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Genotyping and investigation of antibiotic resistance genes of *Acinetobacter baumannii* isolates isolated from raw meat using RAPD-PCR method

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ABSTRACT

Acinetobacter baumannii is a well-known pathogen linked to hospital infections and has recently been detected in raw meat from livestock and poultry, raising concerns over antibiotic resistance transmission to humans. Considering the extensive prevalence of multidrug-resistant *A. baumannii* in food sources, this study focused on genotyping *A. baumannii* isolates from meat and poultry samples and examining the presence of essential antibiotic resistance genes. As part of a cross-sectional study conducted in August 2023, researchers gathered 125 raw meat samples from livestock and poultry at various sites throughout Isfahan province, Iran. The *A. baumannii* isolates were identified by standard microbiological producers and examined for antimicrobial susceptibility using the disk diffusion technique. Their resistance patterns were analyzed through RAPD-PCR. Totally, 22 *A. baumannii* isolates from raw meat were recovered. All isolates exhibited resistance to at least two antibiotics. Strains derived from livestock showed high resistance to tetracycline (72.7%), whereas strains from poultry exhibited the highest resistance to both tetracycline (90.9%), and gentamicin (81.8%). In both poultry and livestock isolates, *tetA* was the most frequently detected resistance gene at 90.1 and 81.8%, respectively. While, the $bla_{\text{oxa-24-like}}$ genes were present at one isolate with a rate of 9.1%. Genotyping grouped the isolates into five RAPD profiles, with genetic similarities ranging from 74%-100%, indicating notable diversity despite widespread resistance. These findings highlight the emergence of antibiotic-resistant *A. baumannii* in meat products and underscore the potential risk of resistance gene transfer from animals to humans, emphasizing the need for further research and public health strategies.

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1. Introduction

Consuming fresh or undercooked food is widely identified as a significant contributor to foodborne illnesses globally and is often linked to recurring outbreaks of bacterial pathogens. Meat, regarded as a key protein source, is particularly noteworthy for its susceptibility to spoilage and its suitability for the proliferation of various microorganisms [1]. Even during the processing of meat products considered healthy, contamination with harmful bacteria, such as species from the *Acinetobacter* genus, can occur [2]. *Acinetobacter*, classified as saprotrophic bacteria, is widely present in diverse environments and has emerged as a prevalent microorganism in healthcare settings due to its adaptability to various hospital conditions [3]. Among its species, *Acinetobacter baumannii* stands out as a significant hospital-associated microorganism, particularly common in intensive care units (ICUs). It plays a critical role in causing infections such as pneumonia, septicemia, urinary tract infections, nosocomial meningitis, and skin infections.

Surprisingly, this bacterium can also be detected in various food items, including fruits, raw vegetables, unpasteurized milk, and meat [4]. Genotypic methods offer greater accuracy but are often costly, complex, and time-consuming for identifying bacterial pathogens, highlighting the limitations of traditional cultural and biochemical techniques, which may miss certain microorganisms. [5,6]. The studies emphasizes Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) as a promising alternative for identifying *A. baumannii*, valued for its simplicity, speed, and effectiveness in strain typing. Despite its advantages, RAPD-PCR can suffer from reproducibility issues if not properly standardized, and is currently used as a complementary tool for preliminary identification and validation of other typing methods [7].

A. baumannii is a highly resistant and persistent bacterial species that poses a serious threat in healthcare settings due to its ability to survive in hospital environments and resist multiple antibiotics. It has also been found in food-producing animals, raising concerns about its role as a reservoir for human infections [8]. Compared to other *Acinetobacter* species, *A. baumannii* shows greater resistance and is classified into multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) categories [9]. Over the past three decades, it has increasingly adapted to new antibiotics, complicating treatment efforts. Its capacity to acquire resistance genes has led to rising mortality rates and made it a major public health concern. Furthermore, its potential to spread through the food chain underscores its broader impact on both human and animal health [6, 10]. Considering the extensive prevalence of MDR *A. baumannii* in food, this study focused on genotyping *A. baumannii* isolates from meat and poultry samples using RAPD-PCR and examining the presence of essential antibiotic resistance genes. By revealing the genetic diversity and resistance profiles of these isolates, the research enhances understanding of food's potential role in the transmission of MDR *A. baumannii* and offers valuable insights for developing strategies to combat the escalating threat of antibiotic resistance.

