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RESEARCH ARTICLE

Molecular Characterization and Drug Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Poultry Meat and Meat Products

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ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is a multidrug-resistant (MDR) environment associated microorganism that causes infections in animals and human. The present study aimed to determine antibiotic susceptibility patterns and molecular characteristics of *P. aeruginosa*. The molecular typing of *P. aeruginosa* isolates was also performed. Poultry meat and meat product samples (n=110) were collected. The samples were inoculated on MacConkey and cetrimide agar for cultural identification and isolation. Gram staining and biochemical tests were performed for confirmation. The confirmed isolates of *P. aeruginosa* were then used for antibiotic susceptibility testing against commonly used antibiotics including meropenem, amikacin, aztreonam, azithromycin, enrofloxacin, and gentamicin. The virulence genes including *exoU, exoS, lasR, rhlR, algD, pslD,* and bla^{TEM} were detected in selected MDR isolates of *P. aeruginosa*. Among the poultry meat samples, *P. aeruginosa* was more prevalent (50%) in fresh meat samples than others [frozen meat (40%) and meat products (8%)]. Confirmed isolates were Gram-negative, catalase positive, oxidase positive, beta-hemolytic, and citrate-positive while colorless growth on MacConkey's agar and green color growth on cetrimide agar was observed. In antibiotic sensitivity testing, results indicated that in poultry meat and meat product isolates, all *P. aeruginosa* isolates were highly resistant to azithromycin (83%). High resistance rate was also observed for aztreonam (67%), gentamicin (58%) and enrofloxacin (50%) and all *P. aeruginosa* isolates were highly sensitive to meropenem (84%) followed amikacin (75%). In molecular detection of virulence genes, poultry meat samples, *exoU, algD,* and their genes showed comparable prevalence (80%). The prevalence of drug resistance genes in *P. aeruginosa* from poultry meat samples was as follows; bla^{TEM} (95%), *tetR* (0%), e*xoU* (25%), e*xoS* (30%), *algD* (80%), *pslD* (85%), *lasR* (85%) and *rhlR* (80%). In summary, the presence of virulent genes in *P. aeruginosa* isolates enables them to harbor antibiotic resistance and acts as an MDR public health pathogen with the potential to transfer to humans via the food chain.

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INTRODUCTION

The degradation of food occurs by the opportunistic Gram-negative bacterium *Pseudomonas aeruginosa* (*P. aeruginosa*), which can be detected by the production of slime and mal-odor, off-flavors, as well as pigment secretion. Because there are inadequate processing and refrigeration technologies in developing countries, food spoiling due to this bacterium is a consequential global problem. Many food-borne infections linked to *P. aeruginosa* are being reported (Rezaloo *et al*., 2022).

This specific bacterium causes infections due to a variety of virulent factors. The precursor proteins that encode the three phenazine molecules are secreted by the phenazine operons (*phzH*, *phzM*, and *phzS*). These substances are the cause of the rise in intracellular oxidative effects. Exoenzymes (*exoS, exoT, exoU*, and *exoY*), alkaline protease (apr), toxins A and B (*toxA* and

toxB), elastase genes A and B (*lasA* and *lasB*), alginate-encoded genes (algD and algU), hemolytic and nonhemolytic phospholipase C (plcH and plcN), pilus genes (*pilA* and *pilB*), and pyoverdine encoded gene (*pvd*) are additional factors that contribute to *P. aeruginosa* virulence. These factors are more often involved in attachments and adhesions, inflammatory responses, and ultimately host cell invasion (Lianou *et al.*, 2017).

Antibiotic resistance is a pivotal component of *P. aeruginosa*-related infections. The resistance of *P. aeruginosa* to various antibiotic drugs evolves with time. For an inclusive period, resistant strains produce many complex diseases that result in significant financial losses from treatment and management (Wang and Wang, 2020). Antimicrobial resistance in *P. aeruginosa* has been identified against a variety of drugs, primarily β-lactams, aminoglycosides, tetracyclines, quinolones, and macrolides (Hassan *et al*., 2020). In the past few years, *P. aeruginosa* strains on plasmids frequently carrying additional genes that encode the resistance to non-beta-lactam antibiotics have given rise to extended-spectrum beta-lactamase (ESBL) types in hospital and community settings. These strains are resistant to all beta-lactam antibiotics, except for carbapenems (Morohoshi *et al*., 2022).

