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China antimicrobial resistance surveillance network for pets (CARPet), 2018 to 2021

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Abstract

China Antimicrobial Resistance Surveillance Network for Pets (CARPet) was established in 2021 to monitor the resistance profiles of clinical bacterial pathogens from companion animals. From 2018 to 2021, we recovered and tested 4,541 isolates from dogs and cats across 25 Chinese provinces, with *Escherichia coli* (18.5%) and *Staphylococcus pseudintermedius* (17.8%) being the most predominant bacterial species. The Enterobacterales were highly susceptible to tigecycline, meropenem, colistin, and amikacin (70.3%–100.0%), but showed moderate resistance to ampicillin, ceftriaxone, doxycycline, florfenicol, levofloxacin, enrofloxacin, and trimethoprim-sulfamethoxazole (29.3%–56.7%). About 66.3% of *Acinetobacter* spp. were resistant to florfenicol, with relatively low resistance to another 11 antibiotics (1.2%–23.3%). The *Pseudomonas* spp. showed high susceptibility to colistin (91.7%) and meropenem (88.3%). The coagulase-positive *Staphylococcus* spp. showed higher resistance rates to most antimicrobial agents than coagulase-negative *Staphylococcus* isolates. However, over 90.0% of *Staphylococcus* spp. were susceptible to linezolid, daptomycin and rifampin, and no vancomycin-resistant isolates were detected. *E. faecium* isolates demonstrated higher resistance rates to most antimicrobial agents than *E. faecalis* isolates. *Streptococcus* spp. isolates showed low resistance to most antimicrobial agents except for doxycycline (78.2%) and azithromycin (68.8%). Overall, the tested clinical isolates showed high rates of resistance to commonly used antimicrobial agents in companion animals. Therefore, it is crucial to strengthen the monitoring of bacterial resistance in pets. By timely and effectively collecting, analyzing, and reporting antimicrobial resistance dynamics in pets, the CARPet network will become a powerful platform to provide scientific guidance for both pet medical care and public health.

Keywords Antimicrobial resistance, Surveillance network, Pets, China, Susceptibility testing

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Introduction

Antimicrobial resistance (AMR) has been recognized a major threat to public health [1]. AMR surveillance is crucial in supporting the development of prudent treatment strategies for bacterial infections, as well as promoting antimicrobial stewardship. The Global Action Plan on Antimicrobial Resistance has acknowledged the urgent need for more coordinated and harmonized surveillance systems to monitor AMR at national, regional, and global levels across the medical, veterinary, and agricultural sectors [2, 3]. To address this need, countries and organizations have implemented a set of bacterial resistance surveillance systems, such as the Global Antimicrobial Resistance and Use Surveillance System (GLASS) [4], the European Antimicrobial Resistance Surveillance Network (EARS-Net) [5], and national antimicrobial resistance monitoring systems in the United States [6] and Japan [7, 8]. In China, two well-established bacterial resistance surveillance systems for human clinical isolates have been in place since 2005: the China Antimicrobial Resistance Surveillance System (CARSS) and the China Antimicrobial Surveillance Network (CHINET) [9–11]. Additionally, in 2008, the Ministry of Agriculture and Rural Affairs launched an antimicrobial resistance surveillance program for food animals, including mainly pigs, chickens, ducks and cattle [12].

Although the bacterial resistance surveillance systems have been well-established for human and farm animals, little attention has been given to antimicrobial surveillance in bacteria from pets. As human companions and an important part of family life, pets are being increasingly raised, with more than 878 million pets kept worldwide in 2021, including 112 million dogs and cats in China [13]. Pet medical care, primarily for the diagnosis and treatment of diseases in veterinary clinics, constitutes the second largest market in the pet economy in China [13]. As a result of the growth of the pet medical industry, antimicrobial agents are increasingly being used in pet clinics. For example, the China market size for pet anti-infective drugs (including antimicrobial agents) was US$221.9 million in 2020 and is predicted to reach US$662.15 million by the end of 2027, expanding at a compound annual growth rate of 16.97% over the period 2021–2027 [14]. However, due to the extensive use of antimicrobial agents, AMR is becoming prevalent among bacteria of pet origin. In addition, the close contact between humans and their pets may increase the risk of transmission of antimicrobial-resistant bacteria between them. For instance, the clonally related *mcr-1* positive *Escherichia coli* was detected from a worker and four dogs in a pet store [15]. Moreover, indistinguishable carbapenemase- and extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates were observed from dogs and family members in the same household [16, 17], indicating the potential exchange of antimicrobial-resistant bacteria between pets and humans. Thus, the threat of antimicrobial-resistant bacteria in pets should not be underestimated, and monitoring of AMR in bacteria from pets is urgently needed.

In 2021, we established the China Antimicrobial Resistance Surveillance Network for Pets (CARPet), which includes Chinese companion animal hospitals. In this manuscript, we analysis and present the antimicrobial resistance profile of clinical isolates from dogs and cats collected by central laboratory of CARPet from 2018 to 2021.

Results

Distribution of pet clinical isolates

A total of 4,541 isolates were obtained from pet clinics in 25 Chinese provinces and municipalities between 2018 and 2021, with the majority (90.4%, $n=4,107$) from pets in Beijing and the rest (9.6%, $n=434$) from pets in other 24 regions (Fig. 1A). Overall, the proportion of Gram-negative bacteria (53.6%) was sightly higher than that of Gram-positive bacteria (46.4%). Canine and feline isolates accounted for 69.8% (n = 3,171) and 30.2% (n = 1,370), respectively. In regards to the specimens, most of our isolates (85.1%, n = 3,865) were from the urinary tract (45.9%, n = 2,085), the skin (26.4%, n = 1,199) and the respiratory tract (12.8%, n = 581) (Fig. 1B–D).

Isolates of *Staphylococcus* spp., *Escherichia* spp. and *Enterococcus* spp., were the most frequently detected in both dogs and cats, accounting for more than half of the total isolates (Figs. 2A, S1A and S2A). Among the *Staphylococcus* spp. isolates, the most frequently detected isolates were *S. pseudintermedius* (70.8%, Fig. 2B), accounting for 81.5% of the canine isolates, and 34.9% of the feline isolates (Figs. S1B and S2B). All *Escherichia* spp. isolates were *E. coli* (Fig. 2C), which were also the most common isolates from cats ($n=233$, 17.0%) at the species level, and ranked second ($n=607$, 19.1%) in isolates from dogs, lower than the number of *S. pseudintermedius* ($n=716$, 22.6%). Among the *Enterococcus* spp., *Enterococcus faecium* (45.2%) and *Enterococcus faecalis* (45.0%) were the major species, with a higher percentage (57.7%) of *E. faecium* among cats and a higher percentage (54.1%) of *E. faecalis* among dogs (Figs. 2D, S1D and S2D).

