Prevalence of Carbapenem Resistance Genes in *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* from Meat Samples

Running Title: Carbapenem resistance genes in bacteria transmitted from meat

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Abstract

Background: Infections caused by bacteria transmitted from food, including Enterobacteriaceae, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Acinetobacter baumannii* (*A. baumannii*) resistant to carbapenems, are spreading, and this has caused concerns in the field of treatment. This study investigated the frequency of carbapenem resistance genes in Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii* isolated from raw chicken meat.

Method: In this cross-sectional study, 100 samples of raw chicken meat were collected from Isfahan. Bacterial infection was evaluated and confirmed using biomedical tests. Antibiotic sensitivity tests were performed using disc diffusion for *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter baumannii* isolates. The frequency of genes encoding resistance to carbapenems (*OXA-181, OXA-48, VIM*, *NDM, IMP*, and *KPC*) was determined through polymerase chain reaction (PCR) analysis.

Results: Out of 100 samples, 70 were positive for bacterial infection, of which 30 were infected with *Escherichia coli* (*E. coli*), 14 samples with *Klebsiella pneumoniae* (*K. pneumoniae*), 9 samples with *Salmonella typhimurium* (*S. typhimurium*), 11 samples with P. *aeruginosa*, and 6 samples were infected with *A. baumannii*. The highest amount of antibiotic resistance was estimated to be tetracycline, cotrimoxazole, gentamicin, trimethoprim, and streptomycin, and the lowest amount was azithromycin and rifampin. Among genes encoding resistance to carbapenem, *NDM* and *OXA-48* genes were the most commonly expressed, with a frequency of 60% and 28.24%, respectively.

Conclusion: The study found significant bacterial contamination, especially for Enterobacteriaceae, with notable antibiotic resistance to tetracycline. Carbapenem resistance genes *NDM* and *OXA-48* were prevalent, indicating the urgency of addressing antibiotic resistance.

Keywords: Enterobacteriaceae, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Carbapenems

Introduction

As the global population expands, ensuring food security becomes increasingly pressing. It is imperative to ascertain the capacity of food production systems to accommodate this growth with high quality and consider safe protocols. Animal-derived food products, such as meat, are an important part of the human diet, yet their safety and sustainability are threatened by various factors [1, 2]. Infections caused by Gram-positive and Gram-negative bacteria transmitted from food are spreading, and this has caused concerns in the field of treatment [3]. The prevalence of antibiotic resistance in the food chain poses a significant threat to human health, as antibiotic-resistant bacteria can enter the food supply through various pathways, including the use of antibiotics in livestock for growth promotion and disease prevention [4, 5]. Among the diverse array of resistance mechanisms, the rise of carbapenem resistance in clinically relevant pathogens represents a pressing concern due to the limited treatment options available [6, 7]. The mechanisms of antibiotic resistance, such as the acquisition of resistance genes and the production of carbapenemase enzymes that render carbapenem antibiotics ineffective highlight the complexity of this issue, as these enzymes can spread among bacterial species, complicating treatment options [8, 9].

Carbapenems, including imipenem and meropenem, constitute crucial medications renowned for their extensive and enduring spectrum of activity in contrast to beta-lactamases [10]. *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* are notable bacterial species known for acquiring and disseminating resistance genes, including those conferring resistance to carbapenems [8, 11-14]. The most important carbapenems are commonly categorized into three classes: Class A (*KPC*); Class B, encompassing metallo β-lactamases such as *IMP*, *VIM*, and *NDM*; and Class D, represented by oxacillinase, notably *OXA-48*, of which *OXA-48* has been reported frequently across the world for their role in the rapid spread of infection [15, 16].

Meatborne diseases related to bacterial infections are more common in children [17]. Numerous bacterial pathogens, including *P. aeruginosa, A. baumannii, Klebsiella pneumoniae* (*K. pneumoniae*), *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica*, toxin-producing species like *Staphylococcus aureus* (*S. aureus*), and *Bacillus cereus*, contribute to meat borne diseases either through animal infection or through contamination during meat processing or handling [18–20]. Consuming contaminated meat risks contracting various diseases, categorized into gastrointestinal and extra- gastrointestinal diseases [21]. In the current study, we investigated the frequency of carbapenem resistance genes in *Enterobacteriaceae, P. aeruginosa*, and *A. baumannii* isolated from raw chicken meat in Isfahan, Iran.