The genes bla^{SHV}, bla^{TEM}, bla^{DHA}, bla^{OXA}, bla^{VEB}, and bla^{CTX-M} that encode ESBL enzymes in *P. aeruginosa* are the most crucial and clinically relevant ones. Furthermore, it has been observed that *P. aeruginosa* clinical isolates are becoming more resistant to quinolones (nalidixic acid, ciprofloxacin, ofloxacin) point mutations in the genes encoding the DNA gyrase subunit GyrA and the topoisomerase subunit ParC are primarily contributing in drug resistance (Edris *et al*., 2023).

The objective of this study was to determine the prevalence of *P. aeruginosa* bacteria isolated from poultry meat and its products, as well as the phenotypic and genotypic patterns of antibiotic resistance and virulence factors distribution. This was done due to the increased portion of meat consumption day by day in Pakistan and the significance of *P. aeruginosa* as a food-spoiling agent.

MATERIALS AND METHODS

Sampling and phenotypic characterization of *P. aeruginosa*: Sampling was done from poultry meat and meat products, a total of 110 samples were collected. Poultry meat samples were taken from fresh meat, frozen meat, as well as other meat products. Sterile swabs were utilized for the collection of samples and after proper labeling, transferred to the laboratory under 4°C for further processing. Samples were processed further for the isolation of *P. aeruginosa.* For this, the samples were first enriched in nutrient broth and then incubated at 37°C for 24 hours (Abbas *et al.*, 2022). Then samples having turbidity were inoculated on MacConkey agar. One more time, the MacConkey agar inoculated plates were incubated at the same previous conditions. After 24 hours of incubation, the growth was observed. Pale-colored colonies (non-lactose fermenting) were further inoculated on cetrimide agar for selective growth of *P. aeruginosa* and on blood agar followed by incubation at 37°C for 18hrs. After that, growth was noticed, and the results were recorded. The identification of *P. aeruginosa* was demonstrated by the use of microscopic morphology, Gram-staining, oxidase, urease, catalase test, Triple Sugar Iron test, indole, and citrate utilization tests (Poursina *et al*., 2023).

Antibiotic susceptibility test: The isolated strains were further tested for antimicrobial sensitivity testing using a modified Kirby-Bauer disc diffusion method (Ishaq *et al.*, 2022). Muller Hinton agar was used, and the results were elucidated according to the clinical laboratory standards (CLSI) guidelines. The frequently used antibiotics including amikacin (30µg), meropenem (10µg), enrofloxacin (5µg), azithromycin (15µg), gentamicin (10µg), and aztreonam (25µg) were tested. Mueller Hinton agar media was prepared, and sterility was evaluated before antibiotic sensitivity testing. The inoculum of each isolate was prepared in normal saline followed by inoculation of the sample on agar plates. The antibiotic discs were placed and kept for incubation for 24 hours at 37°C. After the incubation, the zone of inhibition (around each disc) was evaluated. Based on this, the isolates were described as resistant or sensitive following CLSI guidelines.

Molecular typing of virulence genes: The virulence genes were detected by further processing of *P. aeruginosa* isolates. For this, 12 isolates of *P. aeruginosa* from poultry meat and its products were collected (Abbas *et al.*, 2022). The genes that were detected include; e*xoU, exoS, lasR, rhlR, algD, pslD,* bla^{TEM}, and *tetR*. The primers were purchased from Macrogen (Korea) with the sequence as shown in Table 1. The specific set of primers and their condition of amplification were used. The primers were prepared through Macrogen (Korea).

