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Original Research

Transmission of ceftazidime-avibactam-resistant *Escherichia coli* among pets, veterinarians and animal hospital environment

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ABSTRACT

Ceftazidime-avibactam (CZA) is a recently approved combination synthetic β-lactamase inhibitor used in human clinical medicine. Cases of CZA resistance in humans have already been reported, but limited research has investigated CZA resistance in pets. This study explored the prevalence and transmission of CZA-resistant *Escherichia coli* (CZAREC) among pets, their owners, veterinarians, and the environment in animal hospitals. A total of 5,419 clinical samples were collected from dogs and cats, along with samples from the environment (n = 5,843), veterinarians (n = 557), and pet owners (n = 368) in animal hospitals. From these samples, 760 *Escherichia coli* (*E. coli*) isolates were obtained, out of which 60 were identified as CZAREC. These included 34 isolates from the environment (9.14 %, n = 372), three from veterinarians (8.11 %, n = 37), and 23 from animals (6.82 %, n = 337). No CZAREC isolates were found in pet owners. The predominant sequence types of CZARECs were ST156 (n = 20), ST410 (n = 19) and ST101 (n = 7). Bayesian analysis revealed six clusters comprising 47 isolates from the hospital environment, pets, and veterinaries, displaying genetic relatedness of less than 100 core genome single nucleotide polymorphisms (cgSNPs) between any two isolates in each cluster. Some CZAREC isolates with high genetic similarity persisted in the same animal hospital for four to six months. Moreover, discriminant analysis of principal components indicated that most isolates from different hosts shared a genetic source in the human/dog/cat merged cluster. Overall, evidence of CZARECs transmission was found among pets, the environment, and veterinarians in animal hospitals. The findings emphasize the importance of monitoring CZARECs in the veterinary clinical setting to ensure the health of both pets and humans.

1. Introduction

The emergence and spread of carbapenemase-producing Gram-negative bacteria, including *Enterobacterales*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii*, highlight the urgent need for new antimicrobial agents [1]. Ceftazidime-avibactam (CZA) is a novel combination antimicrobial agent comprising ceftazidime, a third-generation cephalosporin, and avibactam, a novel non-β-lactam β-lactamase inhibitor. CZA exhibits efficient antimicrobial activity against *Enterobacterales* producing extended-spectrum β-lactamase (ESBL), AmpC β-lactamase, *Klebsiella pneumoniae* carbapenemase (KPC), and OXA-48 and drug-resistant *P. aeruginosa* isolates [2]. Therefore, CZA was approved for clinical usage by the Food and Drug Administration in the United States in 2015 and the National Medical Products Administration in China in 2019, primarily for treating complicated urinary tract and intra-abdominal infections caused by multi-drug resistant bacteria [3,4].

Several global and regional resistance monitoring programs reported that over 90 % of *Enterobacterales* collected from humans in clinics between 2012 and 2018 were susceptible to CZA [5–7]. However, the susceptibility of certain isolates to CZA, such as carbapenem-resistant *Enterobacterales*, has shown a decline over time in several countries [4,5,7]. Recent studies have revealed numerous CZA-resistant Gram-negative isolates in clinical settings [5–9]. CZA resistance primarily arises from amino acid substitutions in the

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HIGHLIGHTS

Scientific question

This study investigates the prevalence and transmission dynamics of ceftazidime-avibactam-resistant *Escherichia coli* (CZAREC) in animal hospitals, pets, and veterinarians.

Evidence before this study

The recent approval and use of ceftazidime-avibactam (CZA) in clinical settings have resulted in cases of CZA resistance in humans. However, the understanding of CZAREC in pets and its transmission dynamics remains limited.

New findings

Our study revealed that CZAREC isolates were prevalent among pets, the environment, and veterinarians in animal hospitals. Bioinformatics analysis indicated that several closely related isolates showed correlated temporal and spatial distribution patterns, persisting for four to six months within the same animal hospital. Furthermore, these isolates could potentially be transmitted between pets and veterinarians.

Significance of the study

This research offers critical insights into the prevalence, transmission dynamics, and genetic relatedness of CZAREC in animal hospitals. These results underscore the importance of monitoring CZAREC in veterinary clinical settings using a One Health approach to mitigate the spread of antimicrobial-resistant bacteria between animals and humans.

β-lactamase [10]. Additionally, the resistance can be influenced by various factors, including genetic mutations of the target, alternative target expression [11,12], reduced expression or loss of outer membrane proteins, as well as increased efflux pump expression [13].

Human-derived CZA-resistant isolates are under continuous surveillance, but limited research has been performed on such isolates in animals, especially in pets. Considering that pets are important members of many families, sharing the same living space and having close contact with their owners, they may play a role in facilitating the transmission of bacteria between humans and animals. Previous studies have reported the transmission of multidrug-resistant (MDR) bacteria, including ESBL-producing *Klebsiella pneumoniae* (*K. pneumoniae*) and carbapenem-resistant *Escherichia coli* (*E. coli*), between pets and their owners [14,15], suggesting the importance of monitoring CZA-resistant isolates in pets. Notably, the hospital environment, including animal hospitals, can serve as a reservoir for antimicrobial-resistant bacteria. Similar to human hospitals, animal hospitals contain a significant amount of resistant bacteria in their environment, which may potentially result in nosocomial infections [16,17]. Consequently, the prevalence of CZA-resistant *E. coli* (CZAREC) among pets in hospitals was explored, as well as among veterinarians, pet owners, and the environment in animal hospitals. Furthermore, the correlation between CZAREC isolates from different hosts was analyzed.