Methods

The study involved the collection of 100 raw chicken meat samples sourced from various chicken farms to ensure a representative overview of potential contamination levels across different production settings. The samples were obtained using sterile swabs to prevent any external contamination during collection. Each sample was cultured on Eosin Methylene Blue (EMB) medium, adhering to standard microbiological protocols to facilitate the growth of Gram-negative bacteria. Following the initial confirmation of Gram-negative bacilli, positive lactose colonies were further cultured on Triple Sugar Iron Agar medium specifically designed for the isolation of *Enterobacteriaceae*. For the identification of *Pseudomonas* species, suspicious colonies were cultured on Pseudomonas Cetrimide Agar (PCA), while colonies suspected to be *A. baumannii* were cultured on blood agar. Phenotype and biochemical tests were applied to indemnify the isolates as follows: for *Enterobacteriaceae*, Indole, Methyl Red, Voges.p, and Citrate tests were

utilized to differentiate diagnosis between *E. coli*, *S. typhimurium, and K. pneumoniae*; *P. aeruginosa* isolated were confirmed through Lactose, Citrate, Indole, Oxidase, DNase, and hemolysis tests; and *A. baumannii* isolates were confirmed by Lactose, Oxidase, Catalase, Pigmentation, Urease, and IMVIC tests.

The antibiotic susceptibility of all isolates was determined by the Kirby-Bauer method. Applied antibiotics included tetracycline, ceftazidime, ciprofloxacin, trimethoprim-sulfamethoxazole, tobramycin, chloramphenicol, norfloxacin, amikacin, gentamicin, rifampin, cefalotin, streptomycin, trimethoprim, levofloxacin, imipenem, meropenem, azithromycin [22].

DNA extraction was conducted from pure bacterial cultures [23] and was assessed using NanoDrop [24]. Polymerase chain reaction (PCR) to detect *OXA-181, OXA-48, VIM, NDM, IMP* and *KPC* genes expression was performed through the following steps: for *E. coli* (1 for 5 min cycle at 94°C for initial denaturation; 33 cycles included 60 sec at 94°C for denaturation, 45 sec at 58°C for annealing, and 60 sec at 72°C for extension; and 1 cycle for 7 min at 72°C for final extension), for *K. pneumoniae* (1 for 5 min cycle at 94°C for initial denaturation; 35 cycles included 30 sec at 95°C for denaturation, 90 sec at 58°C for annealing, and 90 sec at 72°C for extension; and 1 cycle for 10 min at 72°C for final extension), for *S. typhimurium* (1 for 4 min cycle at 95°C for initial denaturation; 30 cycles included 45 sec at 94°C for denaturation, 60 sec at 58°C for annealing, and 40 sec at 72°C for extension; and 1 cycle for 5 min at 72°C for final extension), for *P. aeruginosa* (1 for 5 min cycle at 94°C for initial denaturation; 25 cycles included 35 sec at 94°C for denaturation, 45 sec at 53°C for annealing, and 60 sec at 72°C for extension; and 1 cycle for 7 min at 72°C for final extension), for *A. baumannii* (1 for 3 min cycle at 94°C for initial denaturation; 30 cycles included 40 sec at 95°C for denaturation, 55 sec at 59°C for annealing, and 60 sec at 72°C for extension; and 1 cycle for 6 min at 72°C for final extension). Multiplex PCR was performed to evaluate the expression of gene coding for resistance to carbapenem compounds in isolates. Utilized primers have been presented in Table 1 [25]. The quality of the product was evaluated using gel electrophoresis [26].

Statistical analysis

Numbers and percentages reported the frequency of data. All data was analyzed using SPSS version 21.

Results

Studied population and bacterial isolates

Among 100 cultured samples, 70 were positive for bacterial infections. Following standard bacteriology tests, 30 isolates were identified as *E. coli*, 14 isolates as *K. pneumoniae*, 9 isolated as *S. typhimurium*, 11 as *P. aeruginosa*, and 6 isolates were identified as *A. baumannii*.

