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PREVALENCE OF ANTIMICROBIAL RESISTANCE AMONG PATHOGENS ISOLATED FROM PET FOODS AND ANIMAL PROTEIN

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Abstract:

The possibility of transmission of microorganisms to both humans and animals is considered a serious threat, due to the dissemination and high prevalence of antibiotic resistance among bacteria isolated from pet foods and animal proteins which presents as a serious issue to public health. The objective of this study was to evaluate the prevalence and pattern of antimicrobial resistance in bacterial pathogens which were isolated from animal proteins, fisheries, and pet foods. A total of 20 samples were collected and analyzed for the presence of bacterial species. Among the isolates, 22 different bacterial species were identified, including both Gram-negative (e.g., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enterica*) and Gram-positive (e.g., *Bacillus cereus*) pathogens. The antimicrobial susceptibility of these isolates was evaluated, revealing significant resistance patterns. Among the Gram-negative isolates, 32% were identified as producers of extended-spectrum beta-lactamases (ESBLs), while 27% exhibited inducible *AmpC* beta-lactamase activity, and 23% were resistant to colistin. The isolates showed high sensitivity to amoxiclav, imipenem, and netilmicin. These results highlight the importance of monitoring antimicrobial resistance in pet food and animal protein sources and underscore the need for stringent regulations on antibiotic use in animal husbandry to mitigate the spread of resistant pathogens.

Keywords: *AMR, Animals, ESBL, AmpC, Food chain*

Introduction:

The growing threat of antimicrobial resistance (AMR) has serious implications for both human and animal health worldwide. This phenomenon occurs when

microorganisms—such as bacteria, fungi, and parasites—develop resistance to antimicrobial drugs, diminishing their effectiveness. A key factor driving AMR is the widespread and improper use of these agents across various fields, including medicine, veterinary care, agriculture, and food production, accelerating the evolution of resistant strains.

Recent research has brought attention to the role of pet foods and animal-derived proteins in the spread of antimicrobial resistance (AMR). These foods, particularly those containing meat-based ingredients, can harbor resistant pathogens, posing a potential risk to pets, their owners, and the surrounding environment. Studies have identified bacteria such as *Salmonella, Escherichia coli, Campylobacter, and Enterococcus* in pet food, with many strains exhibiting resistance to widely used antibiotics (Aarestrup *et al.,* 2008; Cummings *et al.,* 2020). Transmission can occur through direct contact with pets, contaminated food, or environmental exposure, further contributing to the AMR crisis.

The growing concern over antimicrobial resistance (AMR) transmission from pet food to both animals and humans highlights a significant public health risk. Pets consuming contaminated food can become carriers of resistant bacteria, which may be excreted through feces, urine, or saliva, contributing to environmental contamination. Moreover, resistant pathogens have the potential to reach humans through direct pet contact or indirect exposure via the food chain (Lambertini *et al.,* 2018). This issue is particularly worrisome as pets often live near their owners, increasing the likelihood of bacterial transmission within shared spaces.

One of the most alarming aspects of antimicrobial resistance (AMR) in pet foods is the inclusion of animal proteins sourced from livestock. The livestock industry is a well-documented contributor to AMR due to the widespread use of antibiotics for growth enhancement, disease prevention, and treatment (Van Boeckel *et al.,* 2015). While AMR in various animal-derived foods has been extensively studied, research specifically focusing on pet foods remains limited, despite their complex composition of multiple animal-based ingredients that may harbor resistant bacteria (Li *et al.,* 2019). Beyond direct

contamination risks, concerns also arise regarding pet food manufacturing practices and their role in the broader dissemination of resistant microbes.

Examining the presence and diversity of antimicrobial-resistant pathogens in pet food is essential for devising strategies to reduce AMR-related risks. Systematic monitoring of pet food and animal-derived ingredients helps pinpoint resistance hotspots, shape regulatory frameworks, and enhance safety protocols in production. Research indicates fluctuating AMR levels in pet food, with certain studies uncovering worrisome resistance to critical antibiotics used in both human and veterinary medicine (WHO: Geneva ; 2017).

Methodology:

Sample collection:

A total of 20 different samples were collected from different sources which included pet foods, fishes, prawns, beef, chicken and mutton. The samples were collected in a sterile zip-lock cover and they were transported to the laboratory in an ice box for further processing.