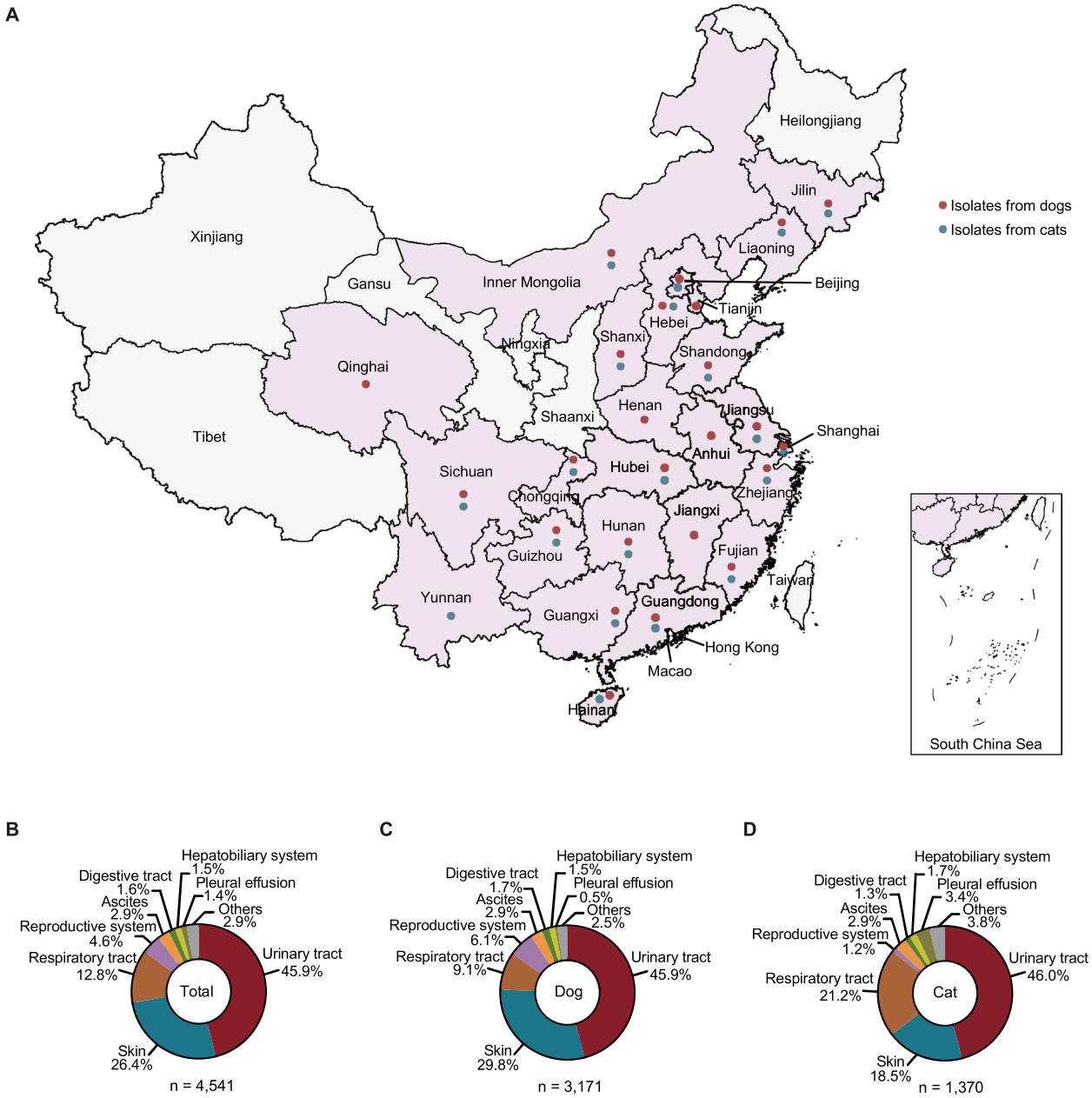

Fig. 1 The geographical distribution of canine and feline isolates (**A**), and the distribution of isolates from different specimens in overall (**B**), dogs (**C**) and cats (**D**) from 2018 to 2021

Susceptibility of Enterobacterales isolates to antimicrobial agents

A total of 1,176 recovered Enterobacterales isolates, including 771 *E. coli*, 167 *Klebsiella* spp., 53 *Enterobacter* spp., and 185 *Proteus* spp. isolates, were tested for their antimicrobial susceptibility.

Escherichia coli

The tested *E. coli* showed the highest resistance rate to ampicillin (56.7%), probably due to its frequent use in pet clinics (Fig. 3). Besides, high resistance rates (30.7%–42.0%) were also observed in several other important antimicrobial agents, including ceftriaxone, cefquinome, levofloxacin, enrofloxacin, florfenicol, trimethoprim-sulfamethoxazole, and doxycycline

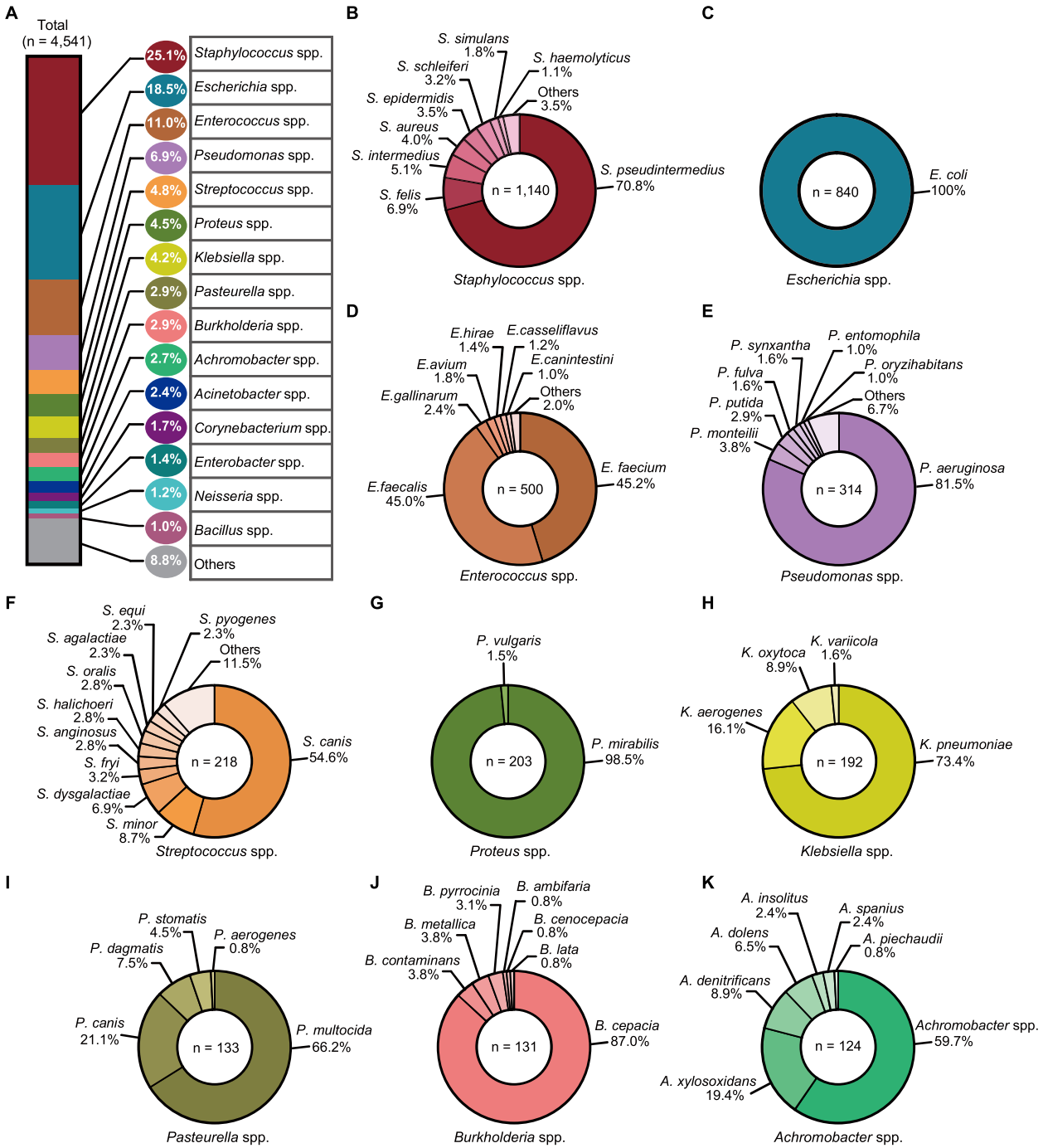

Fig. 2 The distribution of pet clinical isolates from 2018 to 2021. (**A**) a stacked bar plot showing the distribution of the major bacterial genera (≥ 1.0%) of pet isolates. The percentage of each bacterial genus is shown in the corresponding oval shadow. (**B**–**K**) ring plots shows the distribution of the major bacterial species of the top ten bacterial genera. The percentage of each bacterial species within indicated genus is shown in the respective panels B–K