Antibiotic susceptibility test

The highest rates of antibiotic resistance were found in *E. coli* against tetracycline (100%) and streptomycin (93.33%), while *K. pneumoniae* also showed 100% resistance to tetracycline. *S. typhimurium* was resistant to tetracycline (100%) and trimethoprim (88.88%). *P. aeruginosa* exhibited 100% resistance to tetracycline, and *A. baumannii* showed complete resistance (100%) to meropenem and imipenem. Notably, the highest resistance to imipenem was observed in *P. aeruginosa.* (Table 2), Figure 1.

molecular detection of carbapenemase genes

The most frequently reported gene expression of *E. coli, K. pneumoniae,* and *S. typhimurium* was *NDM* (7.33%), *KPC* (71.43%), and *NDM* (66.67%), respectively, which were significantly higher compared to other genes ($P<0.05$). Among *P. aeruginosa* isolates, the frequency of *VIM* (100%)

and *IMP* (90.91%) gene expression was significantly higher compared to others (P=0.018). In *A. baumannii* isolates, the gene expression of *IMP, VIM*, and *OXA-48* was significantly higher than others (P=0.030). Notably, the presence of the *NDM* gene in *S. typhimurium*, which did not show carbapenem resistance, highlights the complexity of antibiotic resistance gene distribution, suggesting that resistance genes should primarily be reported in the context of resistant strains to provide clearer implications for resistance mechanisms (Table 3), Figure 2.

Discussion

The emergence and spread of antibiotic resistance among bacterial pathogens pose significant challenges to public health worldwide. The increasing prevalence of carbapenem resistance genes in various bacterial species has raised concerns about the potential transmission of these resistance genes through the food chain [6, 7]. The expression of carbapenemase genes, which facilitates bacterial survival against carbapenems, raises concerns about the effectiveness of current treatment protocols and the need for novel therapeutic strategies. Strengthening surveillance and implementing targeted infection control measures are essential to mitigate the impact of these resistant strains on public health [27, 28]. In the current study, we investigate the frequency of carbapenem resistance genes in *Enterobacteriaceae*, *P. aeruginosa,* and *A. baumannii* isolated from meat. Among *Enterobacteria*, 42.8% of isolates were *E. coli*, 20% were *K. pneumoniae,* and 12.9% were *S. typhimurium.* The frequency of *P. aeruginosa* was 15.7%, and for *A. baumannii* was 8.6%.

Similarly, Dehkordi et al. reported a prevalence of 45.2% for *E. coli* isolated from raw chicken meat in Iran [29]. Nazari Moghadam et al. demonstrated that 2% of chicken meat samples were positive for *Salmonella,* and all were *S. typhimurium* [30]. It indicates that most antibiotics commonly utilized in the livestock and poultry industries demonstrate ineffectiveness against a significant isolate of *Salmonella*, underscoring their importance in public health considerations. In a study by Mousse et al., about 20% of bacteria isolated from the food were *K. pneumoniae* [31]. Atabay et al. observed that among 484 isolates, 78.92% were *E. coli*, followed by 7.64% *Salmonella*, 3.71% *Proteus*, 3.51% *S. aureus*, 1.85% *Streptococcus*, 1.44% *P. aeruginosa*, 1.23% *Klebsiella*, and 1.03% *S. epidermidis* [32]. Rafei et al. reported different prevalence of *A. baumannii* isolated from meat, raw milk, drinking water, cheese, and domestic animal samples, respectively [33].

We observed that about 70% of selected samples were Gram-negative bacteria from raw chicken meat with high antibiotic resistance to various antibiotics, including tetracycline, cotrimoxazole, gentamicin, trimethoprim, and streptomycin. This high contamination rate likely stems from the excessive use of antibiotics in poultry farming, where these substances are routinely prescribed to control and prevent bacterial infections, treat diseases, and promote growth [1, 34]. Such practices not only contribute to the development of resistant bacterial strains but also pose significant public health risks as these resistant pathogens can be transmitted to humans through the food chain. Moreover, the widespread resistance observed may compromise the effectiveness of treatment options for bacterial infections in humans, necessitating urgent interventions to regulate antibiotic use in livestock and improve food safety standards [35].