2. Materials and Methods

2.1 Sampling

This study involved the collection of 125 raw meat samples from livestock and poultry, comprising beef (25 samples), sheep (25 samples), camel (25 samples), chicken (25 samples), and turkey (25 samples). The samples were obtained from various locations across Isfahan province as part of a cross-sectional study conducted in August 2023. To ensure proper preservation, the samples were stored near ice in sterile plastic bags and promptly transported to the laboratory.

2.2 Material

Tryptose soy broth (TSB) medium, Eosin Methylene Blue (EMB) agar, Blood agar base, Mueller Hinton agar, Urea Agar, sulfide-indole-motility (SIM) medium, Methyl Red (MR) and Voges-Proskauer (VP) medium, oxidation-fermentation medium and triple sugar iron agar were purchased from Merck (Darmstadt, Germany). Hydrogen peroxide was purchased from Oxoid (Basingstoke, United Kingdom). Gentamicin, Amikacin, Tobramycin, Co-trimoxazole, Ceftazidime, Tetracycline, Ciprofloxacin, Levofloxacin, Imipenem and Meropenem purchased from Oxoid (Basingstoke, United Kingdom). Agarose gel purchased from Sigma-Aldrich (St. Louis, UxSA). PCR mixture purchased from Kiagene Fanavar Aria Co. (Tehran, Iran). Nanodrop 2000 spectrophotometer was purchased from Thermo Fisher Scientific (San Jose, CA, USA). The Standard strain of *A.* baumannii (ATCCBAA-747) was purchased from Institute Pasteur Iran.

2.3 Bacterial isolation

A portion of 10 g from each meat and poultry sample was thoroughly blended with 90 mL of TSB medium and incubated at 37°C. Following incubation, the samples were grown for 24 hours at 37°C using EMB and blood agar mediums. The identification of *Acinetobacter* species was carried out through a series of standard biochemical tests, such as TSI, SIM, MR-VP tests, urease test, oxidative-fermentation test, catalase, and oxidase [11].

2.4 Antibiotic susceptibility testing

The disk diffusion technique was employed to determine the antibiotic susceptibility of *A. baumannii* isolates [12]. Initially, the isolates were grown in TSB medium and incubated at 37°C for 24 hours. A bacterial

suspension with turbidity equivalent to 0.5 McFarland standard was then prepared and inoculated onto Mueller Hinton agar medium. The plates were incubated under standard conditions at 37°C for 16-18 hours. Following incubation, the susceptibility of the isolates was evaluated based on the CLSI guidelines (M100-Ed34, 2024) [13]. The study included specific antibiotics for this analysis: Gentamicin (10 µg/disk), Amikacin (30 µg/disk), Tobramycin (10 µg/disk), Cotrimoxazole (23.1-75.25 µg/disk), Ceftazidime (30 µg/disk), Tetracycline (30 µg/disk), Ciprofloxacin (5 µg/disk), Levofloxacin (5 µg/disk), Imipenem (10 µg/disk) and Meropenem (10 µg/disk).

2.5 *DNA extraction*

DNA was extracted using the boiling method [14]. The PCR mixture had a total volume of 25 µL, which included the following components: 2.5 µL of 10X buffer, 0.5 µL of dNTPs (0.2 mM), 1 µL of $MgCl_2$ (1.5 mM), 1 µL of each forward and reverse primer (5 pmd/µL), 0.2 µL of DNA Taq Polymerase enzyme, 2 µL of template DNA, and 16.8 µL of distilled water. The PCR protocol involved initial denaturation at 94°C for 5 minutes, followed by 33 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 7 minutes. PCR products were analyzed using 1% agarose gel. A 208 bp fragment of the 16srRNA gene signified the presence of *A. baumannii* in the samples tested. For the experiment, and *A. baumannii* strain ATCCBAA-747 served as the positive control [15]. PCR was performed to detect key antibiotic resistance genes, including *tetA* and *tetB*, *dfrA1,* bla_{VIM}, bla_{SIM}, and bla_{IMP}, as well as $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24}$ and $bla_{OXA\text{-}58\text{-}like}$ [16].