2. Materials and methods

2.1. Sample collection

A total of 5,419 samples were collected from diseased dogs and cats in 54 animal hospitals in Beijing between September 2018 and September 2020. These samples included urine, skin, pleural effusion and ascites, tracheal wash fluid, hydrohystera, swabs of traumatic wounds, eye secretion, and nasopharyngeal swabs. From these clinical samples, 337 *E. coli* isolates were detected, with 235 originating from dogs and 102 from cats. Subsequently, 13 animal hospitals where *E. coli* was frequently detected in dogs and cats were selected. Environmental samples were collected from these hospitals every two months, spanning from September 2020 to July 2021. The sampling areas included the reception hall, consulting room, treatment area, X-ray examination room, ultrasonic examination room, computed tomography (CT) examination room, dog/cat ward, infection ward, injection room, and observation room. Three to ten samples were collected per room, depending on its size. Each environmental sample was obtained from the surface of facilities, covering an area of approximately 25 cm^2, using a cotton swab soaked in sterile phosphate-buffered saline (PBS). Additionally, samples were also collected from veterinarians and pet owners present in the sampling room. These individuals were sampled using the same method as employed in the environment, targeting exposed skin, cuff, collar, and clothing surfaces.

2.2. Bacterial isolation and identification

All samples were cultured on CHROMID® *E. coli* agar plates (bioMérieux, Marcy I'Etoile, France) at 37 °C for 24 h. Blue colonies were selected following the manufacturer's instructions to identify presumptive *E. coli* isolates. Subsequently, species were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Autobio, Zhengzhou, China) and 16S ribonucleic acid (RNA) gene sequencing [18].

2.3. Antimicrobial susceptibility testing

Susceptibility testing for CZA was conducted using the agar dilution method according to the Clinical & Laboratory Standards Institute (CLSI) guidelines [19]. The minimum inhibitory concentration (MIC) values of CZA were determined by using a fixed avibactam concentration of 4 mg/L and performing a two-fold serial dilution of ceftazidime. Moreover, the MICs of ten other antimicrobial agents, including meropenem, amikacin, ciprofloxacin, minocycline, chloramphenicol, cefepime, amoxicillin-clavulanate, ampicillin-sulbactam, nitrofurantoin, and colistin were tested against CZA-resistant *E. coli* using the agar dilution method [19]. The results were interpreted according to the CLSI VET01S-Ed5, M100-ED31, and EUCAST guidelines (V11.0) [19–21]. Isolates resistant to three or more different classes of antimicrobial agents were classified as MDR [22]. *E.coli* American type culture collection (ATCC) 25922, 35218, and 700603 were used as quality control strains.

2.4. Whole genome sequencing, assembly, and molecular analysis

The deoxyribonucleic acid (DNA) of all CZAREC isolates was extracted using the MAGEN Bacterial DNA Kit according to the manufacturer's instructions. The DNA libraries of CZARECs were prepared using the Annoroad Universal DNA Library Prep Kit and sequenced on the Illumina X Ten platform, and the draft genomes were assembled by SPAdes (version 3.10.1) [23]. Antimicrobial resistance genes were predicted using the resfinder database by Abricate [24]. Furthermore, the multilocus sequence typing (MLST) was identified using PubMLST (https://pubmlst.org/mlst), and the Clermont *E.coli* phylogenetic groups were determined as described previously [25]. All assembled genomes were used for core-genome alignments to produce a phylogenetic tree using snippy (https://github.com/tseemann/snippy), and the core-genome single nucleotide polymorphism (cgSNP) phylogenetic tree was constructed by IQtree [26]. In addition, the lineages of the cgSNP phylogenetic tree were predicted by fastbaps [27]. Subsequently, the phylogenetic tree was visualized using the ggtree soft-

ware package in R 4.1.1 [28]. Finally, the localization of resistance genes was predicted by MOB-suite [29], and the genetic context of resistance genes was annotated by Prokka [30].

2.5. Prediction of the potential source of CZARECs using discriminant analysis of principal components

Discriminant analysis of principal components (DAPC) is a multivariable method designed to identify and describe clusters of genetically related individuals [31]. In this study, the DAPC model was employed to predict the potential genetic source of CZARECs collected from different hosts based on the core-genome data. The metadata files of *E. coli* (n = 66,853) from humans, chickens, pigs, and cattle were downloaded from the National Center for Biotechnology Information (NCBI) database. Additionally, information on *E. coli* from dogs and cats (n = 354) was previously collected in our laboratory. Subsequently, 20 % of *E. coli* from both the NCBI and our laboratory was randomly selected to construct the cgSNP DAPC model using the adegenet package in R 4.1.1 based on their core-genome data [31]. Cross-validation was performed in 30 replicates to determine the main component fraction retained in the model. For the cross-validation, the data was divided into a training set (90.0 % of the data) and a validation set (10.0 % of the data) using stratified random sampling. Finally, the constructed model was employed to predict the potential genetic sources of all CZAREC isolates from pets and their owners, the environment, and veterinarians in hospitals.

2.6. Statistical analysis

Pearson's chi-squared test or Fisher's exact test was carried out to compare categorical variables, with an alpha value of 0.05. These tests were conducted using packages in R 4.1.1.

