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RESEARCH PAPER

Molecular Detection of Escherichia Coli That Isolated from Mouth Samples of Cat and Their Susceptibility to Biosynthesized Selenium Nanoparticles

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ABSTRACT

In this study, we aimed to determine the public and molecular characteristics of *Escherichia coli* in oral swabs obtained from domestic cats in Iraq. A total of 50 oral swabs were collected, constituting 25 males and 25 females. *E. coli* was isolated from 16 samples (5 [20%] from males and 11 [44%] from females), suggesting higher prevalence in female cats than male. The identification of isolates was done by using VITEK® 2 compact system and confirmed by16s rRNA gene sequencing. Ciprofloxacin and Ceftazidime resistance in all *E. coli* isolates was determined by the disc diffusion test. Furthermore, the highest growth inhibition against these isolates was achieved using selenium nanoparticles (SeNPs) biosynthesized by pseudomonas aeruginosa, which were characterized and confirmed using X-ray diffraction (XRD). Phylogenetic analyses inferred from 16S rRNA sequences showed that the Iraqi feline oral isolates were closely grouped with *E. coli* strains previously reported in India, China, Russia and Norway indicating potential genetic relationships on a worldwide scale. These results emphasize the need to monitor antimicrobial-resistant *E. coli* in companion animals as a putative reservoir of zoonotic pathogens.

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INTRODUCTION

Escherichia coli, a definitive Gram-negative bacterium of the Enterobacteriaceae family, has been studied extensively as both a harmless commensal organism and a major opportunistic pathogen across multiple animal species. In recent years, investigations into *E. coli* colonization in companion animals particularly domestic cats (Felis catus) that share close living spaces with human populations have expanded our understanding of pet-associated microbial dynamics [1]. While conventional veterinary research has predominantly prioritized the feline gut microbiota, emerging evidence establishes the oral cavity as a critical and highly complex reservoir for potentially pathogenic bacteria, including *E. coli* [2]. Isolating and characterizing *E. coli* from the oral microbiome of healthy or diseased pet cats provides essential insights into bacterial ecology and transmission routes. Interestingly, host physiological and behavioral traits appear to influence this colonization; recent studies indicate that sex-specific factors heavily impact the feline oral microbiome composition, leading to distinct variations in *E. coli* carriage rates and strain diversity between male and female cats

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[3]. This anatomical colonization becomes highly concerning when evaluating public health risks. *E. coli* strains recovered from companion animals are increasingly exhibiting multidrug resistance (MDR) profiles that closely mimic human clinical isolates [4]. This phenotypic overlap underscores the potential role of domestic pets as active reservoirs for resistant bacteria, elevating the risks of zoonotic interspecies transmission through close daily interactions such as licks or shared environments. To combat the escalating threat of antimicrobial resistance among feline oral pathogens, traditional antibiotics are proving insufficient, driving the exploration of alternative therapeutic materials. Among these, nanotechnology offers a promising frontier. Selenium nanoparticles (SeNPs) have emerged as highly potent antimicrobial and antibiofilm agents due to their unique physical properties, including a high surface-area-to-volume ratio and minimal toxicity to host tissues compared to other metallic nanoparticles [5]. SeNPs exhibit multi-targeted bactericidal mechanics, including the generation of localized reactive oxygen species (ROS), physical disruption of the bacterial cell wall, and cellular deformation [6]. Because SeNPs damage physical cell structures rather than targeting narrow metabolic pathways, they significantly lower the likelihood of bacteria developing structural resistance, making them an ideal candidate for treating resistant Gram-negative strains like *E. coli* isolated from companion animal cavities. Consequently, continuous screening of oral isolates against advanced nanomaterials is highly essential to evaluate their potential for future veterinary topical or systemic applications.

MATERIALS AND METHODS

The investigation utilized crystal violet, Gram's iodine, safranin, ethanol, and various cultures and reagents. The laboratory equipment comprised a compound microscope, microscopic slides, and sterile loops. The Zoonotic Diseases Laboratory at the College of Veterinary Medicine in Baghdad, Iraq, supplied all necessary materials.

