3	0/0/1	50/29/44	20/32/38	30/38/19

R, resistant; I, Intermediate; S, susceptible; MIC, minimum inhibitory concentration.

MIC_{50}, lowest concentration to inhibit 50% of bacteria; MIC_{90}, lowest concentration to inhibit 90% of bacteria.

The dilution ranges tested are those contained in the white area. Values above this range indicate MIC values higher than the highest concentration within the range. Values below this range indicate MIC values lower than the lowest concentration within the range. Breakpoints are employed according to VET01S document. When available, susceptible and resistance breakpoints are indicated in vertical dotted and solid lines respectively. For antibiotics without intermediate zone, a single vertical solid line is indicated. For oxacillin, breakpoint of *S. aureus* is indicated in vertical doubled solid line and breakpoint of *S.* (*pseud*)*intermedius* and *S. hyicus* is in vertical doubled dotted line.

[1]10 isolates from stray cats, 31 isolates from hospital-visiting cat, and 16 isolates from hospital staff

[2]Only CLSI breakpoint for dog isolates available.

[3]Indicates isolates numbers from stray cat, hospital-visiting cat and hospital staff, respectively.

[4]The breakpoints derived from human breakpoints used [22].

[5]No CLSI breakpoint available.

Table 4. MICs of 11 antimicrobial agents against 454 Coagulase-negative Staphylococci[1] isolated in this study.

Antimicrobials	MIC_{50}	MIC_{90}	MIC values (μg/ml)																	
	(μg/ml)	(μg/ml)	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	% R	% I	% S
Ampicillin[2]	0.06/0.25/0.5[3]	1/2/16		8/11/4		25/45/14	10/44/21	8/50/22	5/31/19	4/16/21	4/16/21	0/5/15	0/3/6	0/2/7	0/6/7	0/1/3		20/35/62	0/0/0	80/65/38
Penicillin[4]	0.06/0.25/1	1/2/16	10/19/2		11/13/6	14/36/15	7/23/9	8/51/22	3/32/21	5/16/18	3/18/24	1/7/16	1/6/9	1/5/6	0/1/6	0/3/6		34/60/80	0/0/0	66/40/20
Oxacillin[4]	0.25/0.25/2	2/8/≥ 64		2/5/0		14/28/8	15/47/24	11/44/14	7/34/10	8/21/13	3/14/14	0/7/27	1/8/19	1/5/6	0/8/4	2/9/21		34/46/71	0/0/0	66/54/29
Amox/clav	0.12/0.12/0.5	1/0.5/2			27/48/16	1/3/1	19/73/21	10/51/34	3/26/28	2/19/33	0/2/11	2/3/8	0/3/3	0/0/2	0/2/3			6/13/38	5/11/18	89/76/45
Cephalexin[5]	2/2/8	16/16/32				0/2/2		0/1/3	2/3/1	17/41/10	31/102/38	7/39/19	3/13/19	2/11/31	1/6/20	1/3/3	0/9/9			
Gentamicin[4]	≤ 0.06/≤ 0.06/1	16/16/≥ 64				54/158/61	0/2/0	3/14/12	0/5/4	1/2/4	1/3/1	0/6/9	0/14/9	3/5/10	1/13/20	1/8/30		8/11/38	0/6/6	92/83/57
Clindamycin[2]	0.12/0.12/0.12	1/0.5/2		1/12/7		26/67/49	19/90/70	5/21/4	9/14/6	2/8/5	0/2/4	0/6/4	1/2/2	1/0/2		0/8/7		3/7/9	3/4/6	94/89/85
Enrofloxacin	0.12/0.12/0.25	0.25/1/4		2/681		25/58/24	21/89/50	14/39/23	2/14/14	0/12/26	0/4/4	0/2/3		0/0/7	0/4/8	0/2/0		0/4/11	0/7/19	100/90/70
Marbofloxacin	0.25/0.05/0.25	0.5/1/8			0/3/5	0/1/0	16/30/7	42/134/76	6/35/25	0/16/28	0/3/4		0/1/7	0/2/6	0/3/5	0/2/0		0/4/11	0/1/3	100/95/86
Chloramphenicol[4]	4/4/4	8/8/8							0/2/1		10/37/	42/125/94	11/54/42	0/1/2	1/3/1	0/5/4	0/3/0	2/5/3	0/0/1	98/95/96
Tetracycline[2]	0.25/0.25/2	16/32/≥ 128				9/21/1		32/96/40	17/58/24	1/15/7	1/5/12	0/4/3	0/0/4	1/2/5	2/7/9	1/15/25	0/7/30	9/24/59	27/25/15	64/51/26

R, resistant; I, Intermediate; S, susceptible; MIC, minimum inhibitory concentration.