(Fig. 3). Regarding the critically important antimicrobial agents in human medicine, such as meropenem, colistin, and tigecycline, more than 96.0% of the isolates were susceptible (Fig. 3). Further analysis identified no significant differences in the resistance to most antimicrobial agents between canine and feline isolates, except for florfenicol (41.5% vs. 30.4%, $p < 0.01$) and amikacin (5.5% vs. 10.3%, $p < 0.05$) (Fig. 3). The resistance rates

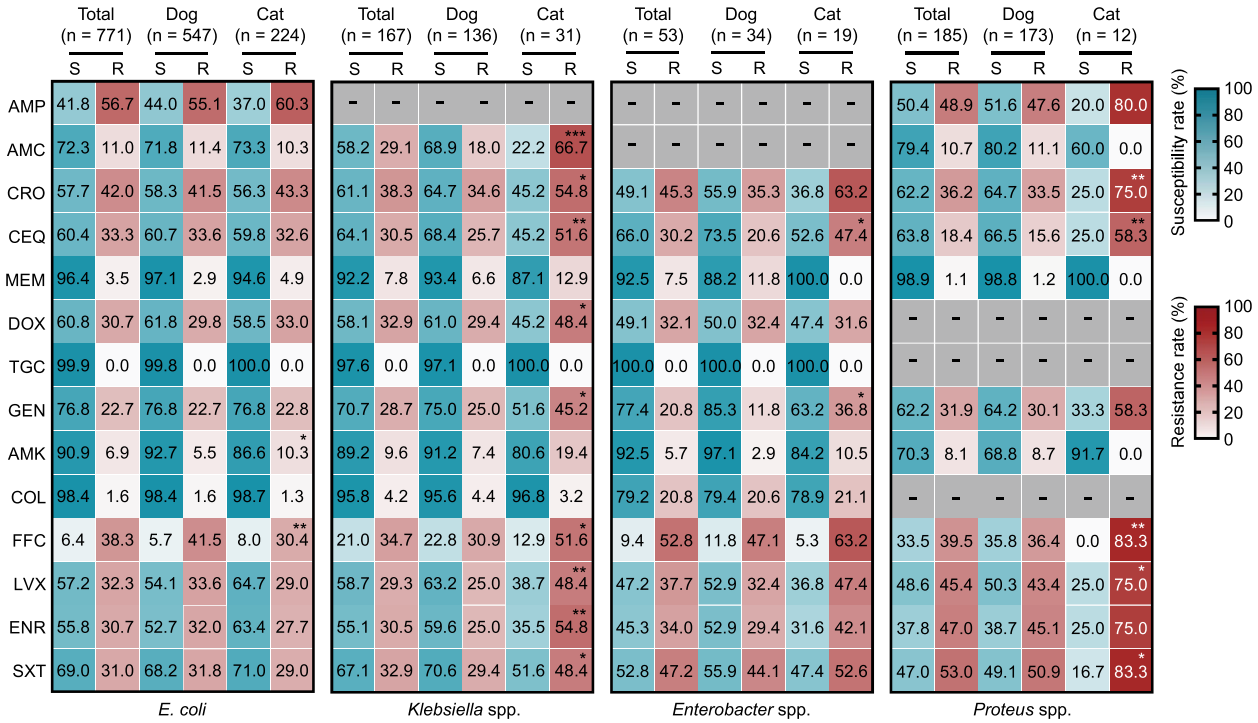

Fig. 3 Heatmaps showing the susceptibility of pet-derived Enterobacterales isolates including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. to antimicrobial agents. Abbreviations: ampicillin (AMP), amoxicillin-clavulanate (AMC), ceftriaxone (CRO), cefquinome (CEQ), meropenem (MEM), doxycycline (DOX), tigecycline (TGC), gentamicin (GEN), amikacin (AMK), colistin (COL), florfenicol (FFC), levofloxacin (LVX), enrofloxacin (ENR), trimethoprim-sulfamethoxazole (SXT). The short black horizontal line in grey cells indicates that bacteria of the respective species are intrinsically resistant to the corresponding antimicrobial agents. The statistical differences in resistance rates to the corresponding antimicrobial agents between canine and feline isolates are indicated by asterisks. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) are considered as statistically significant. For ampicillin and amoxicillin-clavulanate, only the susceptibility of isolates from urinary tract samples is reported, with the number of *E. coli*, *Klebsiella* spp. and *Proteus* spp. being 462, 79, and 131 in total, 316, 61 and 126 in dogs, and 146, 18 and 5 in cats, respectively. For the remaining antimicrobial agents, the number (n) of all isolates is indicated in the figure

of *E. coli* isolates to the tested antimicrobial agents were relatively stable from 2018 to 2021, with a slight increase in β-lactam resistance, among which only the third generation cephalosporins showed a significant increase in resistance from 35.9% in 2018 to 47.1% in 2021 ($p = 0.04$), appealing for a potential need to introduce more prudent supervision of their use (Fig. 4A, Table S1).

Klebsiella spp.

Similar to the resistance profiles of the *E. coli* isolates, *Klebsiella* spp. showed high resistance rates to ceftriaxone, cefquinome, enrofloxacin, florfenicol, trimethoprim-sulfamethoxazole, and doxycycline (30.5%–38.3%) and high susceptibility rates to meropenem, colistin and tigecycline (92.2%–97.6%) (Fig. 3). However, the feline isolates showed higher resistance rates than the canine isolates to most antimicrobial agents (Fig. 3). The overall trends of resistance rates against most antimicrobial agents increased from 2018 to 2021, except for colistin, which decreased from 10.0% in 2018 to 0.0% in 2021 (Fig. 4B, Table S1).

Enterobacter spp.

The top seven antimicrobial agents to which *Enterobacter* spp. showed high resistance rates (30.2%–52.8%) were consistent with those in *E. coli* excluding ampicillin. The highest resistance rate was recorded for florfenicol (52.8%). In contrast to *E. coli* and *Klebsiella* spp., the susceptibility of *Enterobacter* spp. to colistin was less than 90.0% (Fig. 3). Among the antimicrobial agents tested, only gentamicin and cefquinome showed significant differences in the resistance rates between canine and feline isolates (Fig. 3). The resistance rates of *Enterobacter* spp. to more than half of the antimicrobial agents tested in 2019 and 2021 seemed to be higher than those tested in 2018 and 2020 (Fig. 4C, Table S1).

Proteus spp.