3. Results

3.1. Prevalence of CZAREC isolates

A total of 12,187 samples were collected, comprising 5,419 from pets, 5,843 from animal hospital environment, 557 from veterinarians, and 368 from pet owners. From these samples, 337 (6.22 %), 372 (6.37 %), 37 (6.64 %), and 14 (3.80 %) *E. coli* isolates were recovered respectively (Table 1). The overall positive rate of CZARECs among these identified *E. coli* was 7.89 % (60/760), among which 6.82 % (23/337) was obtained from pets, 9.14 % (34/372) from the environment, and 8.11 % (3/37) from veterinarians. No *E. coli* collected from pet owners (0/14) was found to be resistant to CZA (Table 1). No significant difference in CZA resistance rate was observed among *E. coli* from pets, environment, veterinarians, and pet owners (P = 0.486). However, the prevalence of CZARECs in *E. coli* from different infection sites of pets showed a significant difference (P = 0.010), with relatively high rates in tracheal wash fluid (2/4, 50.00 %), eye secretion (1/3, 33.33 %), and nasopharyngeal swab (12/43, 27.91 %). In animal hospitals, the positive rates of CZARECs displayed significant variation among different wards (P = 0.002). The highest positive rate was observed in the intensive care unit (ICU) (3/5, 60.00 %), followed by the pharmacy (1/2, 50.00 %), respiratory ward (1/3, 33.33 %), central treatment area (7/26, 26.92 %), observation room (1/4, 25.00 %), and dog and cat ward (3/19, 15.78 %) (Table S1). Although all three CZAREC isolates from veterinarians were obtained in dog/cat wards, no significant difference in the prevalence of CZARECs was observed among veterinarians working in different wards (P = 1.000). However, these results should be interpreted with caution owing to the considerable variation in sample size between groups, with some groups having relatively small sample sizes.

3.2. Antimicrobial susceptibility profiles of CZAREC isolates

Most CZAREC isolates (n = 51, 85.00 %) demonstrated high-level resistance to CZA, with MIC of ≥ 128 mg/L; in contrast, the remaining nine isolates (15.00 %) showed an MIC of 16 mg/L. Furthermore, the CZAREC isolates also exhibited high resistance rates to other β-lactams, including ampicillin-sulbactam (100 %), cefepime (100 %), amoxicillin-clavulanate (88.14 %), and meropenem (86.44 %) (Fig. 1). Moreover, CZAREC isolates also displayed high resistance rates to ciprofloxacin (98.31 %) and chloramphenicol (66.10 %). However, the resistance rates of amikacin (28.81 %), minocycline (28.81 %), nitrofurantoin (8.47 %), and colistin (1.69 %) were relatively low. The overall percentage of MDR was 33.33 % (20/60), with one isolate displaying resistance to all tested antimicrobial agents except nitrofurantoin.

3.3. Molecular characteristics of CZAREC isolates

All 60 CZAREC isolates were classified into four phylogenetic subgroups (B1, B2, C, and D), with the majority belonging to groups B1 (n = 30, 50.00 %) and C (n = 19, 31.67 %). Only three and two isolates were clustered into Groups B2 and D, respectively. Subsequently, MLST analysis revealed that these 60 CZAREC isolates were assigned to 13 serotypes (STs). The most prevalent STs were ST156 (n = 20, 33.3 %), ST410 (n = 19, 31.67 %), and ST101 (n = 7, 11.67 %), accounting for 76.67 % of all 60 isolates (Fig. 1). These three predominant STs were observed in samples from multiple sources, including the hospital environment, pets, and/or veterinaries. Bayesian analysis of population structure (BAPS) divided the 60 CZAREC isolates into six different lineages (L1-L6), with 81.67 % belonging to three predominant lineages, namely L1 (n = 20), L2 (n = 10) and L3 (n = 19). All isolates of ST156 belonged to L1, while ST410 belonged to L3. L2 contained all seven isolates of ST101, and one isolate of ST359, ST1706, and ST297 (Fig. 1). In the phylogenetic tree, six clusters (1–6) were further identified, comprising a total of 47 isolates with cluster sizes of 19, 3, 4, 13, 6, and 2 isolates. Within each cluster, the isolates displayed a high genetic similarity, with less than 100 cgSNPs. Notably, in Cluster 1, nearly 90 % of the isolates from pets, veterinarians, and the environment demonstrated only 1–11 cgSNPs (Fig. 1).

3.4. Spatial and temporal distribution of CZAREC isolates

Further analysis revealed that some genetically similar isolates of pets, animal hospital environment, and/or veterinarians within each cluster, originated from the same animal hospital and persisted for several months. These findings implied highly correlated temporal and

Table 1
Samples collection, *E. coli* and CZAREC isolates information from pets, animal hospital environment, veterinarians and pet owners.

Type of samples	No. of samples	No. of *E. coli*	No. of CZAREC	Rates of CZAREC among *E. coli*
Pet	5,419	337	23	6.82
Animal hospital environment	5,843	372	34	9.14
Veterinarian	557	37	3	8.11
Pet owner	368	14	0	0.00
Total	12,187	760	60	7.89

Abbreviations: *E. coli*, *Escherichia coli*; CZAREC, ceftazidime-avibactam-resistant *Escherichia coli*.