MIC_{50}, lowest concentration to inhibit 50% of bacteria; MIC_{90}, lowest concentration to inhibit 90% of bacteria.

The dilution ranges tested are those contained in the white area. Values above this range indicate MIC values higher than the highest concentration within the range. Values below this range indicate MIC values lower than the lowest concentration within the range. Breakpoints are employed according to VET01S document. When available, susceptible and resistance breakpoints are indicated in vertical dotted and solid lines respectively. For antibiotics without intermediate zone, a single vertical solid line is indicated.

[1]10 isolates from stray cats, 31 isolates from hospital-visiting cat, and 16 isolates from hospital staff

[2]Only CLSI breakpoint for dog isolates available.

[3]Indicates isolates numbers from stray cat, hospital-visiting cat and hospital staff, respectively.

[4]The breakpoints derived from human breakpoints used [22].

[5]No CLSI breakpoint available.

Table 5. MICs of 13 antimicrobial agents against 278 *Enterobacteriaceae*[1] isolated in this study

Antimicrobials	MIC_{50}	MIC_{90}	MIC values (µg/ml)																			
	(µg/ml)	(µg/ml)	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	% R	% I	% S
Ampicillin[2]	4/4/4[3]	≥128/≥128/≥128					0/2/0		0/0/1		0/1/1	6/37/9	23/62/14	2/16/2	1/6/6	1/9/1	2/5/5	4/51/11		100/99/98	0/0/0	0/1/2
Amox/clav	8/8/16	32/32/64				0/1/1		0/2/0	0/0/1	1/1/0	0/1/1	0/13/1	15/50/12	13/65/4	4/25/7	3/13/3	0/8/17	3/10/3		97/98/96	3/1/0	0/2/4
Cephalexin[4]	8/8/32	≥128/≥128/≥128									0/2/1	1/1/0	5/31/1	21/81/12	7/35/7	1/9/11	1/1/11	3/29/7				
Cephalothin[2]	8/8/32	≥64/≥64/≥64						0/1/0		0/1/1	0/1/1	1/5/1	10/38/3	20/81/9	3/20/3	0/8/3	5/34/29			72/76/88	26/20/6	3/4/6
Cefoxitin[5]	4/4/16	16/≥128/≥128									1/1/0	5/16/2	14/88/8	13/46/9	1/7/13	0/3/2	0/3/1	5/25/15		13/16/36	3/4/26	85/8038/
Ceftiofur[6]	0.5/0.5/0.5	2/4/2				0/3/1		2/3/0	6/40/17	22/76/14	6/38/12	2/8/3	0/2/0	0/3/1	0/1/0	1/0/2	0/1/0	0/14/0		3/10/6	0/1/0	97/89/94
Ceftriaxone[5]	≤0.015	1/4/0.12			22/124/36		13/28/8	1/10/2	1/1/0	0/3/0	1/2/0		0/3/0	0/1/1	0/1/2	1/0/0	0/16/1			3/11/8	0/0/0	97/89/92
Gentamicin[2]	1/1/0.5	2/2/1				0/3/1			1/0/1	4/18/25	30/124/18	3/29/1	0/6/1		0/16/0		1/1/3	0/5/0		3/5/6	0/3/2	97/92/92
Imipenem[5]	0.25/0.25/0.5	0.5/0.5/1			0/6/3		2/3/0	15/58/3	14/84/13	7/23/18	0/9/11	0/6/1	1/0/1							3/0/2	0/3/2	97/97/96
Meropenem[5]	≤0.015/≤0.015/0.06	0.12/0.25/0.25			27/132/16		4/15/18	5/16/3	3/21/8	0/1/3	0/1/0	0/0/1		0/3/1						0/2/2	0/0/2	100/98/96
Enrofloxacin	0.015/0.03/0.06	0.25/0.5/0.5			19/77/9	14/57/16	2/14/14	0/15/5	1/7/0	1/3/2	1/5/2	0/0/1	1/0/0		0/8/1	0/3/0				3/6/2	33/3/6	95/92/92
Marbofloxacin	0.015/0.015/0.03	0.12/0.5/0.5	0/5/0		24/104/16	10/37/10	2/7/16	0/4/1	1/12/1	1/6/3	0/2/2	1/1/1	0/1/0	0/6/0	0/1/0	0/3/0				0/6/0	3/1/2	97/94/98
Tetracycline[5]	2/2/2	16/128/128					0/1/1		0/2/0	1/3/1	14/53/5	21/85/18	1/3/1	0/1/2		0/1/1	1/10/13	0/14/7	1/16/1	5/22/44	0/1/4	95/78/52