Sampling

All oral pet cat swabs used in this study were collected from different locations in Baghdad province, Iraq, between October 2024 and April 2025. In total there were fifty samples. The samples were subjected to bacterial isolation and identification, which are standard procedures. The isolated bacteria were then used for antibiotic sensitivity testing using VITEK to find out the sensitivity of the bacteria.

Animal Ethics

All cat-related procedures adhered to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine (Protocol number 1052, dated 4/5/2024). The animals were maintained in enhanced environments, and we employed a sophisticated handling protocol to mitigate stress.

Isolation of Escherichia coli

Feline oral swab specimens were analyzed utilizing established culture techniques for enterobacteria. After a 24-hour incubation at 37ºC, the collected swabs were immediately streaked over MacConkey agar and injected into nutrient broth for enrichment. To get pure, well-isolated lactose-fermenting colonies, presumptive positive cultures were successively re-cultured on fresh MacConkey agar plates. The collected colonies were then streaked onto Eosin Methylene Blue (EMB) agar to detect the distinctive green metallic sheen characteristic of Escherichia coli, followed by incubation at 37ºC for 24 hours [7].

Biochemical traits and the diagnostic Vitek-2 System

A number of routine biochemical assays, including methyl red, indole synthesis, urease, oxidase, Voges-Proskauer, and motility tests, were conducted to verify the identity of the isolates. Simultaneously, automated biochemical profiling of the presumptive positive E. coli isolates was performed utilizing the Vitek-2 compact system (bioMérieux, France) in full compliance with the manufacturer's operational guidelines [7,8].

Antimicrobial susceptibility testing

The susceptibility profiles of the verified E. coli isolates to a selection of essential veterinary and human antibiotics were assessed using the Kirby-Bauer disk diffusion technique. The evaluated antimicrobials comprised imipenem, ceftriaxone, cefixime, aztreonam, ciprofloxacin, cefotaxime, tetracycline, ceftazidime, and sulfamethoxazole-trimethoprim. All resultant zones of inhibition were quantitatively quantified and classified as susceptible, intermediate, or resistant according

to the Clinical and Laboratory Standards Institute criteria [9,10].

PCR-test

PCR amplification of the bacterial 16S rRNA gene was conducted utilizing the universal primer set for final molecular identification.Forward primer 27F: 5'-AGAGTTTGATCCTGGCTCAG-3'Reverse primer 1492R: 5'-TACGGTTACCTTGTTACGACTT-3'This design produces a diagnostic amplicon of approximately 1.5 kb [11,12]. The PCR products were sequenced using Sanger sequencing on an automated capillary DNA sequencer. Gene sequences were compared to reference databases utilizing the Basic Local Alignment Search Tool (BLAST) on GenBank, and a phylogenetic tree was created employing the Neighbor-Joining approach to evaluate the evolutionary relationships of the feline isolates [11].

Biological Synthesis of Selenium Nanoparticles (SeNPs)

The biogenic production of selenium nanoparticles was accomplished by a green synthesis method utilizing Pseudomonas aeruginosa.

Bacterial cultivation

A loopful of P. aeruginosa was introduced into 250 mL of nutritional broth and cultured for 24 hours at 37°C. Bacterial cells were eliminated using centrifugation at 6,000 rpm for 10 minutes,

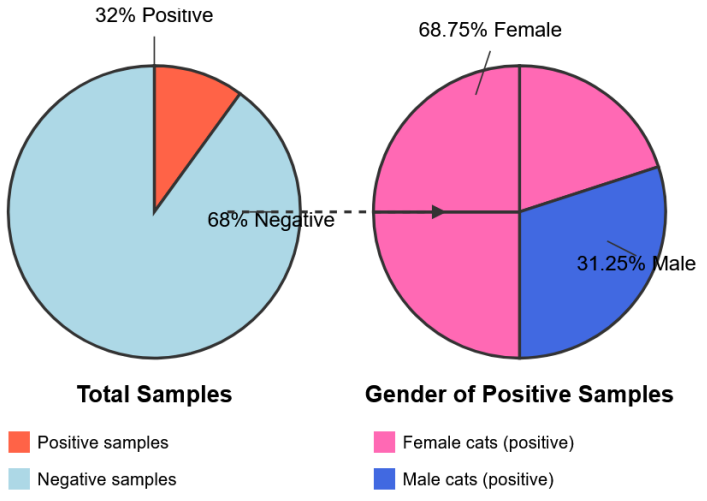

Fig. 1. Growth percentage from all samples collected. Figure 4.1A: Positive growth from all samples shown as a chart. Figure 4.1B: Percentage of *E. coli* from male and female positive growths.