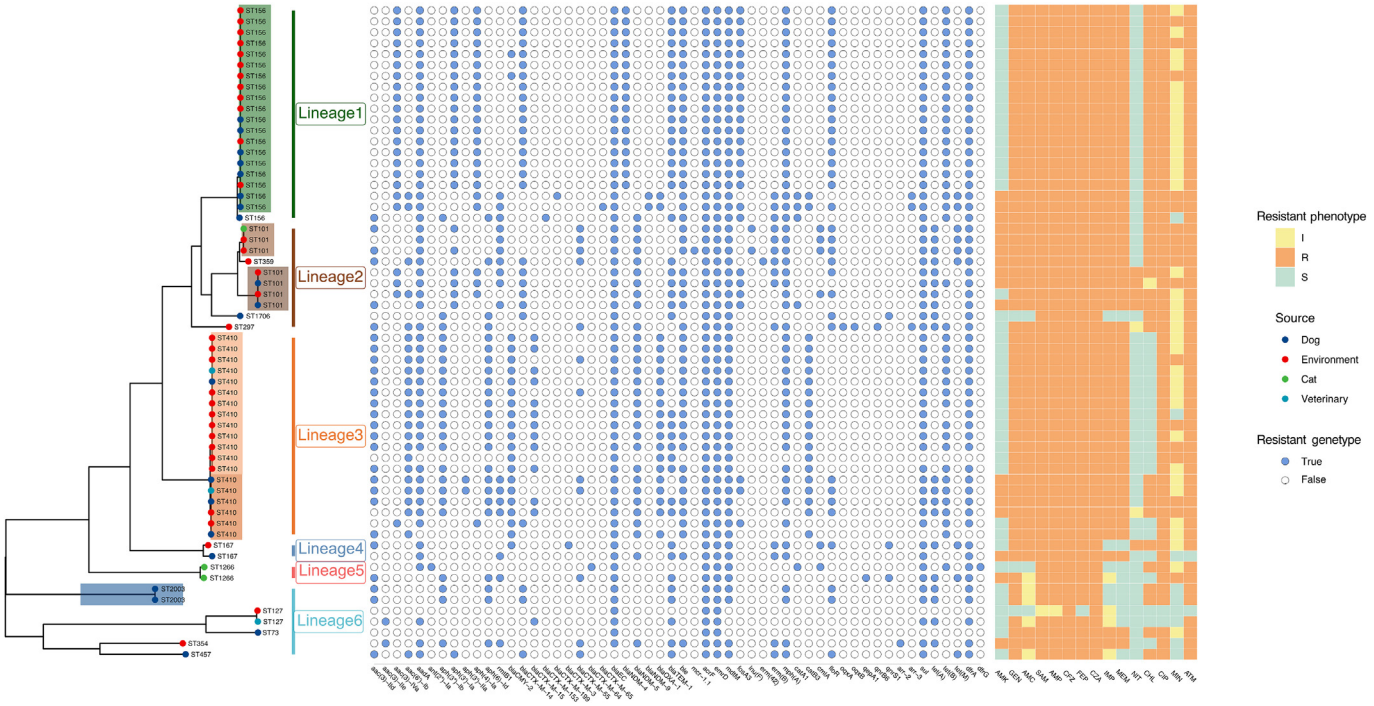

Fig. 1. Core-genome phylogenetic tree of CZARECs. Core-genome phylogenetic tree of 60 ceftazidime-avibactam-resistant *E.coli* isolated from pets, veterinarians, and animal hospital environment. Lineages: Bayesian analysis of population structure. Resistant phenotype: Antimicrobial resistance phenotype of CZARECs. Resistant genetype: Antimicrobial resistance genes carried by CZARECs. Cluster 1–6: The different clusters (cgSNP <100) are represented by shades of different colors. Abbreviations: CZAREC, ceftazidime-avibactam-resistant *Escherichia coli*; cgSNP, core genome single nucleotide polymorphisms.

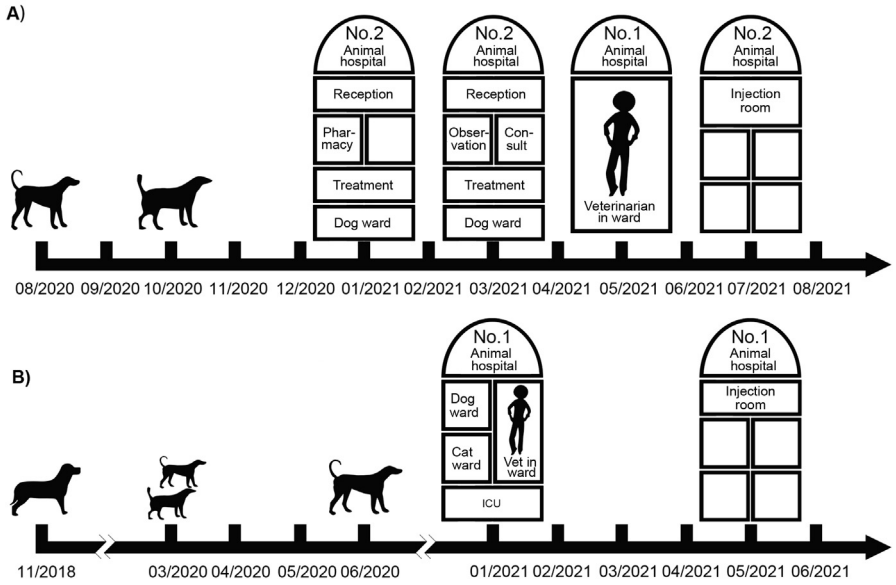

Fig. 2. Spatial and temporal distribution of CZARECs. A) The isolation time and location of the 19 isolates in Cluster 1. B) The isolation time and location of the 13 isolates in Cluster 4. Notably, two dog-derived isolates in Cluster 1 were obtained from animal hospitals other than No.1 and No.2. In addition, the four dog-derived isolates in Cluster 4 originated from animal hospital No.1. Abbreviation: CZAREC, ceftazidime-avibactam-resistant *Escherichia coli*.