R, resistant; I, Intermediate; S, susceptible; MIC, minimum inhibitory concentration.

MIC_{50}, lowest concentration to inhibit 50% of bacteria; MIC_{90}, lowest concentration to inhibit 90% of bacteria.

The dilution ranges tested are those contained in the white area. Values above this range indicate MIC values higher than the highest concentration within the range. Values below this range indicate MIC values lower than the lowest concentration within the range. Breakpoints are employed according to VET01S document. When available, susceptible and resistance breakpoints are indicated in vertical dotted and solid lines respectively.

[1]39 isolates from stray cats, 189 isolates from hospital-visiting cat, and 50 isolates from hospital staff

[2]Only CLSI breakpoint for dog isolates available.

[3]Indicates isolates numbers from stray cat, hospital-visiting cat, and hospital staff, respectively.

[4]No CLSI breakpoint available.

[5]The breakpoints derived from human breakpoints used [22].

[6]Only CLSI breakpoint for cattle isolates available.

Table 6. MICs of 10 antimicrobial agents against 226 *Enterococcus* spp.[1] isolated in this study

Antimicrobials	MIC_{50}	MIC_{90}	MIC values (µg/ml)															
	(µg/ml)	(µg/ml)	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	% R	% I	% S
Ampicillin	2/2/1[2]	8/64/4		0/4/1		8/10/4	11/44/5	30/62/7	2/9/3	1/4/0	0/1/0		0/2/0	0/4/0	5/9/0	9/11/0	0/0/0	91/89/100
Penicillin	4/4/4	64/32/8			3/7/1		5/6/3	5/13/2	23/58/9	12/38/4	3/8/1	0/6/0	1/2/0	5/11/0		16/18/5	0/0/0	84/82/95
Vancomycin	1/1/1	4/4/2		0/7/1		14/22/5	30/63/7	7/28/5	1/21/1	5/8/1						0/0/0	9/5/5	91/95/95
Erythromycin	8/2/2	≥256/≥256/4		16/34/7		1/9/1	5/17/0	4/18/4	2/13/3	1/1/2		4/1/0	0/1/0	0/3/0	24/52/3	51/39/25	19/32/35	30/29/40
Chloramphenicol	8/8/4	64/32/16				0/2/1		5/15/0	20/49/9	8/38/7	12/19/2	5/15/0	7/11/1			21/17/5	21/13/10	58/70/85
Ciprofloxacin	1/2/1	4/4/2			14/35/7		22/39/7	12/45/4	4/4/2	1/5/0		1/6/0	3/0/0			16/20/10	21/30/20	63/50/70
Tetracycline	32/32/1	≥256/128/128		8/29/3		4/18/6	1/4/1	1/2/0	2/1/0	2/2/0	1/4/1	13/25/4	6/18/1	11/36/4	8/10/0	68/62/50	4/1/0	28/36/50
Minocycline	16/16/0.25	32/32/16	14/42/9		2/5/2	1/10/1	0/1/1		3/5/0	3/4/0	15/42/5	19/39/2	0/1/0			60/55/35	5/3/0	35/42/65
Streptomycin[3]							52 (< 2,000)						5 (> 2,000)			9/9/5		
Gentamicin[4]							47 (< 500)						10 (> 500)			18/17/5		

R, resistant; I, Intermediate; S, susceptible; MIC, minimum inhibitory concentration.

MIC_{50}, lowest concentration to inhibit 50% of bacteria; MIC_{90}, lowest concentration to inhibit 90% of bacteria.