2.6 *RAPD-PCR*

The RAPD-PCR method utilized the DAF4 primer (5' CGGCAGCGCC 3') previously developed by Wiedmann-al-Ahmad et al. [17]. The PCR reaction was conducted with a total volume of 25 µL, consisting of 2.5 µL of 10X buffer, 2 mM $MgCl_2$, 25 mM dNTPs, 2 µm of DAF4 primer, 2 units of Taq polymerase enzyme, and 5 µL of template DNA. The thermal cycling protocol included an initial cycle at 94°C for 2 minutes, followed by 45 cycles of denaturation at 94°C for 40 seconds, annealing at 45°C for 40 seconds, extension at 72°C for 40 seconds, and a final cycle at 72°C for 5 minutes. The experiment was repeated at least three times to ensure reproducibility, and a consistent set of bands was defined as the profile.

2.7 *Statistical analysis*

The study's data were processed using SPSS version 26 software (IBM Corp, Armonk, NY., USA), with a 95% confidence level applied to the analyses. A p-value of <0.05 was regarded as the threshold for statistical significance. Band patterns generated from RAPD-PCR were evaluated with Bionumeric software using the Dice algorithm, and clone clustering was carried out through the UPGMA method. Isolates were classified into clusters or types based on an 80% similarity coefficient.

3. Results

3.1 *Bacteria isolation and identification*

Of the 125 raw meat specimens examined, *A. baumannii* was detected in 3 beef samples (12%), 5 sheep samples (20%), 3 camel meat samples (12%), 7 chicken samples (28%), and 4 turkey samples (16%). In total, 22 samples accounting for 17.6% of the overall collection were found to be contaminated with *A. baumannii*. Statistical evaluation revealed a notable difference ($p = 0.032$) in contamination rates between sheep and poultry samples compared to other meat types.

3.2 *Antibiotic susceptibility*

The antibiotic resistance profiles of 22 *A. baumannii* isolates were assessed using the disk diffusion technique across commonly used antibiotic agents. Each isolate exhibited resistance to at least two antibiotics. Among livestock-derived samples, tetracycline showed the highest resistance rate at 72.7%, while meropenem and ceftazidime had the lowest resistance rates at 9.1%. Detailed resistance data can be found in Tables 1 and Tables 2.

3.3 *Antibiotic resistance genes*

PCR analysis in this study verified the presence of a 208 base pair fragment associated with the 16srRNA gene in 22 *A. baumannii* isolates, confirming their identity. In both poultry and livestock isolates, *tetA* was the most frequently detected resistance gene at 90.1 and 81.8%, respectively. While, the $bla_{oxa\text{-}24\text{-}like}$ genes were present at one isolate with a rate of 9.1% (Table 3 and 4).

Table 1. Antibiotic resistance pattern in *A. baumannii* strains isolated from beef, sheep and camel meat samples

Sample/Frequency	Gentamicin No. (%)	Amikacin No. (%)	Tobramycin No. (%)	Co-trimoxazole No. (%)	Ceftazidime No. (%)
Beef/3	2 (66.7)	1 (33.3)	0 (0)	1 (33.3)	0 (0)
Sheep/5	1 (20)	1 (20)	1 (20)	3 (60)	1 (20)
Camel/3	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)
Sample/Frequency	Ciprofloxacin	Levofloxacin	Imipenem	Meropenem	Tetracycline
Beef/3	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)	2 (66.7)
Sheep/5	3 (60)	1 (20)	1 (20)	0 (0)	3 (60)
Camel/3	0 (0)	0 (0)	0 (0)	1 (33.3)	3 (100)

Table 2. Antibiotic resistance pattern in *A. baumannii* strains isolated from turkey and chicken meat samples