Rezaloo et al. found that 9.16% of meat samples were *P. aeruginosa*. These isolates exhibited pronounced antibiotic resistance, particularly to ampicillin, penicillin, and tetracycline, and the lowest resistance was reported to imipenem and trimethoprim [36]. Studies reported that *K. pneumoniae* and *E. coli* isolated from meat were more likely to be multidrug-resistant and resistant [37, 38]. *K. pneumoniae* isolated from meat was more resistant to tetracycline and gentamicin than

those isolated from the gastrointestinal tract [37]. Among detected *E. coli*, *K. pneumoniae*, *S. typhimurium*, and *P. aeruginosa* had the highest resistance to tetracycline. Yang et al. reported that among *Salmonella* isolated from meat, *S. typhimurium* isolates were more prevalent than others with the highest resistance to tetracycline [39]. Consequently, the prescription of antibiotics, and inevitably, the emergence of antibiotic resistance in poultry farms, is exacerbated. The primary source of contamination in livestock and poultry stems from the transmission of antibiotic-resistant bacterial strains from humans to meat, leading to an escalation in the prevalence of strains isolated from livestock and poultry that are resistant to antibiotics used in human medicine [40, 41].

Alhazmi et al.'s study on *K. pneumoniae* isolates showed a high frequency of *blaOXA-48* and *blaNDM* carbapenemase genes. Among their isolates, only one isolate each harbored *blaVIM* and *blaKPC* genes, while no *blaIMP*-producing isolates were detected [42]. In the current study, KPC was the most prevalent carbapenemase gene expression in *K. pneumoniae,* while *OXA-48* had the lowest frequency. It should be considered that differences in geographical location and sample origin between studies may influence the variations of the genes. Bakhtiari et al. demonstrated that 24% of *K. pneumoniae* isolates were resistant to imipenem, 35.45% represented the *blaKPC* gene, and 16.36% expressed the *blaVIM-1* gene [43]. Amini et al. demonstrated that among *K. pneumoniae* isolates, expression of *blaVIM* and *blaIMP* were higher than other carbapenemase gene [44]. Li et al. showed that among carbapenem-resistant *A. baumannii*, common carbapenemase-positive genes included *blaOXA-51*-like, *blaOXA-23*-like, *blaNDM-1*, and *blaOXA-58* [45]. The current study reported gene expressions of *IMP, VIM*, and *OXA-48* genes in *A. baumannii* isolates. Another study by Ghaffoori Kanaan et al. demonstrated that the most common gene expression among carbapenem-resistant *S. enteritidis* isolates was *blaIMP*, *blaOXA−48−*like, and *blaNDM*. In contrast, the *blaKPC* and *blaVIM* genes were undetected [46]. We detected *S. typhimurium* and *E. coli* from collected meat, with the highest frequency of *NDM* expression.

The differences observed in the levels of antibiotic resistance and the prevalence of specific genes across various studies are multifactorial. These include the indiscriminate use of antibiotics in different countries, variations in patterns of antibiotic administration, the emergence of novel resistance mechanisms, the site of infection acquisition, genetic variations among bacterial strains, and geographical conditions, among others [47, 48]. The emergence of antibiotic resistance poses a significant burden on healthcare systems globally, leading to substantial economic costs. Apart from inappropriate antibiotic usage, factors such as aggressive treatment approaches, immunocompromised patients, and non-compliance with hygiene protocols also contribute to the problem. In the agricultural sector, adherence to health regulations, proper animal nutrition, modern slaughtering practices, and maintaining the integrity of the cold chain during transportation play pivotal roles in infection control efforts.

This study has some limitations that should be acknowledged. First, the sample size was relatively small, which may affect the generalizability of our findings. Additionally, the geographical bias inherent in our sampling could limit the applicability of the results to broader contexts, as antibiotic resistance patterns may vary significantly across different regions due to variations in antibiotic usage and regulations. Finally, the potential for changes in resistance patterns over time, influenced by evolving antibiotic practices, cannot be ruled out. Future research with larger, more diverse samples is essential to provide a more comprehensive understanding of carbapenem resistance in these pathogens.