The antimicrobial list with high resistance rates (> 30.0%) in *Proteus* spp. was mostly identical to that in *E. coli*,

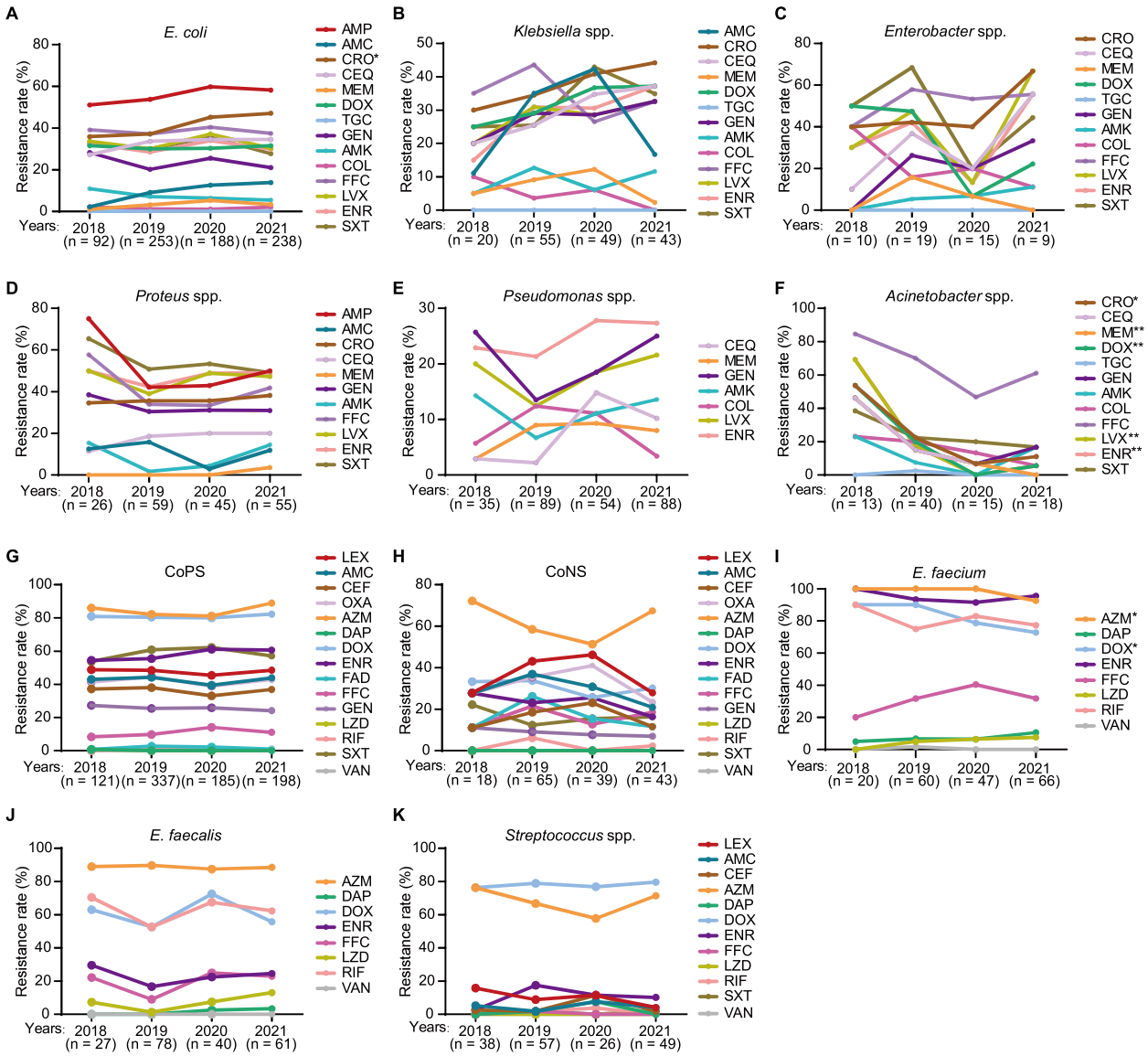

Fig. 4 Line charts showing the variation of overall resistance rates in tested isolates including *E. coli* (**A**), *Klebsiella* spp. (**B**), *Enterobacter* spp. (**C**), *Proteus* spp. (**D**), *Pseudomonas* spp. (**E**), *Acinetobacter* spp. (**F**), CoPS (**G**), CoNS (**H**), *E. faecium* (**I**), *E. faecalis* (**J**), and *Streptococcus* spp. (**K**) from 2018 to 2021. Abbreviations: ampicillin (AMP), amoxicillin-clavulanate (AMC), ceftriaxone (CRO), cefquinome (CEQ), meropenem (MEM), doxycycline (DOX), tigecycline (TGC), gentamicin (GEN), amikacin (AMK), colistin (COL), florfenicol (FFC), levofloxacin (LVX), enrofloxacin (ENR), trimethoprim-sulfamethoxazole (SXT), cephalexin (LEX), ceftiofur (CEF), oxacillin (OXA), azithromycin (AZM), daptomycin (DAP), fusidic acid (FAD), linezolid (LZD), rifampin (RIF), vancomycin (VAN), coagulase-positive *Staphylococcus* (CoPS), coagulase-negative *Staphylococcus* (CoNS). Asterisks represent the statistical significance of the variation trends in the resistance rates of the corresponding antimicrobial agents from 2018 to 2021. $p < 0.05$ (*) and $p < 0.01$ (**) are considered as statistically significant. The breakpoints for *S. aureus* from bovine mastitis to ceftiofur and for *S. pseudintermedius* from dogs to doxycycline were used to determine the resistance of *Staphylococcus* spp. from dogs and cats in this study. As florfenicol breakpoints for *Staphylococcus* spp., *Enterococcus* spp. and *Streptococcus* spp. from dogs and cats have not been established, the florfenicol breakpoints approved for *Streptococcus suis* from pigs were used to determine florfenicol resistance in the aforementioned pathogens this study

except for gentamicin showing lower resistance in the latter (Fig. 3). *Proteus* spp. isolates showed the highest resistance to trimethoprim-sulfamethoxazole (53.0%) and the highest susceptibility to meropenem (98.9%). To be noted, higher resistance rates were observed in isolates from cats compared to those from dogs, showing consistency with the situation among *Klebsiella* spp. isolates (Fig. 3). For most antimicrobial agents, the