spatial distribution characteristics. For instance, out of the 19 CZAREC isolates in Cluster 1, 16 were recovered from environmental samples across seven different wards in animal hospital No.2 over six months. Within two months (01/2021 and 03/2021), 12 environmental CZARECs were isolated from the same reception room, treatment room, and dog wards in hospital No.2, suggesting the potential persistence of CZARECs in hospital environment (Fig. 2A). Within Cluster 4, four dog isolates were collected from animal hospital No. 1 (two from urinary tract samples, one from upper respiratory tract, and one from trauma samples), along with one isolate from a veterinarian and nine isolates from the environment across four different wards at the same hospital over the past four months (Fig. 2B). Notably, eight out of the nine environment-derived isolates were found in the ICU, cat wards, and dog wards during the same period. The results suggested the potential spread of CZAREC isolates within the hospital environment. Similarly, the veterinarian-derived isolate was also identified during the same period, suggesting a possible transmission between the veterinarian and their work environment (Fig. 2B).

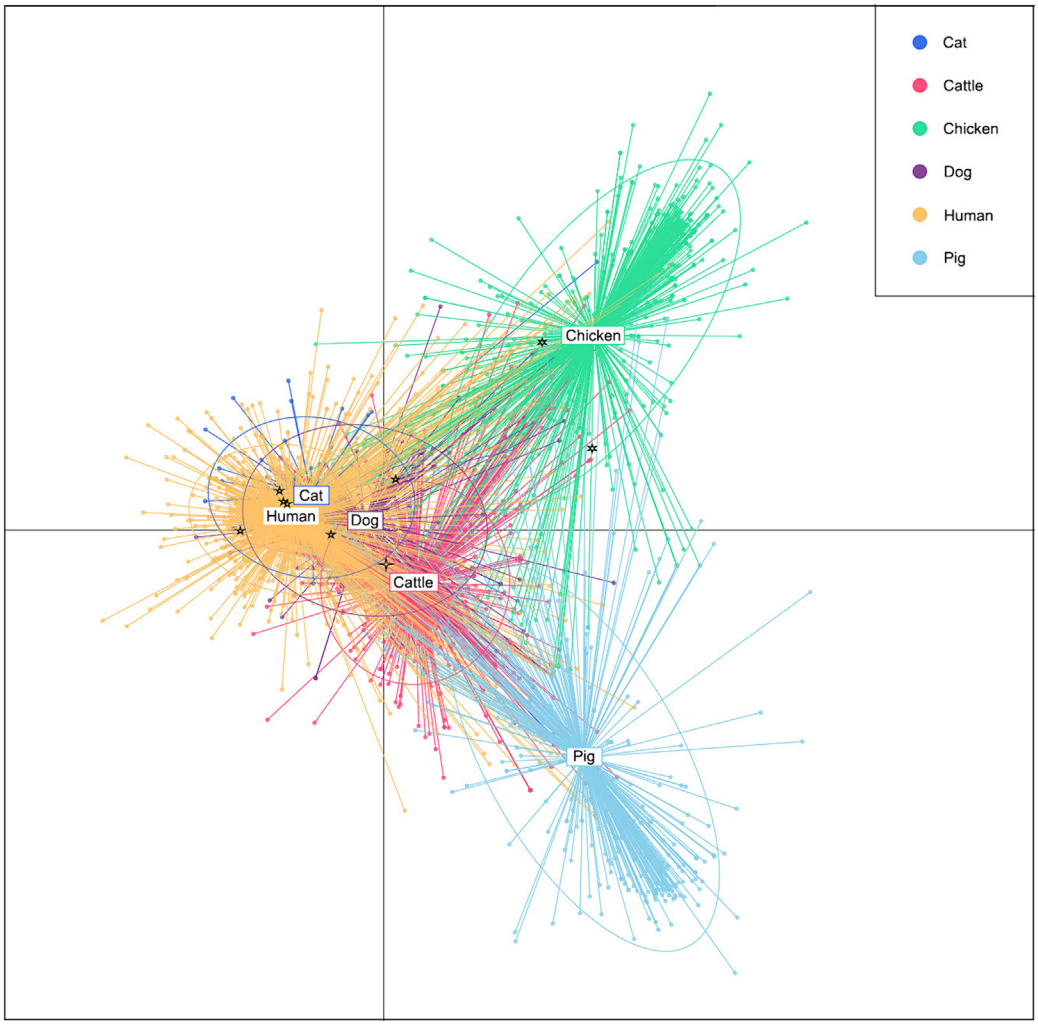

Fig. 3. Source predictions for CZARECs isolated from animal hospital environment and veterinarians using the DAPC model. Scatter plots represent individuals, and inertial ellipses represent groups. The asterisks in the figure represent two-dimensional projections of predicted results in the model of the animal hospital environment and veterinary isolates in this study. The five-pointed stars represent human strains, the six-pointed stars represent chicken strains, and the four-pointed stars represent cattle strains. Abbreviations: CZAREC, ceftazidime-avibactam-resistant *Escherichia coli*; DAPC, discriminant analysis of principal components.