The dilution ranges tested are those contained in the white area. Values above this range indicate MIC values higher than the highest concentration within the range. Values below this range indicate MIC values lower than the lowest concentration within the range. The breakpoints derived from human breakpoints used. When available, susceptible and resistance breakpoints are indicated in vertical dotted and solid lines respectively. For antibiotics without intermediate zone, a single vertical solid line is indicated.

[1]57 isolates from stray cats, 149 isolates from hospital-visiting cat, and 20 isolates from hospital staff

[2]Indicates isolates numbers from stray cat, hospital-visiting cat, and hospital staff, respectively.

[3]High-level resistance to streptomycin (2,000 µg/ml).

[4]High-level resistance to gentamicin (500 µg/ml).

Table 7. MICs of 11 antimicrobial agents against 110 *mecA*-positive staphylococci isolated in this study.

Antimicrobials	stray cats (7)					hospital-visiting cats (32)					veterinary staff (71)				
	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	% R	% I	% S	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	% R	% I	% S	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	% R	% I	% S
Ampicillin[1]	2	32	85.7	0	14.3	2	32	84.4	0	15.6	2	16	88.7	0	11.3
Penicillin[2]	1	≥ 64	85.7	0	14.3	2	16	96.9	0	3.1	2	32	98.6	0	1.4
Oxacillin[2]	2	≥ 64	100	0	0	16	≥ 64	100	0	0	4	≥ 64	100	0	0
Amox/clav	1	8	57.1	28.6	14.3	1	8	53.1	37.5	9.4	1	4	66.2	19.7	14.1
Cephalexin[3]	16	64				16	64				16	64			
Gentamicin[2]	0.25	32	28.6	0	71.4	8	≥ 64	40.6	18.8	40.6	32	≥ 64	60.6	5.6	33.8
Clindamycin[1]	0.5	16	14.3	28.6	57.1	0.12	4	12.5	0	87.5	0.12	8	15.5	5.6	78.9
Enrofloxacin	0.25	2	0	14.3	85.7	0.25	4	12.5	15.6	71.9	0.5	16	14.1	32.4	53.5
Marbofloxacin	0.25	2	0	14.3	85.7	0.5	16	12.5	0	87.5	0.5	8	14.1	5.6	80.3
Chloramphenicol[2]	4	32	14.3	0	85.7	4	8	3.1	0	96.9	4	8	2.8	1.4	95.8
Tetracycline[a]	32	64	57.1	28.6	14.3	1	64	53.1	25	21.9	64	≥ 128	88.7	5.6	5.6

R, resistant; I, Intermediate; S, susceptible; MIC, minimum inhibitory concentration.

MIC_{50}, lowest concentration to inhibit 50% of bacteria; MIC_{90}, lowest concentration to inhibit 90% of bacteria.

Breakpoints are employed according to VET01S document.

[1]Only CLSI breakpoint for dog isolates available.

[2]The breakpoints derived from human breakpoints used [22].

[3]No CLSI breakpoint available.

Table 8. The most prevalent resistance profile per antimicrobial category found in Coagulase-negative Staphylococci isolated in this study based on CLSI human clinical breakpoint data.

No. antimicrobial category	No. of isolates (%)	Resistance pattern (no. of isolates)		
		stray cats (n=64)	hospital-visiting cats (n=230)	veterinary staff (n=160)
All susceptible	115 (25.3)	31	64	20
1	109 (24.0)	OXA (11)	BLA (37)	BLA (18)
2	80 (17.6)	BLA-OXA (4)	BLA-OXA (38)	BLA-OXA (15)
3	54 (11.9)	AMG-BLA-OXA (1)	BLA-BLI-OXA (14)	BLA-BLI-OXA (9)
4	38 (8.4)	BLA-BLI-OXA-TET (2)	AMG-BLA-OXA-TET (3)	AMG-BLA-OXA-TET (8)
5	39 (8.6)	-	AMG-BLA-BLI-OXA-TET (4)	AMG-BLA-BLI-OXA-TET (24)
6	18 (4.0)	AMG-BLA-BLI-OXA-LIN-TET (1)	AMG-BLA-BLI-OXA-FQN-TET (2)	AMG-BLA-BLI-OXA-FQN-TET (6)
7	3 (0.7)	-	AMG-BLA-BLI-OXA-LIN-PNC-TET (2)	AMG-BLA-BLI-OXA-FQN-LIN-TET (2)
Non-MDR	304 (67.0)	57 (89.1[1])	179 (77.8[1])	68 (42.5[1])
MDR	150 (33.0)	7 (10.9[1])	51 (22.2[1])	92 (57.5[1])