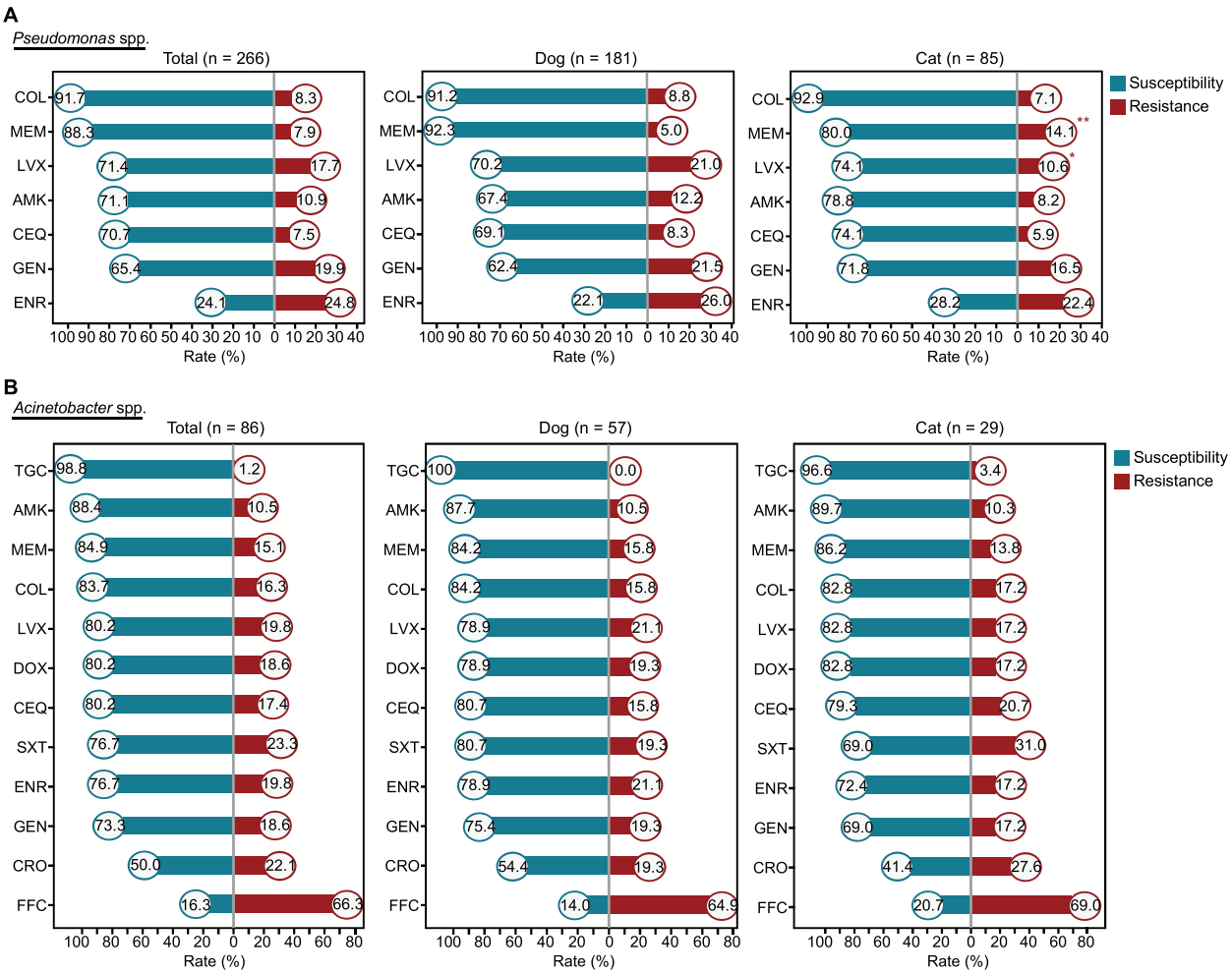

Fig. 5 Diverging bar plots showing the susceptibility of *Pseudomonas* spp. (**A**) and *Acinetobacter* spp. (**B**) isolates to antimicrobial agents. Abbreviations: colistin (COL), meropenem (MEM), levofloxacin (LVX), amikacin (AMK), cefquinome (CEQ), gentamicin (GEN), enrofloxacin (ENR), tigecycline (TGC), doxycycline (DOX), trimethoprim-sulfamethoxazole (SXT), ceftriaxone (CRO), florfenicol (FFC). Statistical differences in the resistance rates to the corresponding antimicrobial agents between canine and feline isolates are indicated by asterisks. $p < 0.05$ (*) and $p < 0.01$ (**) are considered as statistically significant. In case that the intermediate category was defined for tested antimicrobial agents by the interpretive criteria applied, the percentages of isolates falling into this category can be retrieved by subtracting the sum of the percentages of resistant and susceptible isolates from 100%

highest resistance rates were seen in 2018, and relatively stable trends were observed in the following three years (Fig. 4D, Table S1).

Susceptibility of non-Enterobacterales Gram-negative isolates to antimicrobial agents

A total of 266 *Pseudomonas* spp. and 86 *Acinetobacter* spp. isolates of non-fermentative Gram-negative bacilli were recovered and tested for antimicrobial susceptibility.

Pseudomonas spp.

In general, the resistance rates of *Pseudomonas* spp. to the tested antimicrobial agents were less than 25.0%, with enrofloxacin showing the highest resistance rate (24.8%) and cefquinome the lowest (7.5%) (Fig. 5A). There was no significant difference in the prevalence of resistance to most antimicrobial agents between canine and feline isolates, except for meropenem (5.0% vs. 14.1%, $p < 0.01$) and levofloxacin (21.0% vs. 10.6%, $p < 0.05$) (Fig. 5A). An overall increasing trend in the resistance rates of most antimicrobial agents was observed from 2019 to 2021, except for colistin, which showed a decreasing trend from 12.4% in 2019 to 3.4% in 2021 (Fig. 4E, Table S2).