Isolation of bacteria:

1 gm of the sample was taken and a fine paste was made with sterile saline with the help of a sterile mortar and pestle. A loopful of this was inoculated onto on Nutrient Agar (NA), Mannitol Salt Agar (MSA), Blood Agar (BA) and MacConkey Agar (MAC). The plates were incubated at 37°C for 24 hours. Next day the colonies were picked up and gram staining, motility, catalase and oxidase were done. The bacterial isolates were identified as per the standard protocols.

Antibiotic sensitivity testing:

Once the bacterial isolate was identified, the antibiotic sensitivity testing was carried out by Kirby Bauer disc diffusion method (Bauer *et al*., 1996) for the following antibiotics- cefotaxime, cefotaxime/clavulinic acid, imipenem, cefoxitin, ceftriaxone, amoxyclav, imipenem and netilin.

Method:

1. Standard suspension of the isolates was made and the turbidity was matched to Mc Farland standard 0.5. The inoculum was spread evenly on the agar surface using a sterile cotton swab and allowed to dry for 5-10 minutes.
2. Antibiotic discs were placed on the Mueller Hinton agar surface with appropriate spacing. Plates were inverted and incubated at 37°C.
3. After the incubation, the diameter of the zone of inhibition was measured and interpreted according to the CLSI zone size interpretive chart.

Criteria for deciding an organism as ESBL producers:

Zone diameter of various third generation cephalosporin,

Cefotaxime 30mcg =< 27mm or

Ceftazidime 30mcg =< 22mm or

Ceftriazone 30mcg =< 25mm

Increase in zone size with addition of an inhibitor (ESBL) by >5mm.

ESBL screening tests

Disc diffusion test: (Wayne, 2009)

This test requires use of Cefotaxime (30mcg) alone and in combination with Cavulinic acid (10mcg). Cefotaxime & with Cefotaxime/Clavulanic acid (30mcg/10mcg) discs were placed with distance between the two discs 10mm edge to edge on MHA plate inoculated with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture and was incubated overnight at 37^{o}C. An increase in the zone diameter by > 5mm of Cefotaxime versus its zone when tested in combination with Clavulanic acid is considered as an ESBL producer.

Disc Antiagonism Test (*AmpC* β-lactamase inducibility):

In this test, lawn culture of test isolate (0.5 Mc Farland) was made over Muller-Hinton agar plate (MHA) and ceftazidime (30µg) and Cefoxitin (30µg) disc were placed 20mm apart from centre to centre. Plates were incubated for 18-24 hours at 37^{o} C. *AmpC* β-lactamase inducibility was recognized by isolates showing blunting of ceftazidime zone of inhibition adjacent to cefoxitin disc and were considered screen positive. (Sanders *et al.*,1982)

Phenotypic detection of colistin resistance:

In this test, lawn culture of test isolate (0.5 Mc Farland) was made over Muller-Hinton agar plate (MHA) and Colistin (10µg) disc was placed. Plates were incubated for 18-24 hours at 37° C. ≤11 mm is proposed to be interpreted as resistance, whilst an inhibition zone ≥11 mm indicates sensitivity.

Results:

Collection of samples:

A total of 20 samples were collected from pet food, fisheries, and animal proteins to assess the level of bacterial pathogens. (Table 1)

Table 1: Source of Samples collection

S.No	Source of sample collection	Number of samples collected
1	Pet foods	7
2	Chicken	6
3	Mutton	2
4	Prawn	3
5	Fish	1
6	Beef	1

Prevalence of bacterial isolates from pet food, fisheries and animal proteins:

A total of 32 different bacterial species were identified from different pet foods, fisheries and animal proteins. Among the isolates, 22 were found to be gram negative bacteria and 10 were found to be gram positive rods.

Prevalence of gram-negative bacteria:

A total of 22 different bacterial species were identified from different pet foods, fisheries and animal proteins. The prevalence of bacterial isolates was as follows- *Pseudomonas aeruginosa, Enterobacter amnigenus, Kluyvera ascorbata, Klebsiella pneumoniae, Enterobacter intermedium, Serratia grimesii, Citrobacter rodentium, Salmonella enterica and Klebsiella oxytoca.* (Table 2).