3.5. Tracing the source of CZAREC isolates

DAPC was performed to determine the potential original host of the 60 CZAREC isolates from the animal hospital environment, veterinarians, and pets. Core-genome data of 4,850 *E. coli* isolates from humans (n = 2,600), cats (n = 100), dogs (n = 200), chickens (n = 600), pigs (n = 450), and cattle (n = 900) were obtained from NCBI and our previous studies for model construction. The DAPC model was built using the first 1,000 principal components (99.70 % of total variance) and five discriminant functions. Within the model, chickens-, pigs- and cattle-derived isolates were clearly distinguished from each other. However, *E. coli* isolates from dogs, cats, and humans clustered closely together, and could not be easily separated (Fig. 3). The average prediction accuracy rate of the model based on 30 cross-validations for the testing set of 4,850 *E. coli* isolates was only 52.98 %. Therefore, the dogs-, cats- and humans-derived isolates were merged into one cluster, which increased the average prediction accuracy rate to 70.00 %. Using the new model, the potential genetic source of our 60 CZAREC isolates was traced. All CZAREC isolates derived from pets (n = 23) and veterinarians (n = 3) were predicted to be sourced from the merged human/dog/cat cluster. Similarly, the majority of environmental CZARECs (n = 30, 88.23 %) were also predicted to originate from the merged cluster. Nonetheless, the remaining four environmental CZARECs were predicted to be sourced from the chicken (n = 3) and cattle (n = 1) clusters, respectively (Fig. 3).

3.6. Antimicrobial-resistant genotype and genetic environment of CZARECs

All 60 CZAREC isolates carried β-lactamases genes, with 83.33 % (n = 50) carrying class B β-lactamases genes ($bla_{\text{NDM-4, 5, 9}}$), which conferred high-level CZA-resistance with an MIC of ≥ 128 mg/L (Fig. 1). The remaining ten isolates only carried class A ($bla_{\text{CTX-M}-3, 14, 15, 55, 64, 123}$, bla_{TEM}), class C ($bla_{\text{EC-5, 8, 13, 15, 18}}$, $bla_{\text{CMY-2}}$) or class D ($bla_{\text{OXA-1}}$) β-lactamases genes (Table S2). In addition, nine out of the ten bla_{NDM}-negative isolates exhibited AmpC β-lactamase mutations, leading to amino acid substitution in the Ω-loop, R2-loop, or H-11 helix regions (Fig. 4). Despite certain mutations in these domains being associated with CZA resistance, whether the newly identified mutations can confer such resistance remains obscure. Notably, no other known mutations in β-lactamase genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{CMY}, and bla_{OXA}) linked to CZA resistance [3] were found in these ten bla_{NDM}-negative isolates. Further analysis revealed that out of the 50 bla_{NDM}-positive isolates, the bla_{NDM} gene was detected on plasmids of 49 isolates, while one isolate harbored the gene within the chromosome. The genetic environment of bla_{NDM} genes was classified into eight different types (Fig. 5). Within Cluster 1, which consisted of 19

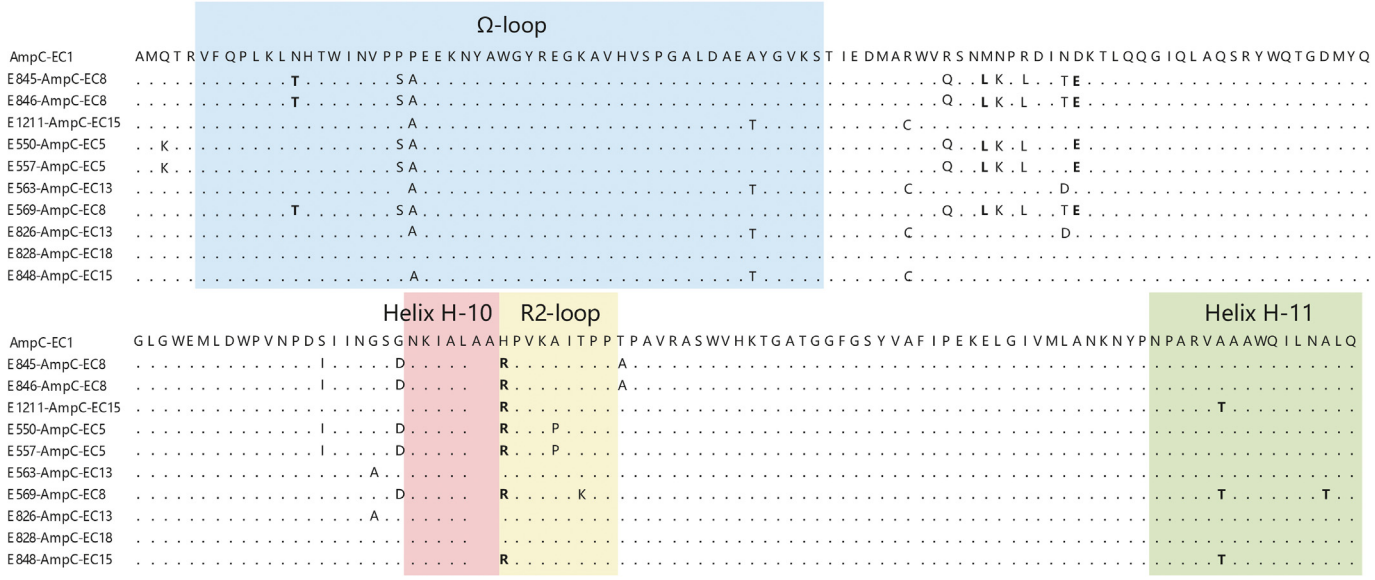

Fig. 4. Alignment of amino acid sequences of the *E. coli* narrow-spectrum AmpC β-lactamase AmpC-EC1 [38] and the AmpC β-lactamase of ten CZARECs without NDM. Abbreviations: CZAREC, ceftazidime-avibactam-resistant *Escherichia coli*; NDM, New Delhi metallo-β-lactamase.