Table 1. Distribution of *E. coli* isolates according to collected swabs

Gender	Total number of swabs	*E. coli* isolates	Total isolates
Female oral swabs	25	11	68.75%
Male oral swabs	25	5	31.25%
Total	50	16 (32%)	
Chi-square	3.308		
P-value	0.05		

and the resultant cell-free supernatant, including active reducing biomolecules, was collected [13].

Synthesis of selenium nanoparticles

For nanoparticle manufacturing, the produced bacterial culture medium containing the active supernatant was augmented with sodium selenite Na2SeO3 to attain a final concentration of 2 mM. The combination was incubated in a shaking incubator at 37°C and 200 rpm for a duration of 72 hours. Throughout incubation, the alteration of the culture media from a pale yellowish tint to a deep orange-red hue clearly indicated the effective reduction of poisonous selenite (Se^{+4}) to stable, elemental selenium (Se^{0}) nanoparticles [13,14]. Following a 72-hour period, the colloidal SeNPs were harvested using centrifugation at 6,000 rpm for 15 minutes, subsequently washed twice with sterile deionized distilled water to eliminate residual salts, transferred to sterile Petri plates, and dried at 37 °C to get pure dry SeNPs.

X-ray diffraction (XRD)

(XRD) measurements were conducted using a Shimadzu-6000 X-ray diffractometer (Japan) to ascertain the structural phase, purity, and crystalline characteristics of the dried biomimetic nanoparticles, running at specified voltage and current settings [13].

RESULTS AND DISCUSSION

Isolation of E. coli

Fifty oral swabs were obtained from pet cats, categorized into two groups: 25 swabs from males and 25 from females, gathered from various veterinary clinics in Baghdad city between October 2024 and April 2025. Following the completion of morphological, microscopic, and biochemical analyses, together with the installation of the VITEK® 2 System, the findings are shown in Table 1. The total positive growth from all swabs was 16 (32%), with 11 (68.75%) positive results from female swabs and 5 (31.25%) positive results from male swabs (Fig. 1).

Physical features were employed to identify the *E. coli* isolates. The isolate appeared as vibrant pink colonies on MacConkey agar, but on EMB medium, the colonies exhibited a green metallic sheen. The presence of bile salts and crystal violet in MacConkey agar facilitates the proliferation of Gram-negative bacteria while inhibiting the development of Gram-positive bacteria.

Identification utilizing the VITEK-2 system

The automated Vitek-2 system identification method is an effective technology for the rapid identification of Gram-negative bacteria. Vitek-2 cards (bioMérieux) were employed for the detection of Gram-negative colonies. The results indicated that all 35 isolates of suspected *E. coli* yielded good outcomes.

Molecular Characterization and Prevalence of Escherichia coli

Sixteen feline oral swab samples produced positive, viable isolates of *Escherichia coli.* Genomic

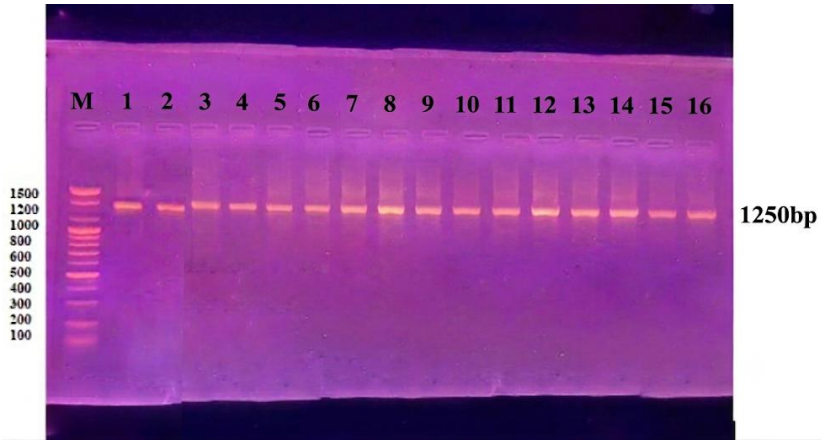

Fig. 2. Agarose gel electrophoresis of the genomic DNA extract of *E. coli* isolates has successfully extracted genomic DNA; M: DNA ladder (1500).