Table 2: Prevalence of gram-negative bacteria (n =22)

S.No	Name of the organisms	Percentage of isolation
1	*Pseudomonas aeruginosa*	7(31%)
2	*Enterobacter amnigenus*	1(5%)
3	*Kluyvera ascorbata*	2(9%)
4	*Klebsiella pneumoniae*	2(9%)
5	*Klebsiella oxytoca*	2(9%)
6	*Enterobacter intermedium*	1(5%)
7	*Serratia grimesii*	2(9%)
8	*Citrobacter rodentium*	1(5%)
9	*Salmonella enterica*	4(18%)

Prevalence of gram-positive bacteria:

A total of 10 gram-positive rods were identified from different pet foods, fisheries and animal proteins. The prevalence of bacterial isolates was as follows- *Bacillus cereus, Bacillus polymyxa, Bacillus brevis and Bacillus firmus.* (Table 3)

Table 3: Prevalence of gram-positive bacteria (n =10)

S.No	Name of the organisms	Percentage of isolation
1	*Bacillus cereus*	5(50%)
2	*Bacillus polymyxa*	2(20%)
3	*Bacillus brevis*	2(20%)
4	*Bacillus firmus*	1(10%)

Antibiotic sensitivity testing:

ESBL screening test among gram-negative bacteria:

A total of 22 gram-negative isolates were obtained in the present study and among the 22 isolates, 7 (32%) were found to be positive to produce ESBL by disc diffusion method using cefotaxime and with cefotaxime/clavulanic acid and 15 (68%) were found to be ESBL non-producers. (Table 4)

Table 4: Cefotaxime antibiotic sensitivity pattern of gram-negative bacteria

Total number of gram-negative bacteria	Cefotaxime sensitive Isolates	Cefotaxime resistant isolates
22	15(68%)	7 (32%)

Disc Antiagonism Test (*AmpC* β-lactamase inducibility):

The disc antagonism test was done to detect inducible *AmpC* β-lactamase for all the isolates. 6/22 (27%) bacterial isolates were found to be positive for inducible *AmpC* β-lactamase. The isolates showed blunting of the ceftazidime zone of inhibition adjacent to the cefoxitin disc or the isolates showed no zone of inhibition around the cefoxitin disc. (Table 5)

Table 5: *AmpC* β-lactamase screening of gram-negative bacteria

Total number of gram-negative bacteria	Cefoxitin sensitive isolates	Cefoxitin resistant isolates
22	16 (73%)	6 (27%)

Phenotypic detection of colistin resistance:

The phenotypic detection of colistin resistance was done for all the isolates. 5/22 (23%) bacterial isolates were found to be positive colistin. (Table 6)

Table 6: Screening of colistin resistance among gram negative bacteria

Total number of gram-negative bacteria	Colistin sensitive isolates	Colistin resistant isolates
22	17 (77%)	5 (23%)

All the gram-negative bacterial isolates showed a high level of sensitivity 86% towards amoxiclav followed by 82% by imipenem and netilin.

Discussion:

The rise of antimicrobial-resistant pathogens in pet foods and animal-derived proteins presents a growing challenge to both veterinary and human health. As antimicrobial resistance (AMR) continues to escalate globally, it is vital to investigate how resistant bacteria enter the food chain, particularly through pet food, which remains largely under examined in surveillance efforts. The routine use of antibiotics in otherwise healthy food animals, even in the absence of disease, has played a major role in accelerating the spread of AMR worldwide (FAO, 2018). Antibiotics in animal agriculture are not solely used for treatment but also for other purposes, further exacerbating the issue.

In livestock production, antimicrobials play a critical role beyond therapeutic use, being widely administered for metaphylaxis (targeted treatment of entire herds or flocks following exposure to infected individuals), prophylaxis (preventive intervention to inhibit bacterial colonization and subsequent infection), and as antibiotic growth promoters (AGPs), which alter the gut microbiome to enhance nutrient absorption and accelerate growth—examples include avilamycin, bambermycin, efrotomycin, and ionophores (McEwen & Fedorka-Cray, 2002). Global epidemiological data suggest that antimicrobial application in food animals is more prevalent for growth enhancement than for treating infectious diseases. This widespread practice is primarily driven by the rising demand for protein-dense animal products, particularly in rapidly industrializing nations where evolving dietary patterns favor increased consumption of high-quality animal proteins.

This study detected 22 distinct bacterial species across various sources, including pet food, fisheries, and animal-derived proteins. The broad diversity and prevalence of these microorganisms highlight the potential health risks they pose to both animals and humans. The identified bacteria comprise both Gram-negative and Gram-positive pathogens, many of which are linked to antimicrobial resistance, zoonotic transmission, and foodborne diseases, underscoring their significance in public and veterinary health.