Sample/Frequency	Gentamicin No. (%)	Amikacin No. (%)	Tobramycin No. (%)	Co-trimoxazole No. (%)	Ceftazidime No. (%)
Turkey/4	2 (50)	1 (25)	1 (25)	2 (50)	1 (25)
Chicken/7	7 (100)	4 (57.1)	2 (28.6)	5 (71.4)	2 (28.6)
Sample/Frequency	Ciprofloxacin	Levofloxacin	Imipenem	Meropenem	Tetracycline
Turkey/4	1 (25)	1 (25)	0 (0)	0 (0)	3 (75)
Chicken/7	4 (57.1)	3 (42.9)	4 (57.1)	2 (28.6)	7 (100)

Table 3. Frequency of resistance genes in *A. baumannii* strains isolated from livestock samples

Sample	*tetA* No. (%)	*tetB* No. (%)	*dfrA1* No. (%)	bla_{VIM} No. (%)	bla_{SIM} No. (%)
Beef	3 (100)	3 (100)	2 (66.7)	1 (33.3)	1 (33.3)
Sheep	3 (60)	3 (60)	3 (60)	1 (20)	0 (0)
Camel	3 (100)	1 (33.3)	3 (100)	0 (0)	1 (33.3)
Sample	bla_{IMP} No. (%)	bla_{OXA-23} No. (%)	bla_{OXA-24} No. (%)	bla_{OXA-58} No. (%)	
Beef	1 (33.3)	1 (33.3)	0 (0)	2 (66.7)	
Sheep	4 (80)	1 (20)	1 (20)	1 (20)	
Camel	0 (0)	0 (0)	0 (0)	0 (0)	

Table 4. Frequency of resistance genes in *A. baumannii* strains isolated from poultry samples

Sample	*tetA* No. (%)	*tetB* No. (%)	*dfrA1* No. (%)	bla_{VIM} No. (%)	bla_{SIM} No. (%)
Turkey	3 (75)	3 (75)	3 (75)	0 (0)	0 (0)
Chicken	7 (100)	5 (71.4)	5 (71.4)	3 (42.9)	2 (28.6)
Sample	bla_{IMP} No. (%)	bla_{OXA-23} No. (%)	bla_{OXA-24} No. (%)	bla_{OXA-58} No. (%)	
Turkey	1 (25)	1 (25)	1 (25)	1 (25)	
Chicken	5 (71.4)	1 (14.3)	0 (0)	3 (42.9)	

3.4 RAPD-PCR

To determine the genetic similarities, isolates showing over 80% similarity were grouped into what were referred to as Type-RAPD profiles. In total, 22 isolates were analyzed and categorized into five distinct RAPD profiles, labeled A through E. Interestingly, four of the five profiles (A, B, C, and E) included both poultry and livestock isolates hinting at potential cross-species transmission or shared environmental sources (Table 5). These findings provide meaningful insights into the genetic relationships among *A. baumannii* isolates from different animal hosts. The presence of genetically similar strains in both poultry and livestock suggests potential epidemiological links that merit further investigation especially in the context of antimicrobial resistance and disease transmission between species.

Table 5. Profile details and isolate clustering *A. baumannii*

Profile	Isolates source and assigned number
A	Poultry: 19, 20, 21; Livestock: 8, 2
B	Livestock: 1, 14; Poultry: 22; Livestock: 3
C	Poultry: 18; Livestock: 4, 6, 7, 10, 5, 16
D	Poultry: 12, 15
E	Poultry: 17, 13; Livestock: 11, 9