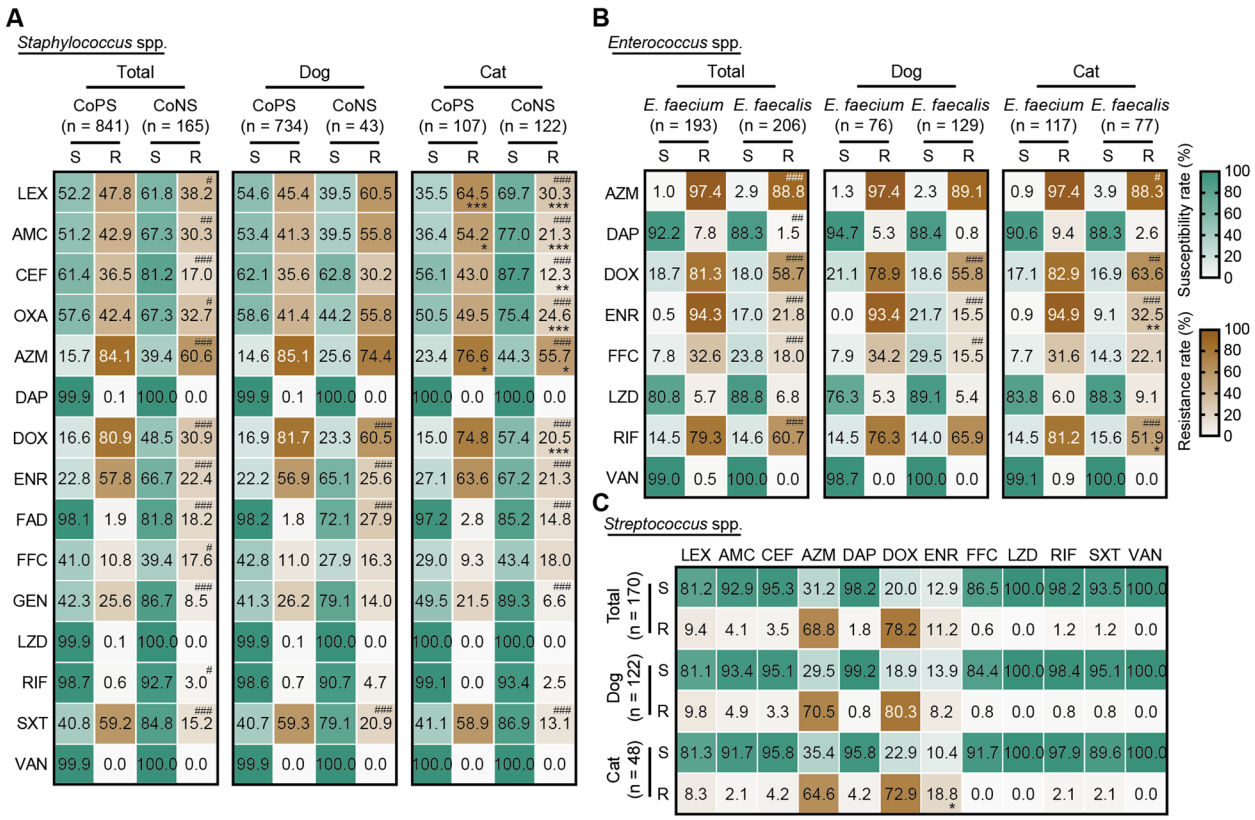

Fig. 6 Heatmaps showing the susceptibility of *Staphylococcus* spp. (**A**), *E. faecium* and *E. faecalis* (**B**), and *Streptococcus* spp. (**C**) isolates to various antimicrobial agents. Abbreviations: coagulase-positive *Staphylococcus* (CoPS), coagulase-negative *Staphylococcus* (CoNS), cephalexin (LEX), amoxicillin-clavulanate (AMC), ceftiofur (CEF), oxacillin (OXA), azithromycin (AZM), daptomycin (DAP), doxycycline (DOX), enrofloxacin (ENR), fusidic acid (FAD), florfenicol (FFC), gentamicin (GEN), linezolid (LZD), rifampin (RIF), trimethoprim-sulfamethoxazole (SXT), vancomycin (VAN). The statistical differences in the resistance rates to the corresponding antimicrobial agents between CoPS and CoNS or between *E. faecium* and *E. faecalis* in each group (including total, dog and cat) are indicated by hashtags (#). The statistical differences in resistance rates to the corresponding antimicrobial agents between canine and feline isolates in each group (including CoPS, CoNS, *E. faecium* and *E. faecalis*) are indicated by asterisks (*). $p < 0.05$ (# or *), $p < 0.01$ (## or **) and $p < 0.001$ (### or ***) are considered as statistically significant

Acinetobacter spp.

The resistance rate of *Acinetobacter* spp. isolates to florfenicol was the highest (66.3%), with the remaining antimicrobial agents showing relatively low resistance rates ranging from 1.2% to 23.3% (Fig. 5B). No marked difference were detected in the resistance rates between canine and feline isolates. A noticeable trend of decrease in the resistance rates to almost all antimicrobial agents was observed from 2018 to 2021, with statistical significance in ceftriaxone, meropenem, doxycycline, levofloxacin, and enrofloxacin ($p < 0.05$, Fig. 4F, Table S2).

Susceptibility of Gram-positive cocci isolates to antimicrobial agents

Staphylococcus spp.

A total of 1,006 isolates of the genus *Staphylococcus* were recovered, comprising 841 (83.6%) coagulase-positive *Staphylococcus* (CoPS) and 165 (16.4%) coagulase-negative *Staphylococcus* (CoNS, Table S3). The resistance rates of CoPS to most commonly used antimicrobial agents were significantly higher than those of CoNS. Nonetheless, CoNS exhibited higher resistance to florfenicol, fusidic acid, and rifampin than CoPS (Fig. 6A). Notably, more than 90.0% of the *Staphylococcus* spp. isolates were susceptible to rifampin, vancomycin, linezolid, and daptomycin (Fig. 6A). The differences in the resistance profiles between CoPS and CoNS among isolates from cats resembled those in the overall isolates as described above (Fig. 6A). In contrast, only the resistance rates of doxycycline, trimethoprim-sulfamethoxazole, enrofloxacin, and fusidic acid showed significant differences between CoPS and CoNS from dogs ($p < 0.001$, Fig. 6A). In canine CoNS isolates, the resistance rates to most antimicrobial agents were higher than those in feline CoNS isolates, which was not the case in CoPS isolates (Fig. 6A). All the resistance rates of CoPS isolates remained at the same level from 2018 to 2021 (Fig. 4G, Table S4). While the majority of CoNS isolates showed some fluctuations, the overall

trends of the resistance rates remained stable (Fig. 4H, Table S4).

Enterococcus spp.

A total of 193 *E. faecium* and 206 *E. faecalis* isolates were recovered and tested. Both *E. faecalis* and *E. faecium* showed high susceptibility (> 80.0%) to daptomycin, vancomycin and linezolid. Overall, *E. faecium* showed higher resistance to most antimicrobial agents than *E. faecalis*, irrespective of whether the isolates originated from dogs or cats (Fig. 6B). Further analysis revealed no significant difference in the resistance rates of *E. faecium* between isolates from dogs and cats, and only a higher resistance to rifampin and a lower resistance to enrofloxacin were identified in *E. faecalis* isolates from dogs when compared with those from cats (Fig. 6B). From 2018 to 2021, we only observed a significantly decreasing trend in the resistance rates of *E. faecium* to azithromycin and doxycycline ($p < 0.05$), and no remarkable alterations were found in the resistance rates of *E. faecalis* (Fig. 4I, J and Table S4).

Streptococcus spp.

A total of 170 *Streptococcus* spp. were recovered and tested for their antimicrobial susceptibility, most of which were *S. canis* ($n = 108$), *S. dysgalactiae* ($n = 13$) and *S. minor* ($n = 10$). In general, *Streptococcus* spp. showed low resistance (0.0%–11.2%) to the tested antimicrobial agents, with the exception of doxycycline (78.2%) and azithromycin (68.8%). There was no significant difference in the resistance rates of *Streptococcus* spp. isolates from dogs and cats to most antimicrobial agents, except for enrofloxacin (8.2% vs. 18.8%) (Fig. 6C). Moreover, we observed no remarkable alterations in the resistance rates of *Streptococcus* spp. from 2018 to 2021 (Fig. 4K and Table S4).

Multidrug resistance

Among Enterobacterales isolates, 51.0% of *E. coli*, 40.7% of *Klebsiella* spp., 52.8% of *Enterobacter* spp., and 48.6% of *Proteus* spp. exhibited multidrug resistance (MDR) (Figure S3). In particular, the MDR rates of *Enterobacter* spp. and *Proteus* spp. isolates were higher in feline isolates than those in canine isolates, i.e., 73.7% vs. 41.2% and 83.3% vs. 46.2%, respectively. In general, there was a relatively low MDR rate (6.4%) in *Pseudomonas* spp. isolates and a moderate MDR rate (27.9%) in *Acinetobacter* spp. isolates (Figure S3). The MDR rate of CoPS was much higher than that of CoNS (72.5% vs. 41.2%). In addition, the canine CoNS isolates were resistant to more antimicrobial agents compared to the feline isolates, with the MDR rates being 62.8% and 33.6%, respectively (Figure S3). As for *Enterococcus* spp. isolates, the overall MDR level of *E. faecium* was significantly higher than that of *E. faecalis* (92.7% vs. 52.9%, $p < 0.05$), implying a more severe occurrence of AMR in *E. faecium*. The percentage of MDR in *Streptococcus* spp. isolates was at a relatively low level (12.4%, Figure S3).

Discussion

Antimicrobial resistance is a significant challenge in the treatment of infectious diseases, with no exception in pet clinics. To address this issue, it is crucial to develop timely and effective surveillance systems with a comprehensive antimicrobial resistance database, which will have significant implications for pet medical care and public health. To this end, we have introduced the CARPet (carpet.cau.edu.cn) surveillance network, which aims to collect, analyze, and report AMR data from pets across different regions of China. The database provides users with information on sampling time and locations, sample type, size, as well as the species distribution and resistance rates of corresponding pet-derived clinical pathogens. CARPet is proven to be a powerful platform that can (i) provide scientific guidance for the diagnosis and treatment of bacterial infections, (ii) give insight into the occurrence of antimicrobial-resistant bacteria, (iii) predict potential threats of bacterial pathogens, and (iv) support the development of relevant policies or interventions for the national and local administrations of antimicrobial agents in pet clinics.