DNA was isolated from each isolate and underwent polymerase chain reaction (PCR) amplification aimed at the [insert target gene, e.g., uidA or 16S rRNA] gene.Electrophoretic examination of the PCR results consistently demonstrated a singular, unique amplicon band at about 1250 bp (Fig. 2), therefore verifying the molecular identification of all 16 isolates.The identified Iraqi *E. coli* exhibited 99% similarity with other global isolates, as seen in Fig. 3.

Characterization of Selenium nanoparticles

X-ray diffraction (XRD) examination was conducted to assess the crystalline structure and phase purity of the produced nanoparticles. Fig. 4 demonstrates that the XRD pattern of the sample (designated "45-nano") displays clear, well-defined reflection peaks, affirming the extremely crystalline characteristics of the produced material.The diffraction pattern displays three significant peaks at 2\theta values of roughly 43.5, 50.8, and 74.3. The highest peak (100% relative intensity) occurs at 2\theta approx 43.5, indicating the principal crystalline reflection plane of the structure. Furthermore, two small peaks of diminished intensity are seen within the region of 36 - 39.The acute and slender contour of the diffraction peaks signifies a substantial level of crystallinity. The average crystallite size, determined using the Scherrer equation, was found to be in the nanometer range (about 35 - 45 nm), corroborating the effective production of nanoscale particles. The distinct, well-defined reflections indicate a well ordered and systematically arranged atomic structure inside the produced nanostructure.

Antibiotic Resistance in Bacteria

Sixty *E. coli* isolates were evaluated for 17 antibiotic discs with the disc diffusion method, revealing resistance to ciprofloxacin drugs (Fig. 5). The clear zones found prevented microbial growth against selenium nanoparticles. The SeNPs substance demonstrated notable activity as an antibacterial agent against *Escherichia coli* germs. Fig. 6 illustrates the antibacterial efficacy of SeNPs, Various concentrations of SeNP were synthesized (100, 50, and 25 µg/ml) for each plate, evidenced by distinct zones indicating bacterial inhibition in the designated region, with the width of the inhibition zone expanding as the quantity of SeNPs increases.

The results of the present study reveal a significant disparity in the isolation rate of Escherichia coli from the oral cavities of domestic cats, with a notably higher frequency in females (68.75%) compared to males (31.25%). While E. coli is regarded as an opportunistic colonizer in