Detection of virulence antibiotic resistance genes: Extraction of DNA was performed utilizing a bacterial genomic DNA extraction kit (Thermo Scientific, USA) following the guidelines of the manufacturer. The amount and quality of extracted DNA were evaluated using a spectrophotometer (A260/A280) and electrophoresis on a 2% agarose gel (Kolbeck *et al*., 2021). The remaining purified DNA was stored at -20°C till further use (Genomic DNA purification protocols). The purified DNA was quantified by applying a Nanodrop lite spectrophotometer (NAS-99 software) and the values were recorded. A 20µL PCR volume consisted of 4µL Master Mix (TaKaRa, 2.0 plus dye, Premix Taq, Cat. No. RR902, lot # AKG3093A) with 1µL (0.5µM) of each primer, 4µL genomic DNA and 10µL molecular biology grade water (Invitrogen) was used (Eraky *et al*., 2020).

PCR amplification of all genes was done using 35 cycles of denaturation at 95°C for 30sec. Other conditions were as follows; initial denaturation at 95°C for 5 min, denaturation at 95°C for 30sec (35 cycles). Annealing temperature for bla^{TEM},*tetR, algD,pslD, exoU, exoS, lasR* and *rhlR* genes was 60, 95, 58, 56, 61, 55, 50, and 52°C for 1min, 30sec,30sec, 30sec, 30sec, 1min, 30sec and 30sec respectively. Extension at 72°C for 30sec, and final extension for five minutes at 72°C was set in all the reactions. A 1.5% agarose gel was used for the analysis of the amplified products. For size comparison, a 100-base pair (bp) DNA ladder (DL 500 DNA) was utilized (Abbas *et al*., 2022).

Statistical analysis: Results were analyzed by calculation of percentage positivity of *P. aeruginosa* isolated from poultry meat and meat products (fresh meat, frozen meat, and meat products). The drug resistance pattern of *P. aeruginosa* isolates from all sources was determined using percentage positivity.

RESULTS

Prevalence of *P. aeruginosa* in poultry meat: Comprehensively, 110 samples from poultry meat and meat products were cultured for the isolation and identification of *P. aeruginosa*. All the positive poultry meat and meat products samples (n=12) showed colorless colonies on MacConkey agar plates, and green color colonies on cetrimide agar plates showed the production of a green pigment named pyocyanin. This is the specific characteristic of positive *P. aeruginosa* on cetrimide agar. On blood agar, all positive (n=12) *P. aeruginosa* isolates from poultry meat and meat products showed beta-hemolysis.

All of the collected samples were processed for determination of *P. aeruginosa* prevalence. The results depicted the highest prevalence of *P. aeruginosa* in frozen poultry meat samples. Among the poultry meat samples (12/110), *P. aeruginosa* was more prevalent (50%) in fresh meat samples than others [frozen meat (40%) and meat products (8%)]. Data analysis indicated significant difference between the types of samples (poultry meat, and meat products) and the prevalence of *P. aeruginosa* (Fig. 1). Fresh meat was found to be highly positive (50%) for *P. aeruginosa* compared with other meat types.

Table 1: List of primers of virulence genes of *P. aeruginosa* along with product length

Genes	Primers	Product length
tetR	F: CCGAATGCGTATGATTCTCC R: CGCTTTACTGGCACTTCAGC	888 bp
rhlR	F: CAATGAGGAATGACGGAGGC R: CTT CAG ATG AGG CCC AGC	730 bp
lasR	F: ATGGCCTTGGTTGACGGTT R: GCAAGATCAGAGAGTAATAAGACCCA	725 bp
exoS	F: CGTCGTGTTCAAGCAGATGGTGCTG R: CCGAACCGCTTCACCAGGC	444 bp
algD	F: CGTCTGCCGCGAGATCGGCT R: GACCTCGACGGTCTTGCGGA	313 bp
exoU	F: TACCAGGTACGGCCATGTTC R: ACGCTCTGAAGCCTGAAGAC	575 bp
pslD	F: CTCATGAAACGCACCCTCCT R: TGCGACCGATGAACGGATAG	295 bp
bla^{TEM}	F: ATGAGTATTCAACATTTCCG R: GACAGTTACCAATGCTTAATCA	516 bp