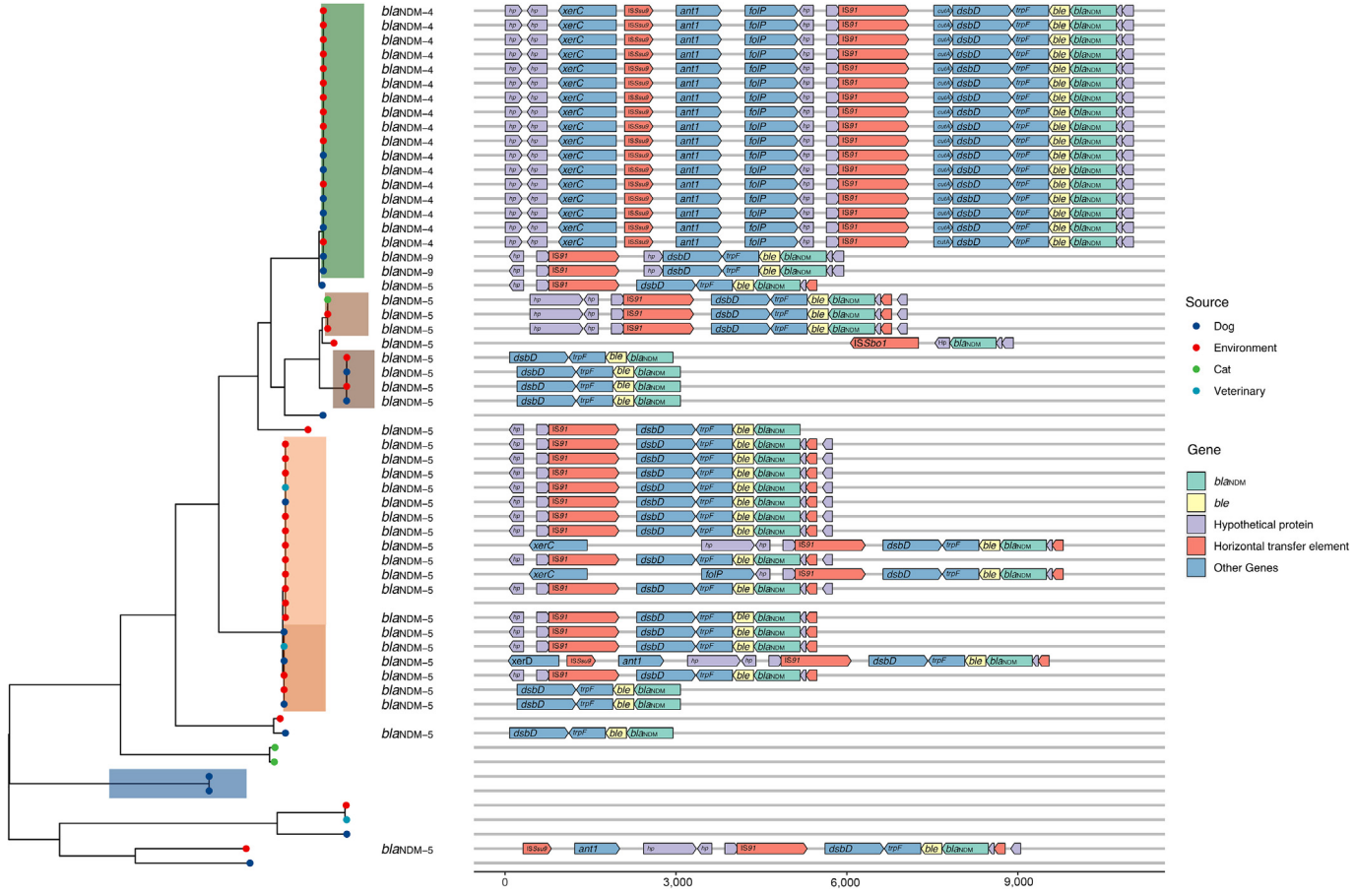

Fig. 5. Genetic environments of bla_{NDM} genes among CZAREC isolates. Abbreviation: CZAREC, ceftazidime-avibactam-resistant *Escherichia coli*.

bla_{NDM}-positive isolates, 17 isolates shared a similar genetic framework surrounding bla_{NDM-4}, while the remaining 2 isolates had a similar genetic background surrounding bla_{NDM-9}. The remaining 30 bla_{NDM}-positive CZARECs all carried bla_{NDM-5} and displayed a high similarity within each cluster of the genetic environment, which greatly varied between clusters (Fig. 5).

4. Discussion

The current study investigated the prevalence of CZARECs in animal hospitals environment, pets, and veterinarians. CZAREC isolates were more prevalent in animal hospitals (7.89 %) compared to rates observed in humans both before (0.1 % to 3.0 %) [5–9] and after

(3.2 % to 4.5 %) the clinical use of CZA in China [32,33]. The increase in resistance may be attributed to the global rise in β-lactamase resistance genes, particularly bla_{NDM}, which confers resistance to CZA by evading avibactam inhibition [34–36]. The results revealed that 83.06 % of CZAREC isolates carried bla_{NDM}. Additionally, mutations in AmpC β-lactamase genes were identified in nine CZAREC isolates, specifically in the Ω-loop, R2-loop, or H-11 helix domain [37,38]. Although whether these mutations can independently reduce CZA susceptibility remains unclear, they may contribute to it. Other mechanisms, such as changes in drug targets, decreased outer membrane permeability, or active efflux, may also be involved in developing CZA resistance [2]. Further investigation is required to comprehensively elucidate the factors contributing to CZA resistance in these isolates.