Conclusion

The current study underscored multifaceted factors influencing antibiotic resistance disparities among studies, including varied antibiotic usage patterns, emergence of resistance mechanisms, and genetic diversity among bacterial strains. Antibiotic resistance poses a substantial global healthcare burden, exacerbated by inappropriate usage and other factors like aggressive treatments and agricultural practices. Addressing these complexities is critical for effective infection control.

Ethics approval

The study was confirmed by the ethical committee of the Falavarjan Branch, Islamic Azad University, Isfahan, Iran (IR.IAU.FALA.REC.1400.033).

Consent for publication

Not Applicable

Availability of data *and material*

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request**.**

Competing interests

The authors declare that they have no competing interests.

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Authorship

All authors made significant contributions to the work reported. M.R and L.H participated in the research design and writing the first draft; M.R and L.H performed the research and analytic tools; M.R and L.H participated in data analysis. All authors reviewed and confirmed the final manuscript. All authors agreed that the corresponding author would act on their behalf for any communication about the paper during the submission, peer review process, and publication.

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Table 1. Forward and reverse primers

Genes/primers name	Sequence (5'->3')	Product size (bp)
16sr RNA (E. coli)	F:AGATTTGATCMTGGCTCAG R:CCGTCAATTCATTTGATTT	919
16S-23SITS (K. pneumonia)	F:ATTTGAAGAGGTTGCAAACGAT R:TTCACTCTGAAGTTTTCTTGTGTTC	130
23sr RNA (S. typhimurium))	F:GCCAACCATTGCTAAATTGGCGCA R:GGTAGAAATTCCCAGCGGGTACTGG	429
Nanl (P. aeruginosa)	F:ATGAATACTTATTTTGATAT R:CTAAATCCATGCTCTGACCC	228
16s-23srDNA (A. baumannii)	F:CATTATCACGGTAATTAGTG R:AGAGCACTGTGCACTTAAG	208
KPC	F:ATTTTCAGAGCCTTACTGCCC R:TATCGTTGATGTCACTGTATCG	901
IMP	F:GGAATAGAGTGGCTTAAYTCTC R:GGTTTAAYAAAACAACCACC	232
VIM	F:GATGGTGTTTGGTCGCATA R:CGAATGCGCAGCACCAG	390
NDM	F:CCGTATGAGTGATTGCGGCG R:GCCCAATATTATGCACCCGG	779
OXA-48 & OXA181	F:TATATTGCATTAGCAAGGG R:CACACAAATACGCGCTAACC	847

Table 2. Antibiotic susceptibility results

Antibiotic	**Resistance** n (%)				
	Escherichia coli *(n=30)*	***Klebsiella pneumonia*** *(n=14)*	***Salmonella typhimurium*** *(n=9)*	***Pseudomonas aeruginosa*** *(n=11)*	***Acinetobacter baumannii*** *n=(6)*
Streptomycin	28 (93.33)	12 (85.71)	6 (66.67)	3 (27.27)	3 (50.00)
Gentamicin	22 (73.33)	10 (71.43)	8 (88.89)	10 (90.91)	6 (100.00)
Ampicillin	14 (46.67)	8 (57.14)	4 (44.44)	5 (45.45)	5 (83.33)
Tobramycin	8 (26.67)	5 (35.71)	2 (2.22)	6 (54.55)	4 (66.67)
Cotrimoxazole	26 (86.67)	10 (71.43)	8 (88.89)	9 (81.82)	3 (50.00)
Cephalotin	14 (46.67)	5 (35.71)	0 (0.00)	8 (72.73)	3 (50.00)
Ceftazidime	16 (53.33)	6 (42.86)	0 (0.00)	9 (81.82)	3 (50.00)
Tetracycline	30 (100)	14 (100)	9 (100)	11 (100)	6 (100)
Trimethoprim	26 (86.67)	10 (71.43)	8 (88.89)	8 (7.273)	3 (50.00)
Ciprofloxacin	8 (26.67)	6 (4.86)	6 (66.67)	6 (54.55)	5 (83.33)
Levofloxacin	9 (30.00)	2 (14.29)	2 (.22)	8 (72.73)	5 (83.33)
Imipenem	8 (26.67)	2 (14.29)	0 (0.00)	9 (81.82)	6 (100)
Meropenem	7 (23.33)	3 (21.43)	0 (0.00)	6 (54.55)	6 (100)
Chloramphenicol	11 (36.67)	8 (7.14)	6 (66.67)	2 (18.18)	2 (33.33)
Nitrofurantoin	9 (30.00)	7 (50.00)	8 (88.89)	0 (0.00)	2 (33.33)
Azithromycin	7 (23.33)	2 (14.29)	0 (0.00)	8 (72.73)	4 (66.67)
Rifampin	8 (26.67)	0 (0.00)	2 (2.22)	0 (0.00)	3 (50.00)