This study identified several dominant Gram-negative bacterial pathogens, including *Pseudomonas aeruginosa, Enterobacter amnigenus, Kluyvera ascorbata, Klebsiella pneumoniae, Enterobacter intermedium, Serratia grimesii, Citrobacter rodentium, Salmonella enterica, and Klebsiella oxytoca.* These microbes are highly adaptable and capable of surviving on various surfaces, particularly those associated with pet food and animal-derived products. Notably, *Salmonella enterica* poses a significant public health risk due to its strong link to foodborne diseases and its ability to develop multidrug resistance (Koutsoumanis *et al.,* 2020). As a major causative agent of gastrointestinal infections in both humans and animals, Salmonella can be transmitted zoonotically, with pets acting as potential carriers, increasing the risk of human exposure through direct contact or consumption of contaminated food (Ferrie *et al.,* 2014).

Similarly, *Klebsiella pneumoniae and Klebsiella oxytoca,* both recovered in this study, are opportunistic pathogens frequently implicated in nosocomial infections, including urinary tract infections, pneumonia, and septicemia. These facultative anaerobic, encapsulated bacteria have demonstrated resistance to multiple antimicrobial agents, notably carbapenems and beta-lactam antibiotics, through the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases (Li *et al.,* 2019). Their detection in pet food and animal-derived proteins raises concerns about the horizontal gene transfer of resistance determinants, potentially facilitating the spread of multidrug-resistant strains via environmental contamination or zoonotic transmission through direct contact with colonized pets.

Another noteworthy isolate was *Pseudomonas aeruginosa,* a multidrug-resistant opportunistic pathogen known for its intrinsic resistance to various antibiotic classes, including aminoglycosides, cephalosporins, and beta-lactams (Bassetti *et al.,* 2018). Pseudomonas species are ubiquitous environmental microorganisms, frequently detected in soil, water, and animal-derived products, including pet food. Their capacity to form biofilms enhances their persistence in hostile conditions, providing protection against antimicrobial agents and disinfectants. This biofilm-forming ability, coupled with their psychrotolerant nature, enables P. aeruginosa to survive in refrigerated

environments, posing a significant challenge to food safety and microbial control strategies.

The study also detected several Gram-positive bacterial pathogens, including *Bacillus cereus, Bacillus polymyxa, Bacillus brevis, and Bacillus firmus,* all of which are widely distributed across terrestrial, aquatic, and food-associated ecosystems. Among these, *Bacillus cereus* is particularly noteworthy due to its implication in foodborne intoxications, primarily gastroenteritis, resulting from the ingestion of toxin-contaminated food (Rana *et al.,* 2020). Its pathogenicity is driven by the secretion of enterotoxins, positioning it as a clinically significant microorganism in both human and veterinary epidemiology.

The widespread occurrence of bacterial pathogens can largely be attributed to suboptimal hygiene and improper handling protocols throughout various stages of food production, storage, and processing. In the context of pet food, insufficient sanitation during manufacturing, ineffective temperature regulation during distribution and storage, and unhygienic handling in pet care environments create favorable conditions for microbial contamination and persistence (Lambertini *et al.,* 2018).

This study highlights alarming trends in antimicrobial resistance (AMR) among Gram-negative bacterial isolates obtained from pet foods, fisheries, and animal-derived protein sources. A total of 22 isolates were characterized, and phenotypic antimicrobial susceptibility assays confirmed the expression of extended-spectrum beta-lactamases (ESBLs), inducible AmpC beta-lactamases, and colistin resistance—major resistance determinants contributing to multidrug resistance (MDR) in both veterinary and human clinical microbiology. The detection of ESBL-producing strains is particularly concerning, as these beta-lactamase enzymes hydrolyze beta-lactam antibiotics, including third-generation cephalosporins like cefotaxime, undermining conventional treatment strategies.

In this study, the detection of ESBL-producing bacteria through the disc diffusion assay utilizing cefotaxime and cefotaxime/clavulanic acid corroborates previous microbiological investigations, which report a high prevalence of these resistant strains in animal-derived food matrices (Kieffer *et al.,* 2017). The horizontal gene transfer of

ESBL-encoding plasmids is strongly influenced by the selective pressure exerted through antibiotic administration in intensive animal husbandry, particularly within poultry and livestock production systems. This anthropogenic factor has been a key driver in the dissemination of ESBL-positive pathogens across the food chain, including pet food microbiomes (Shukla, & Nair, 2021).