4. Discussion

A. baumannii represents a genetically uniform group of organisms that are widely distributed. While other Acinetobacter species are typically recovered from soil environments, *A. baumannii* is frequently identified in a range of food products such as pasteurized milk, frozen items, raw meat and poultry, fruits and vegetables as well as in healthcare settings. Although these bacteria exhibit limited tolerance to harsh environmental conditions, their strong ability to colonize hosts contributes to their role as a leading source of hospital-acquired infections [18]. In our study, *A. baumannii* was found in 17.6% of raw meat samples from various livestock and poultry sources including cattle, sheep, camels, chickens, and turkeys. Sheep meat showed the highest contamination rate at 20%, possibly linked to closer human interaction, limited veterinary oversight in abattoirs, and carcass-to-carcass contact. The likely pathways of contamination include transmission from infected meat to workers, cross-contamination between carcasses, and exposure during slaughter through digestive tract contents or fecal matter. Research from various parts of the world has shown that *A. baumannii* can infect animals, particularly domestic species such as camels, cattle, sheep, and chickens, underscoring its growing relevance in veterinary medicine. For instance, a study conducted in Saudi Arabia detected the bacterium in 55 out of 220 meat samples, with the highest contamination found in sheep (46.55%), followed by chicken (32.5%), cow (15%), and camel (9.68%). These findings emphasize the notable role of *A. baumannii* in livestock and poultry infections, while global studies further confirm its widespread occurrence [6]. A study by Askari *et al.* (2019), on 381 raw meat samples showed that the consumption rate in sheep meat (41.12%) was significantly higher than sheep meat (11.72%) and camel meat (2.26%). The higher consumption rate in sheep meat is assessed to be due to the weakness in monitoring the breeding and slaughtering processes. These findings are consistent with our results, which indicate contamination of sheep

meat. Therefore, improved hygiene and monitoring in sheep meat production are necessary to reduce the risk of transmission of this pathogen [4].

Overall, *A. baumannii* exhibits significant resistance to antimicrobial treatments, stemming from both its natural and acquired defense mechanisms. In this study, strains of *A. baumannii* isolated from livestock and poultry demonstrated marked antibiotic resistance. Among livestock-derived samples, the greatest resistance was observed against tetracycline (72.7%), while the lowest resistance was noted for meropenem and ceftazidime (each at 9.1%). Poultry isolates showed the strongest resistance to tetracycline (90.9%) and gentamicin (81.8%).

In an Iranian study conducted in 2020, researchers investigated the antibiotic resistance profile of A. *baumannii* found in raw meat. Out of 194 raw meat samples analyzed, 39 (20.1%) were found to be contaminated with *A. baumannii* isolates, with ovine raw exhibiting the highest contamination rate at 32.14%. The bacterium showed pronounced resistance to several antibiotics, including gentamicin (87.17%), tetracycline (79.48%), and ciprofloxacin (58.97%). Conversely, the lowest resistance level was observed for imipenem, at 17.9%. These findings confirm that raw meat had the greatest degree of contamination and align with results from the current study regarding antibiotic resistance patterns [2].

Research by Wu et al. (2023) highlighted a swift rise in drug resistance among clinical strains of *A. baumannii*, attributing multidrug resistance to factors such as enzymatic activity, membrane-associated proteins, efflux systems, and genetic mutations [19]. Similarly, in a 2020 investigation by Moosavian et al., 124 clinical samples were examined, revealing that 96.8% of the isolates were resistant to rifampin. The lowest resistance rates were noted for colistin and polymyxin B.

The study also identified the *tetA* and *tetB* resistance genes as the most prevalent. These findings closely align with the current study's observations regarding resistance-related genetic markers [20]. Variations in antibiotic resistance patterns reported across studies can be attributed to several factors, including the types of samples collected, differences in sampling techniques, geographic variations, and the methodologies used to evaluate resistance. In a 2018 study, Liu et al. investigated 88 clinical isolates of *A. baumannii* to assess both their resistance to antibiotics and the presence of virulence-associated genes. Susceptibility tests against 10 widely used antibiotics and analysis of 9 genes linked to pathogenicity revealed that the majority of strains were multidrug resistant and harbored several virulence factors concurrently. These traits underscore the bacteriums heightened pathogenic potential, making effective treatment particularly challenging [21].

Mohammadi et al. (2020) investigated antibiotic resistance trends in Acinetobacter isolates and examined the occurrence of the bla_{VIM} resistance genes (17%). Their findings revealed that the majority of isolates (>70%) exhibited resistance to third-generation cephalosporins as well as amikacin and imipenem, while mostly susceptible to polymyxin B [22].