Antimicrobial categories included: aminoglycosides, AMG (gentamicin); β-lactam groups, BLA (ampicillin and penicillin); Oxacillin, OXA; β-lactam/β-lactamase inhibitor combination, BLI (amoxicillin/clavulanate); fluoroquinolones, FQN (enrofloxacin and marbofloxacin); lincosamide, LIN (clindamycin); phenicol, PNC (chloramphenicol); and tetracycline, TET

[1]Percentages

Table 9. The most prevalent resistance profile per antimicrobial category found in *Enterobacteriaceae* isolated in this study based on CLSI human clinical breakpoint data.

No. antimicrobial category	No. of isolates (%)	Resistance pattern (no. of isolates)		
		stray cats (n = 39)	hospital-visiting cats (n = 189)	veterinary staff (n = 50)
All susceptible	135 (48.6)	29	99	7
1	32 (11.5)	BLA (2)	BLA (9)	BLA (3)
2	40 (14.4)	BLA-BLI (1)	BLA-TET (8)	GC-TET (9)
3	48 (17.3)	BLA-BLI-GC (1)	BLA-BLI-GC (11)	BLA-BLI-GC (9)
4	12 (4.3)	BLA-BLI-GC-CPM (1)	BLA-BLI-GC-3GC (3)	AMG-BLA-BLI-TET (1)
5	9 (3.2)	BLA-BLI-GC-3GC-TET (1)	BLA-BLI-GC-3GC-TET (4)	-
6	2 (0.7)	-	AMG-BLA- GC-3GC-FQN-TET (1)	-
Non-MDR	207 (74.5)	35 (89.7[1])	144 (76.2[1])	28 (56.0[1])
MDR	71 (25.5)	4 (10.3[1])	45 (23.8[1])	22 (44.0[1])

Antimicrobial categories included: aminoglycosides, AMG (gentamicin); β-lactam group, BLA (ampicillin); β-lactam/β-lactamase inhibitor combination, BLI (amoxicillin/clavulanate); 1st and 2nd generation cephalosporins, GC (cephalexin, cephalothin and cefoxitin); 3rd generation cephalosporins, 3GC (ceftiofur and ceftriaxone); fluoroquinolones, FQN (enrofloxacin and marbofloxacin); carbapenems, CPM (imipenem and meropenem); and tetracycline, TET

[1]Percentages

Table 10. The most prevalent resistance profile per antimicrobial category found in *Enterococcus* spp. isolated in this study based on CLSI human clinical breakpoint data.

No. antimicrobial category	No. of isolates (%)	Resistance pattern (no. of isolates)		
		stray cats (n = 57)	hospital-visiting cats (n = 149)	veterinary staff (n = 20)
All susceptible	63 (27.9)	13	43	7
1	50 (22.1)	TET (8)	TET (23)	TET (6)
2	47 (20.8)	MAC-TET (10)	MAC-TET (16)	MAC-TET (6)
3	31 (13.7)	MAC-PNC-TET (5)	MAC-PNC-TET (8)	-
4	14 (6.2)	STM-MAC-PNC-TET (2)	AMG-MAC-PNC-TET (4)	AMG-MAC-PNC-TET (1)
5	12 (5.3)	AMG-BLA-FQN-MAC-TET (2)	AMG-BLA-FQN-MAC-TET (7)	-
6	6 (2.6)	AMG-STM-BLA-FQN-MAC-TET (2)	AMG-STM-BLA-FQN-MAC-TET (2)	-
7	3	-	AMG-STM-BLA-FQN-MAC-PNC-TET (3)	-
Non-MDR	160 (70.8)	39 (68.4[1])	102 (68.5[1])	19 (95.0[1])
MDR	66 (29.2)	18 (31.6[1])	47 (31.5[1])	1 (5.0[1])

Antimicrobial categories included: aminoglycosides except streptomycin, AMG (gentamicin); streptomycin, STM; β-lactam groups, BLA (ampicillin and penicillin); glycopeptide, GLP (vancomycin); fluoroquinolone, FQN (ciprofloxacin); macrolide, MAC (erythromycin); phenicol, PNC (chloramphenicol); and tetracycline, TET

[1]Percentages