Close contact between pets and humans facilitates the transmission of antimicrobial-resistant bacteria, including MDR *Enterobacterales* isolates [14,15,39–41]. Hospital environment also serve as reservoirs and vectors for antimicrobial-resistant bacteria [14,15,39–41]. Our study provides compelling evidence of CZAREC isolate transmission among pets, veterinarians, and the environment in animal hospitals. The key findings include: 1) The majority of isolates from pets (63.64 %), the environment (85.29 %), and veterinarians (100.00 %) belonged to ST156, ST410, or ST101; 2) BAPS analysis revealed a close relationship between some CZAREC isolates from pets, veterinarians, and the environment, exhibiting high genetic similarity within closer clusters (less than 100 cgSNPs); 3) Highly similar isolates within each cluster demonstrated correlated temporal and spatial distribution patterns, with some isolates obtained from pets, veterinarians, and the environment within the same animal hospital, persisting for several months; 4) DAPC analysis predicted that most isolates from the hospital environment (90.91 %), pets (100.00 %), and veterinarians (100.00 %) were sourced to the same merged human/dog/cat cluster; 5) Most CZAREC isolates within the same high genetic clusters carried identical bla_{NDM} genes with similar genetic contexts, despite diverse origins. Therefore, these findings imply the potential transmission of CZAREC isolates among pets, veterinarians, and the environment in animal hospitals, and emphasize the role of close contact and hospital environment in the dissemination of antimicrobial resistance. Notably, isolates from humans were derived from exposed skin surfaces and clothes, which can be described as environmental contamination. No CAZREC isolates originated from pet owners, but three were sampled from veterinarians. This difference may be attributed to several possible reasons. Firstly, veterinarians are significantly more exposed to diseased pets compared to pet owners. In addition, they spent extended periods in hospital environment, where 34 CAZREC isolates were identified. This increased exposure to resistant bacteria confers a higher risk of isolating CAZREC from veterinarians. On the other hand, pet owners primarily come into contact with their pets at home. This limited exposure partially explains why CAZREC isolates were not identified among them.

In addition, our study revealed that CZAREC isolates were more commonly found in hospital wards with concentrated personnel or sick animals, such as the ICU, respiratory ward, observation room, dog/cat ward, and central treatment area. Complete disinfection of these areas remains challenging due to the close contact between humans, animals, and the environment. Consequently, the transmission of antimicrobial-resistant bacteria is facilitated in these settings. Conversely, CZAREC isolates were less frequently detected in areas that involved one-on-one contact and thorough disinfection, including the X-ray examination room, ultrasonic examination room, and consulting room. These areas prioritize individual attention and comprehensive cleaning measures, which contribute to a lower prevalence of CZAREC isolates. The three CZAREC-positive animal hospitals in our study were referral centers with high patient volumes, consistently treating pets from various regions. The frequent interaction between personnel and animals in these hospitals increases the risk of closely related isolates across different animal hospitals. For instance, isolates obtained from hospitals No.1 and No.2 were clustered together in Cluster 1, indicating a very close genetic relationship. Moreover, analysis of global *E. coli* genome data revealed that dog/cat isolates were highly similar to human isolates at the cgSNP level. These results suggest a process of co-evolution and gene exchange between humans and pets over time. Collectively, our findings highlight the impact of concentration and interaction between personnel and sick animals on CZARECs prevalence in hospitals, emphasizing the role of close contact and the unique relationship between humans and pets in transmitting antimicrobial resistance.

Nevertheless, the limitations of the current study should be acknowledged. Firstly, there was a time gap between collecting *E. coli* samples from pets and sampling the environment, veterinarians, and owners in animal hospitals. This might result in an underestimation of the correlation between isolates, although evidence of CZARECs sharing between different hosts was still found. Secondly, the environmental and personnel samples were collected over a one-year period, potentially allowing certain dominant CZAREC clones to persist. This persistence could be attributed to the ability of resistant isolates to survive within hospital environment. However, the specific duration of persistence and disappearance of these clones remains uncertain. Still, continuous monitoring of high-risk CZAREC clones is crucial to prevent their transmission and spread, especially considering the growing number of pets and animal hospitals in cities, which provides increased opportunities for contact with these potential isolates. Therefore, veterinarians should prioritize disinfection, particularly in areas where animals and people congregate. Moreover, both veterinarians and pet owners should employ effective personal protective measures within animal hospitals and ensure sterilization before leaving to minimize the transmission of high-risk isolates.

Ethics statement

Ethical approval was reviewed and given by China Agricultural University Animal Ethics Committee document (AW01017102-2).

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Hegen Dai: Investigation, Formal analysis, Writing – original draft. **Dongyan Shao:** Formal analysis, Visualization. **Yu Song:** Investigation, Resources. **Qi An:** Investigation, Resources. **Zhenbiao Zhang:** Investigation, Resources. **Haixia Zhang:** Investigation, Resources. **Siyu Chen:** Supervision, Writing – review & editing. **Congming Wu:** Supervision, Writing – review & editing. **Jianzhong Shen:** Supervision, Writing – review & editing. **Yanli Lyu:** Supervision, Writing – review & editing. **Yang Wang:** Supervision, Writing – review & editing. **Shizhen Ma:** Methodology, Formal analysis, Writing – original draft. **Zhaofei Xia:** Conceptualization, Methodology, Writing – review & editing.

Supplementary data

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