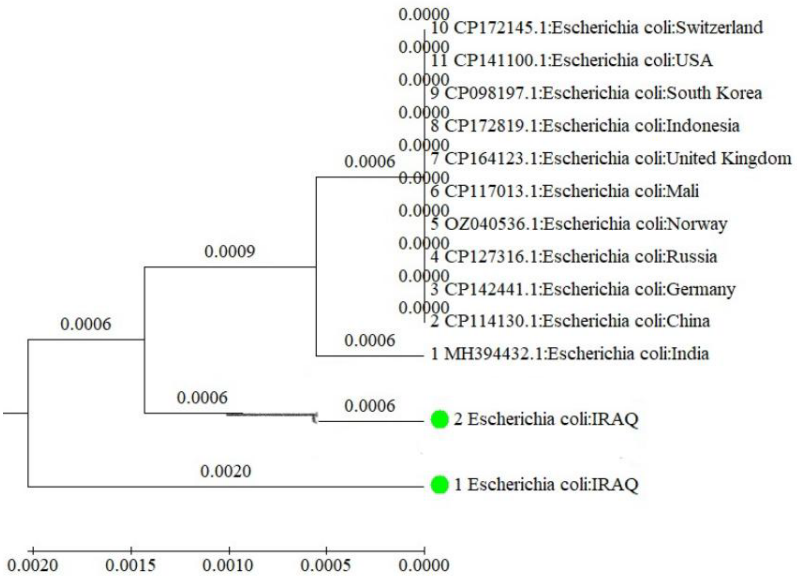

Fig. 3. phylogenic tree of the 16sRNA gene of *E.coli of Iraqi isolates*

the feline oral cavity rather than a predominant commensal species [15,16], this significant gender disparity may be attributed to a confluence of unique biological, anatomical, behavioral, and immunological factors. Female cats often exhibit more frequent and vigorous grooming practices than male cats [17]. Due to E. coli being an enteric bacteria naturally expelled with feces, the meticulous self-grooming of the perineal and perianal areas by females promotes the mechanical transfer of these microflora straight to the oral cavity. This behavioral exposure is exacerbated by significant structural abnormalities. The diminished physical separation between the anus and the urogenital tract in female cats elevates the possibility of localized fecal contamination of the surrounding fur, thus augmenting the likelihood of oral inoculation during habitual self-grooming.

In breeding populations, maternal and caregiving impulses create an additional direct route for oral exposure. In a population of breeding or lactating queens, pronounced maternal and nurturing behaviors particularly the thorough grooming of neonates and the instinctive consumption of their excreta to promote elimination and ensure nest hygiene represent a highly effective pathway for increased oral exposure to enteric pathogens [18]. Maternal-neonatal physical contact and oral exposure are recognized as key factors influencing the early transfer and colonization patterns of intestinal flora between mothers and their kids [19].

Ultimately, these behavioral hazards are supported by systemic physiological, hormonal, and immunological changes. Physiological changes associated with the estrous cycle, gestation, and breastfeeding markedly affect both systemic and localized mucosal immunity in the queen [20]. These hormonal oscillations are known to influence the mucosal habitats of the body, potentially altering the balance of the oral microbiota. Microenvironmental alterations might temporarily weaken mucosal barrier defenses, increasing the susceptibility of the oral cavity to colonization by opportunistic enteric pathogens such as E. coli [16,20], so reinforcing the complex risk profile seen in female cats.

The nucleotide sequence of the Escherichia coli isolate from the oral cavity of pet cats in Iraq has been submitted to the National Center for Biotechnology Information (NCBI) database with the GenBank accession number MH394432, which is crucial for evaluating bacterial diversity, assessing zoonotic risks, and monitoring the spread of antimicrobial resistance within the "One Health" paradigm [21]. Phylogenetic analysis employing evolutionarily conserved genes and specific virulence factors enables precise