Due to the limited availability of local epidemiological data and drug resistance profiles for this pathogen, greater attention is needed regarding its role in hospital-acquired infections. Furthermore, the development and exploration of new antimicrobial agents are vital to effectively manage infections caused by this bacterium.

Santajit et al., conducted a study on *A. baumannii* strains resistant to carbapenems, collected from a hospital in Thailand. Their analysis revealed that 57.56% of the samples exhibited biofilm-forming capabilities. Genotyping through the Rep-PCR technique classified 172 isolates into 36 distinct genetic profiles. The differences observed between their findings and those of the current study likely stem from variations in microbial isolation techniques [23]. In a 2022 investigation, Shali et al. analyzed *A. baumannii* isolates from patients in a burn unit in Iraq, focusing on antibiotic resistance and genetic profiles. The study revealed that 37% of the isolates exhibited multidrug resistance, and ERIC-based genotyping categorized them into six distinct genetic groups [24].

Similarly, in 2019, Sarafan Sadeghi et al. assessed resistance patterns and genotyping of *A. baumannii* strains from patients at Shahrekord Hospital. They found that 71% of the isolates were resistant to ceftazidime and showed greater resistance to other widely used antibiotics. REP-PCR analysis grouped the strains into nine genetic clusters [25].

In this study, *A. baumannii* isolates obtained from raw livestock and poultry meat were genotyped using the RAPD-PCR technique. This dependable and precise method enables researchers to classify microorganisms and explore their genetic relationships. RAPD-PCR works by randomly amplifying genomic regions with short primers, producing DNA fragments whose presence or absence on an electrophoresis gel reveals genetic variation among samples. These variations are typically the result of differences in primer binding sites or structural genetic changes such as insertions, deletions, and inversions.

Some limitations related to this study must be acknowledged. The study was conducted only in August 2023, providing a snapshot in time. Seasonal variations in contamination rates, antibiotic usage in livestock, or environmental factors could influence prevalence and resistance profiles, which this design cannot capture. Also, sampling was limited to Isfahan province in Iran, which may not represent diversity in other regions of Iran or globally. Although RAPD-PCR is rapid and cost-effective, it is known to have issues with reproducibility. It offers lower discriminatory power compared to more robust methods. This study explored how widespread *A. baumannii* is in raw meat from livestock and poultry,

with a special focus on its antibiotic resistance traits and genetic diversity. The results showed that the bacterium is commonly present in these meats and could potentially spread to humans through the consumption of undercooked products, posing a risk of gastrointestinal illness. Notably, the bacterial isolates showed strong resistance to antibiotics like tetracycline, and gentamicin, while remaining more sensitive to carbapenems. There was a clear connection between phenotypic resistance and the resistance genes they carried (genotypic resistance). Genetic fingerprinting using RAPD-PCR revealed a variety of strains, yet there was considerable similarity between those found in livestock and poultry suggesting a shared origin and possible human-to-meat transmission. Overall, the findings emphasize the urgent need to improve practices that minimize contamination and curb antibiotic resistance in the meat supply chain.

Authors' contributions

NAK: Conceptualization, Methodology, Visualization, Formal analysis, Validation, Investigation, Data Curation, Writing - Original Draft. FM: Investigation, Validation, Writing-Review & Editing. MM: Validation, Investigation, Writing - Review & Editing. SMGh: Conceptualization, Resources, Supervision, Project administration. All authors read and approved the final version of manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical declarations

All samples used in this study were obtained from industrial livestock and poultry abattoirs as part of routine operational processes and did not involve experimental procedures on live animals. Because these samples were collected exclusively from post-mortem material that originated from standard commercial slaughter operations, our Institutional Ethics Committee determined that formal ethical approval was not required for this work. Consequently, the authors were exempted from obtaining an ethics approval code by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) of the Shahid Ashrafi Esfahan University, Isfahan, Iran, and an official exemption letter is available with the Editor-in-Chief of the journal upon request. All applicable local, national, and institutional guidelines for the use of animal-derived materials in research were followed throughout the study.

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