Table 3. Frequency of carbapenemase gene expression among bacterial isolates

Bacterial isolates	Frequency of gene expression n (%)						P-value
	KPC	***IMP***	***VIM***	***NDM***	***OXA-48***	***OXA-181***	
Escherichia coli *(n=30)*	14 (46.67)	16 (53.33)	14 (46.67)	22 (73.33)	6 (20.00)	16 (53.33)	0.023
Klebsiella pneumonia *(n=14)*	10 (71.43)	8 (7.14)	6 (42.86)	8 (57.14)	3 (21.43)	6 (42.86)	0.041
Salmonella typhimurium *(n=9)*	3 (33.33)	2 (22.22)	2 (22.22)	6 (66.67)	0 (0.00)	4 (44.44)	0.018
Pseudomonas aeruginosa *(n=11)*	6 (54.5)	10 (90.91)	11 (100)	4 (36.36)	2 .(18.18)	3 (27.27)	0.044
Acinetobacter baumannii *n=(6)*	3 (50.00)	6 (100)	6 (100)	2 (33.33)	6 (100)	2 (33.33)	0.030

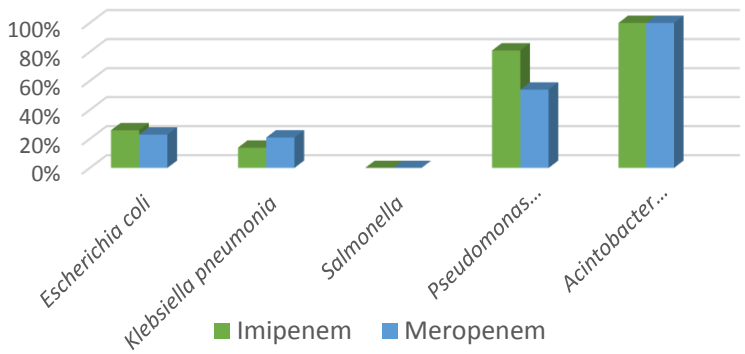
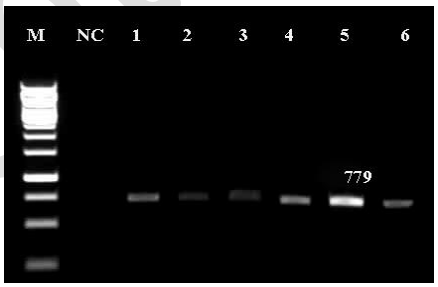


Figure 1. Resistance to carbapenem

A.



B.

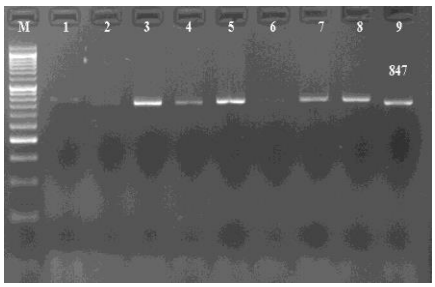


Figure 2. Molecular detection of the most prevalent of carbapenemase genes: A: NDM and B: OXA48 & OXA181.