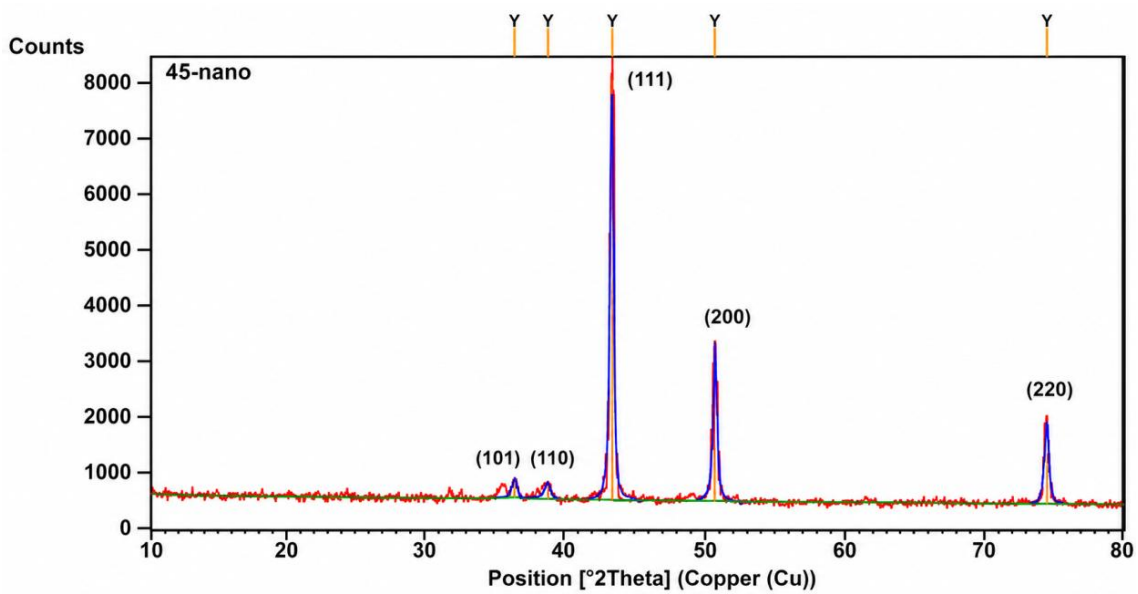

Fig. 4. X-ray diffraction pattern of selenium nanoparticles synthesized in sodium selenite solution using *P. aeruginosa* extract.

identification of the origin of feline oral isolates and their genetic relationship to human clinical infections, especially when categorizing these isolates into core phylogroups such as B2 and D, which are historically linked to extraintestinal pathogenic E. coli (ExPEC) strains that cause severe human infections, including septicemia and urinary tract infections [22]. Moreover, sophisticated sequencing technologies, including whole-genome analysis, facilitate the identification and tracking of identical clonal lineages and common antimicrobial resistance genes present in pet populations and their owners. This offers concrete molecular evidence of the horizontal and reciprocal transmission of these microbes within shared domestic settings, thereby deepening the comprehension of domesticated animals as potential natural reservoirs for bacterial lineages with significant epidemiological implications [23,24].

The structural phase and crystalline nature of the synthesized selenium nanoparticles (SeNPs) were determined using X-ray diffraction (XRD) analysis. The XRD pattern confirmed the successful synthesis and high purity of the nanomaterial, displaying distinct Bragg reflection peaks that correspond directly to the standard hexagonal phase of crystalline selenium.This study's results indicate that synthesized selenium nanoparticles (SeNPs) exhibit significant, dose-dependent antibacterial activity against Escherichia coli sourced from the oral cavities of pet cats, effectively overcoming established antibiotic resistance mechanisms. In our in vitro studies, the optimal concentration of 100 µg/mL proved to be the most effective dose, resulting in greatest zones of inhibition and entirely halting bacterial growth.