Similar AMR surveillance systems have been implemented in Japan and the USA. The Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) monitors the AMR data (MIC values) of bacteria from clinical samples of urine, reproductive tract, ear, and skin of diseased dogs and cats [7]. *E. coli* and *S. pseudintermedius* are the predominant species in pets from JVARM and CARPet. Coincidentally, two US AMR monitoring systems, the FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) and the USDA's National Animal Health Laboratory Network (NAHLN), collect AMR data (MIC values) of clinically relevant *E. coli* and *S. pseudintermedius* of dogs [18]. However, in human clinics, *E. coli* (19.0%) and *K. pneumoniae* (14.1%) account for the major species, according to the annual report of CHINET using the disk diffusion method or Vitek 2 compact automated system [11, 19]. These findings indicate the inconsistency of pathogenic bacteria profiles between pet and human clinics, and highlight the importance and necessity of developing an independent surveillance system for monitoring the prevalence of AMR in bacteria from pets.

Although the above-mentioned surveillance systems are based on different sampling methods and breakpoints, they were all implemented according to the

CLSI recommendations, enabling broad comparisons of these systems with CARPet. Therefore, we compared the pet-derived AMR data from CARPet in China with those from similar systems in the USA and Japan, as well as the corresponding data from human clinics in China. In Enterobacterales, the resistance rates to most commonly used antimicrobial agents of pet-derived *E. coli* were significantly higher ($p < 0.01$) in China than those in both the USA [18] and Japan [20], but lower ($p < 0.001$) than those derived from Chinese human clinics [11, 19] (Table S5). It should be noted that the resistance rates of *E. coli* to both fourth generation cephalosporins and carbapenems were significantly higher ($p < 0.01$) in pets than that in human clinics. In addition, *E. coli* from CARPet presented a significant increase in the resistance to ceftriaxone from 2018 to 2021, consistent with the growing trend from 2011 to 2017 for resistance to another third generation cephalosporin, cefotaxime, in *E. coli* from diseased dogs and cats in Argentina [21]. The higher and increasing resistance to the β-lactams, especially for third- and fourth-generation cephalosporins and carbapenems, prompt the need for stricter controls in the use of these critically important antimicrobial agents in pet clinics. In terms of *Klebsiella* spp., the resistance rates of pet isolates to most antimicrobial agents in China showed no significant difference compared to those in Japan [20] or to the data from Chinese human clinics [11, 19] (Table S5). As for *Enterobacter* spp. and *Proteus* spp., the resistance rates to some antimicrobial agents, such as fourth generation cephalosporins and fluoroquinolones, were significantly higher ($p < 0.05$) in our study than those in human clinics [11, 19] (Table S5).

In *S. pseudintermedius*, pet isolates from CARPet presented higher resistance rates to most antimicrobial agents than those in the USA [18], whereas the CARPet isolates showed higher resistance rates to macrolides and lower resistance rates to fluoroquinolones than those in Japan [20] (Table S6). The resistance rates of *E. faecium* from CARPet to macrolides and linezolid were generally higher than those from pets in Japan [20] and/or those from human clinics in China [11, 19]. Consistent with the observations in *E. faecium*, the resistance rates of *E. faecalis* from CARPet to fluoroquinolones and macrolides were higher than those from pets in Japan [20]. In contrast, their resistance rates to fluoroquinolones and rifampin were lower than those in human clinics [11, 19] (Table S6). Despite the challenges in data comparisons, these findings still suggest the need to establish our own national surveillance system for monitoring the presence of AMR among bacteria from pets.

The overall resistance rates of most bacteria to the tested antimicrobial agents remained largely stable from 2018 to 2021, except for *Acinetobacter* spp. which showed a generally decreasing trend (Fig. 4). We speculate that this may be due to a lower proportion of *Acinetobacter baumannii* isolates for susceptibility analysis in 2019 (25.0%), 2020 (6.7%) and 2021 (27.8%) compared to that in 2018 (69.2%). *A. baumannii* is one of the most clinically significant *Acinetobacter* species with an exceptional capacity to acquire antimicrobial resistance in both human and veterinary medicine [22, 23]. In addition, we observed an extraordinary alteration pattern in the resistance rates of some bacteria (e.g., *Enterobacter* spp.) to certain antimicrobial agents (e.g., cefquinome, doxycycline, levofloxacin, enrofloxacin and trimethoprim-sulfamethoxazole), showing a sharp reduction in one year followed by a rapid increase in the next year, or vice versa (Fig. 4). This may be due to the variation in the proportion of each bacterial species in different years as well as the relatively small number of total isolates within same bacterial genus tested for antimicrobial susceptibility.

The following limitations of CARPet should be taken into consideration. Although the current CARPet platform is based on pet-derived pathogens collected from 25 Chinese provinces/municipalities, there may be potential bias due to the majority of the samples being collected from Beijing, the location of the central laboratory for this network. This may cause a lack of uniformity in sample coverage across the country. However, considering the large diversity and rapid development of clinical AMR information in pets, the current version of CARPet is the first integration of currently collected resources and will continue to be updated and expanded in the coming years. In addition, a few isolates failed to be recovered, even when enrichment media were used, making them unavailable for antimicrobial susceptibility testing. As a result, nearly 8.2%–22.0% of the isolates in each genus were not tested for antimicrobial resistance. Nevertheless, given the low proportion and random dispersion of non-recovered isolates, the AMR data were obtained from the vast majority of the obtained isolates using the Thermo Scientific™ Sensititre AIM™ Automated Inoculation Delivery System with an extremely low-test error rate. Thus, this database provides a solid and comprehensive profile of the presence of antimicrobial-resistant bacteria in pet clinics across China.

Methods

CARPet surveillance system

The CARPet surveillance system has been coordinated by the China Agricultural University Veterinary Teaching Hospital since 2021 with the aim to (i) investigate the occurrence of bacterial pathogens in different types of infections in pets, (ii) detect and analyze

their antimicrobial resistance profiles, and (iii) report the annual and sustainable results in bacteria from pets, including dogs and cats. The central laboratory of CARPet, which is the medical microbiology laboratory of the China Agricultural University Veterinary Teaching Hospital, standardizes the methods of sampling and provides sampling training to the members. The routine clinical samples and corresponding information are sent by provincial companion animal hospitals across China to the central lab. A representative and methodologically unified approach is used to isolate the bacteria and test their susceptibility to selected antimicrobial agents. The antimicrobial susceptibility data of these hospital-based isolates from all CARPet members is submitted to the database annually. In addition, the distribution of pet clinical samples and isolates, and the resistance rates of different genera of bacteria from various infections in the respective years are shown and can be queried on the website of CARPet (carpet.cau.edu.cn). With these efforts, this system is expected to (i) provide scientific medication guidance for veterinarians in each companion animal hospital based on annual and sustainable reports, (ii) identify the national epidemic trends of resistant bacteria in pets, (iii) detect new threats of bacterial pathogens for pets, and (iv) provide timely AMR data for the policies or intervention measure decisions in companion animals made by national and local agricultural-associated administrations.

The medical microbiology laboratory of the China Agricultural University Veterinary Teaching Hospital obtained clinical samples, isolated, identified, preserved bacteria from samples, and recorded the information of samples of any companion animal hospital across China since 2018. Therefore, we collected and recovered all the isolates preserved by the central lab from 2018 to 2021, then tested and analyzed their susceptibility data in this study.