The phenotypic characterization of inducible AmpC beta-lactamase production was performed using the disc antagonism assay, a method that exploits the competitive inhibition of beta-lactamase activity. This observation is consistent with previous microbial resistance studies identifying Enterobacter, Citrobacter, Klebsiella, and other Enterobacteriaceae as frequent reservoirs of AmpC beta-lactamase genes (Bush, 2018). These enzymes, often encoded on chromosomal cassettes or mobilizable plasmids, remain transcriptionally repressed under basal conditions but exhibit inducible expression upon exposure to beta-lactam antibiotics, particularly extended-spectrum cephalosporins. The presence of inducible AmpC producers in bacterial isolates from pet food and animal-derived protein sources poses a significant clinical and epidemiological threat, as their broad-spectrum hydrolytic activity compromises beta-lactam efficacy, restricting antimicrobial stewardship. This resistance mechanism is especially problematic in infections where carbapenemase co-expression further narrows viable treatment options (Jacoby, 2009).

The phenotypic identification of colistin resistance in 5 out of 22 (23%) bacterial isolates underscores a significant challenge in antimicrobial resistance (AMR) surveillance. As a cationic antimicrobial peptide belonging to the polymyxin family, colistin exerts its bactericidal effect by disrupting the integrity of the outer membrane of Gram-negative bacteria through interactions with lipopolysaccharides (LPS). Its designation as a last-resort antibiotic for treating infections caused by extensively drug-resistant (XDR) pathogens, particularly carbapenem-resistant Enterobacteriaceae (CRE), makes the emergence of resistance particularly alarming. The increasing prevalence of colistin-resistant *E. coli, Klebsiella,* and other Enterobacteriaceae is strongly linked to selective pressure from colistin use in food-producing animals, where the horizontal

dissemination of mcr genes via plasmid-mediated transfer has accelerated resistance spread, threatening both veterinary and human medicine (Li *et al.,* 2019).

The antimicrobial susceptibility analysis revealed that all Gram-negative isolates exhibited notable sensitivity to amoxiclav (86%), followed by imipenem (82%) and netilmicin (82%). These findings indicate that despite the rising antimicrobial resistance (AMR), certain antibiotics, particularly β-lactam/β-lactamase inhibitor (BLBLI) combinations and carbapenems, maintain considerable efficacy against these bacterial strains. Amoxiclav, composed of amoxicillin and clavulanic acid, exerts its antimicrobial action by inhibiting peptidoglycan synthesis while neutralizing β-lactamase-mediated degradation, thereby enhancing its spectrum of activity. Its continued effectiveness across veterinary and human medicine highlights its role as a critical agent in combatting Gram-negative infections (Kidsley *et al.,* 2020).

The susceptibility of isolates to netilmicin, an aminoglycoside that exerts bactericidal activity by irreversibly binding to the 30S ribosomal subunit and disrupting protein synthesis, suggests that this class of antimicrobials retains some efficacy against Gram-negative pathogens. However, its clinical application remains restricted due to concentration-dependent nephrotoxicity and ototoxicity, necessitating cautious therapeutic use. The high resistance rates observed among other antibiotic classes, particularly third-generation cephalosporins and colistin, highlight the increasing prevalence of extended-spectrum β-lactamase (ESBL) and plasmid-mediated colistin resistance (mcr genes). These findings reinforce the critical need for robust antimicrobial stewardship programs, continuous resistance surveillance, and molecular epidemiological studies to track the dissemination of resistant determinants in both human and veterinary microbiomes.

Conclusion:

The findings of this study emphasize the intensifying threat of antimicrobial resistance (AMR) in bacterial pathogens isolated from pet foods and animal-derived protein sources. The high prevalence of extended-spectrum β-lactamase (ESBL)-producing bacteria, inducible AmpC β-lactamase producers, and colistin-resistant

Enterobacteriaceae within these isolates signals a significant one health challenge, facilitating the cross-sectoral dissemination of resistance genes. The widespread administration of antimicrobials in animal agriculture, particularly in intensive food production systems, imposes strong selective pressure, driving the horizontal gene transfer (HGT) of resistance determinants through conjugative plasmids, transposons, and integrons. These findings reinforce the role of pet food microbiomes as reservoirs for multidrug-resistant (MDR) pathogens, necessitating stringent antimicrobial stewardship, molecular epidemiological surveillance, and metagenomic approaches to track the genetic mobility of resistance elements across the food chain.

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