This notable nano-mediated suppression is

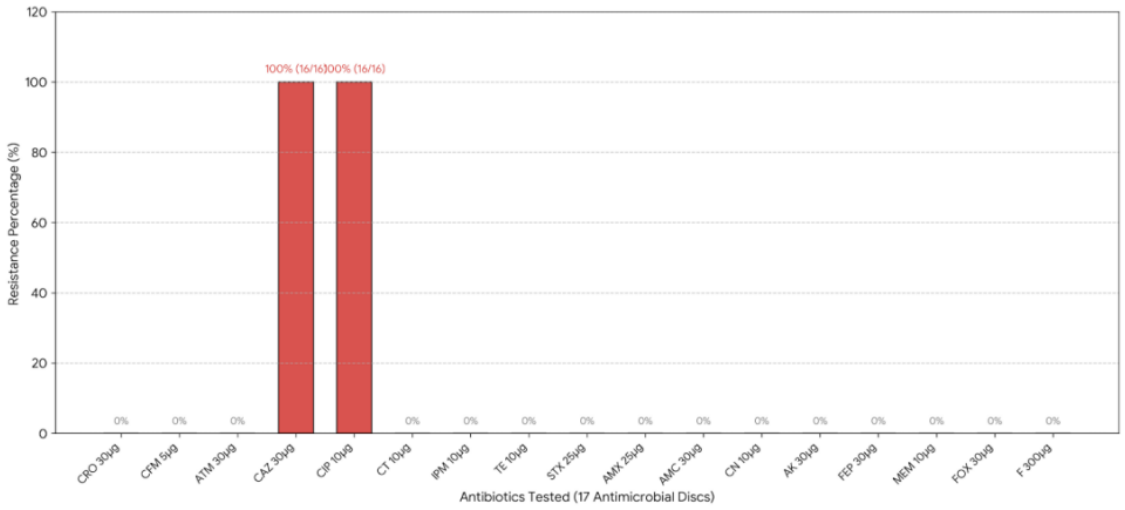

Fig. 5. *E. coli* antibiotic sensitivity (Kirby–Bauer) in oral pet cat

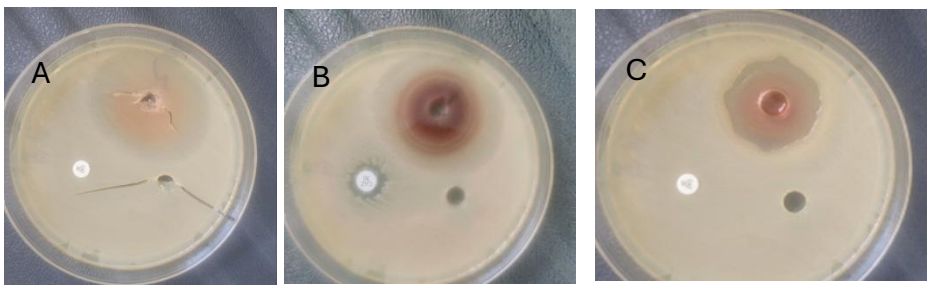

Fig. 6. Agar well diffusion assay of Se nanoparticles at concentrations (A100, B50, C25) against *E. coli*

especially remarkable when compared to the resistance profiles seen with typical treatment agents, including ciprofloxacin and cefotaxime. The oral E. coli isolates from felines demonstrated significant resistance to ciprofloxacin, a fluoroquinolone that inhibits DNA replication, and cefotaxime, a third-generation cephalosporin that targets cell wall synthesis. This observation is consistent with recent global trends indicating an increase in multi-drug resistant (MDR) and extraintestinal pathogenic E. coli (ExPEC) lineages within companion animal reservoirs [22,24].

The ineffectiveness of traditional antibiotics such as ciprofloxacin and cefotaxime against these particular oral isolates highlights the swift development of beta-lactamases and target-site mutations in the feline oral microbiota, likely influenced by intimate domestic interactions and common environmental selective pressures [23]. In contrast, the exceptional effectiveness of SeNPs at 100 µg/mL circumvents these particular biochemical resistance mechanisms owing to their multi-targeted mode of action.

In contrast to traditional single-target pharmaceuticals, SeNPs apply antibacterial pressure by binding to the negatively charged bacterial cell wall, resulting in significant physical membrane disruption, stimulating intracellular reactive oxygen species (ROS) production, and causing irreversible damage to bacterial DNA and metabolic enzymes (Huang et al., 2020). Our findings indicate that SeNPs at an optimal concentration of 100 µg/mL provide a potent alternative therapeutic strategy to mitigate the zoonotic transmission of multi-drug resistant E. coli from companion animals to humans, addressing a critical need in modern veterinary medicine and public health systems.

CONCLUSION

This study demonstrates that the oral cavity of domestic companion cats serves as an active anatomical niche and potential reservoir for multidrug-resistant (MDR) *Escherichia coli*, presenting substantial public health implications under the One Health framework. A clear sex-associated disparity in carriage was established, with female cats showing significantly higher colonization rates (68.75%) than males (31.25%), likely driven by behavioral grooming patterns, anatomical proximity, and maternal-hormonal factors. Molecular characterization via 16S rRNA sequencing and phylogenetic analysis confirmed high genetic homology with global pathogenic lineages (GenBank accession no. MH394432), underscoring the risk of zoonotic transmission of resistant strains to human cohabitants. Importantly, biogenically synthesized selenium nanoparticles (SeNPs) produced via *Pseudomonas aeruginosa* demonstrated high crystallinity, nanoscale dimensions (35–45 nm), and potent, dose-dependent bactericidal efficacy against feline oral *E. coli* isolates that exhibited marked resistance to conventional antibiotics, including ciprofloxacin and cefotaxime. Given their multi-targeted bactericidal mechanisms—including membrane disruption and ROS-mediated cellular damage—SeNPs (particularly at an optimal concentration of 100 µg/mL) represent a promising therapeutic alternative to circumvent target-site resistance and beta-lactamase activity. Future research should prioritize *in vivo* biocompatibility assessments, formulation into veterinary topical dental rinses or systemic regimens, and broader surveillance of companion animal microbiota to mitigate interspecies transmission of antimicrobial resistance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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