Information collection and bacteria recovery and identification

All the bacterial isolates and accompanying information were obtained from the medical microbiology laboratory of the China Agricultural University Veterinary Teaching Hospital. Background information of routine clinical samples of dogs and cats were collected, and the bacteria isolated from these samples from 2018 to 2021 were recorded. To avoid duplicate counts, only one isolate was included when bacteria of the same species were isolated from the same sample based on medical record number and hospital. Bacteria were inoculated on Brain–Heart Infusion Agar containing 5% defibrinated sheep blood and incubated for 18–24 hours at 37 °C. The recovered isolates were purified by picking single colonies from the agar and cultured on a new agar. Species were reconfirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Autobio, Zhengzhou, China) and 16S rRNA gene sequencing. The identified bacteria were stored at −80 °C using Pro-Lab Microbank cryovials (Pro-Lab, Vaughan, ON, Canada) according to the manufacturer's instructions.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing focused on isolates of major bacterial genera including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. in the Enterobacterales family, *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp. and *Streptococcus* spp. using the broth microdilution method with custom-made broth microdilution panels (Thermo Fisher Scientific) according to Clinical and Laboratory Standard Institute (CLSI) documents M07 [24]. Eleven categories of antimicrobial agents, including 14 agents, were selected and arranged on the plates for Gram-negative organisms, such as penicillins (ampicillin), penicillins and β-lactamase inhibitors (amoxicillin-clavulanate), the third and fourth generation cephalosporins (ceftriaxone and cefquinome), carbapenems (meropenem), aminoglycosides (gentamicin and amikacin), fluoroquinolones (levofloxacin and enrofloxacin), folate pathway inhibitors (trimethoprim-sulfamethoxazole), glycylcyclines (tigecycline), phenicols (florfenicol), polymyxins (colistin) and tetracyclines (doxycycline). The plates for Gram-positive organisms contained 15 agents belonging to 15 categories, including penicillins and β-lactamase inhibitors (amoxicillin-clavulanate), isoxazolyl penicillins (oxacillin), first generation cephalosporins (cephalexin), the third generation cephalosporins (ceftiofur), aminoglycosides (gentamicin), ansamycins (rifampin), fluoroquinolones (enrofloxacin), folate pathway inhibitors (trimethoprim-sulfamethoxazole), fusidic acid, glycopeptides (vancomycin), lipopeptides (daptomycin), macrolides (azithromycin), oxazolidinones (linezolid), phenicols (florfenicol) and tetracyclines (doxycycline). The above selected antimicrobial agents are commonly used in veterinary medicine or belong to the critically important antimicrobial agents in human medicine. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619 served as the quality control strains. Results were interpreted mainly according to the breakpoints in CLSI VET01S-Ed5 [25] if appropriate, and the human breakpoints in CLSI M100-Ed31 [26].

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline (V11.0) [27] was applied for the breakpoint of *Staphylococcus* spp. to fusidic acid, as no appropriate CLSI breakpoint was available. The data were analyzed using WHONET software version 2022. The statistical significance of resistance rates of isolates from dogs and cats or isolates of two species within the same genus to the same antimicrobial agent was assessed by a chi-square test on the self-built analysis website (http://123.57.190.207:3838/zhedian3/). Cochran-armitage trend test was used to evaluate the changing trends of resistance rates from 2018 to 2021. For all statistical analyses, a *p*-value less than 0.05 is considered significant. The multidrug resistance (MDR) was defined as resistance to one or more agents in three or more classes of antimicrobial agents as applied in the previous study [28, 29]. For each genus, antimicrobial agents were excluded from the resistance count if the isolates were intrinsically resistant to them [25].

Supplementary Information

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Additional file 1: Figure S1. The distribution of canine isolates from 2018 to 2021. (A) a stacked bar plot showing the distribution of the major bacterial genera (≥ 1.0 %) of canine isolates. The percentage of each bacterial genus is shown in the corresponding oval shadow. (B–K) ring plots shows the distribution of the major bacterial species of the top ten bacterial genera. The percentage of each bacterial species within the indicated genus is shown in the figure. **Figure S2.** The distribution of feline isolates from 2018 to 2021. (A) a stacked bar plot showing the distribution of the major bacterial genera (≥ 1.0 %) of feline isolates. The percentage of each bacterial genus is shown in the corresponding oval shadow. (B–K) ring plots shows the distribution of the major bacterial species of the top ten bacterial genera. The percentage of each bacterial species within the indicated genus is shown in the figure. **Figure S3.** Bubble plots showing the percentages of multidrug resistances in different genera. The grey-shaded same-sized bubbles marked with "ns" indicate that no isolates with the respective numbers of antimicrobial resistances were detected. **Table S1.** Antimicrobial resistance rates of Enterobacterales isolates from 2018 to 2021. Note: For ampicillin and amoxicillin-clavulanate, only the susceptibility of isolates from urinary tract samples is reported, with the number of *E. coli* being 45, 132, 127 and 158 in 2018, 2019, 2020 and 2021, respectively, the number of *Klebsiella* spp. being 9, 20, 26 and 24, and *Proteus* spp. being 16, 38, 35 and 42. For the remaining antimicrobial agents, the number of all isolates is indicated in the parentheses. The short horizontal line in the table indicates that bacteria of the respective species are intrinsically resistant to the corresponding antimicrobial agents. **Table S2.** Antimicrobial resistance rates of *Pseudomonas* spp. and *Acinetobacter* spp. isolates from 2018 to 2021. Note: The short horizontal line in the table indicates that bacteria of the respective species are intrinsically resistant to the corresponding antimicrobial agents. **Table S3.** The species of 1,006 *Staphylococcus* isolates from dogs and cats tested for antimicrobial susceptibility. **Table S4.** Resistance rates of *Staphylococcus* spp., *E. faecium*, *E. faecalis* and *Streptococcus* spp. isolates to the tested antimicrobial agents from 2018 to 2021. Note: The short horizontal line in the table indicates that bacteria of the respective species are intrinsically resistant to the corresponding antimicrobial agents. **Table S5.** Comparison of the antimicrobial resistance rates (%) of Enterobacterales isolates from pets in this study, from pets in the USA [18] and Japan [20], as well as from humans in CHINET [11, 19]. Note: The statistical differences in resistance rates to the corresponding antimicrobial agents between this study and other reported datasets are indicated by asterisks. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) are considered as statistically significant. **Table S6.** Comparison of the antimicrobial resistance rates (%) of *S. pseudintermedius* and *Enterococcus* spp. isolates from pets in this study, from pets in USA [18] and Japan [20], from humans in CHINET [11, 19] to antimicrobial agents. Note: The statistical differences in resistance rates to the corresponding antimicrobial agents between this study and other reported datasets are indicated by asterisks. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) are considered as statistically significant.

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Authors' contributions
J.S., Z.X. and Y.W. designed the study; W.H., Y.L., X.D., Q.A., Y.S., Y.J., X.G., Q.W., Y.Q.S., Y.F.L., D.S., Z.Z., K.Z., L.L., G.Z. and T.Y. collected the data; S.M., S.C., Y.L.L., L.Y., Z.B.Z., H.Z, Y.C., G.L., Y.Q.W., L.W., Z.S., C.W., F.H. and S.S. analysed and interpreted the data; S.M., S.C., Y.L.L. and Y.W. wrote the manuscript. All authors reviewed, revised, and approved the final report.

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Availability of data and materials
All the data supporting the conclusions of this article is included within the article.

Declarations

Ethics approval and consent to participate
Ethical approval was reviewed and given by China Agricultural University Animal Ethics Committee document (No. AW01017102-2).

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no conflicts of interest.

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