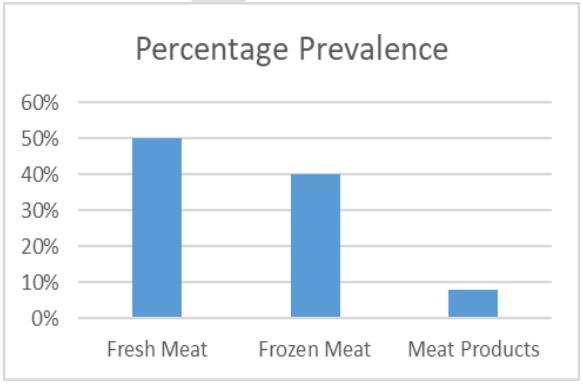

Fig. 1: Prevalence of *P. aeruginosa* among various types of poultry meat samples.

Isolation and identification of *P. aeruginosa* isolated from poultry meat and meat products: A total of 12 positive isolates of *P. aeruginosa* from poultry meat samples were confirmed through culture identification and biochemical tests. These *P. aeruginosa* isolates were further subjected to antibiotic susceptibility testing shown in Fig. 2. In poultry meat and meat product isolates, all *P. aeruginosa* isolates were sensitive to amikacin (84%) and resistant to azithromycin (83%) as shown in Table 2.

Table 2: The antibiotic resistance pattern of *P. aeruginosa* obtained from poultry meat and meat products.

Source of *P. aeruginosa* isolates	Number of isolates resistant to antibiotic					
	EN	AZ	ME	AK	AT	GN
Poultry meat and meat products (n=12)	6 (50%)	10 (83%)	2 (16%)	3 (25%)	8 (67%)	7 (58%)

EN: enrofloxacin, AZ: azithromycin, ME: meropenem, AK: amikacin, AT: aztreonam, GN: gentamicin.

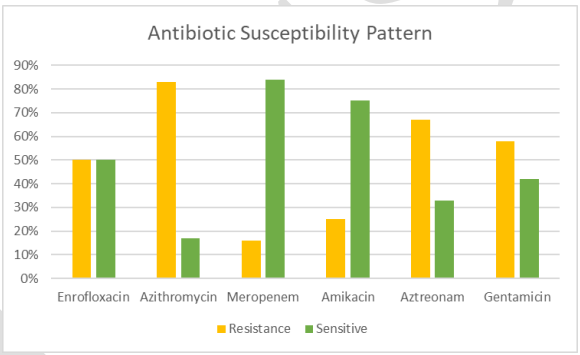

Fig. 2: Overall antibiotic sensitivity pattern of *P. aeruginosa* isolates obtained from poultry meat and meat products samples. The bacterial isolates were tested against antibiotic discs such as enrofloxacin, azithromycin, meropenem, amikacin, aztreonam, and gentamycin.

Gram staining and biochemical characterization of *P. aeruginosa* isolates: The positive isolates of *P. aeruginosa* were further subjected for confirmation through Gram staining and biochemical tests. In Gram staining, Gram-negative rods were observed. In biochemical testing, positive isolates of *P. aeruginosa* were observed as catalase positive and positive for the TSI test (only growth on a slant and not in the butt).

***P. aeruginosa* antibiotic resistance and virulence genes:** A total of 12 positive isolates of *P. aeruginosa* from poultry meat were confirmed through culture identification and biochemical tests. These *P. aeruginosa* isolates were further subjected to antibiotic susceptibility testing. Table 2 indicates the antibiotic resistance pattern of *P. aeruginosa* isolates. The results in Fig. 2 indicated that among poultry meat and meat product isolates, all *P. aeruginosa* isolates were highly resistant to azithromycin (83%). High resistance rate was also observed for aztreonam (67%), gentamicin (58%) and enrofloxacin (50%) and all *P. aeruginosa* isolates were highly sensitive to meropenem (84%) followed amikacin (75%). In overall comparison, all *P. aeruginosa* isolates were highly sensitive to meropenem (84%), followed by amikacin (75%) and enrofloxacin (50%). The least sensitivity was observed for azithromycin (17%), aztreonam (33%) and gentamicin (42%). The highest resistance rate was for azithromycin (83%) and highest sensitivity for meropenem (84%).

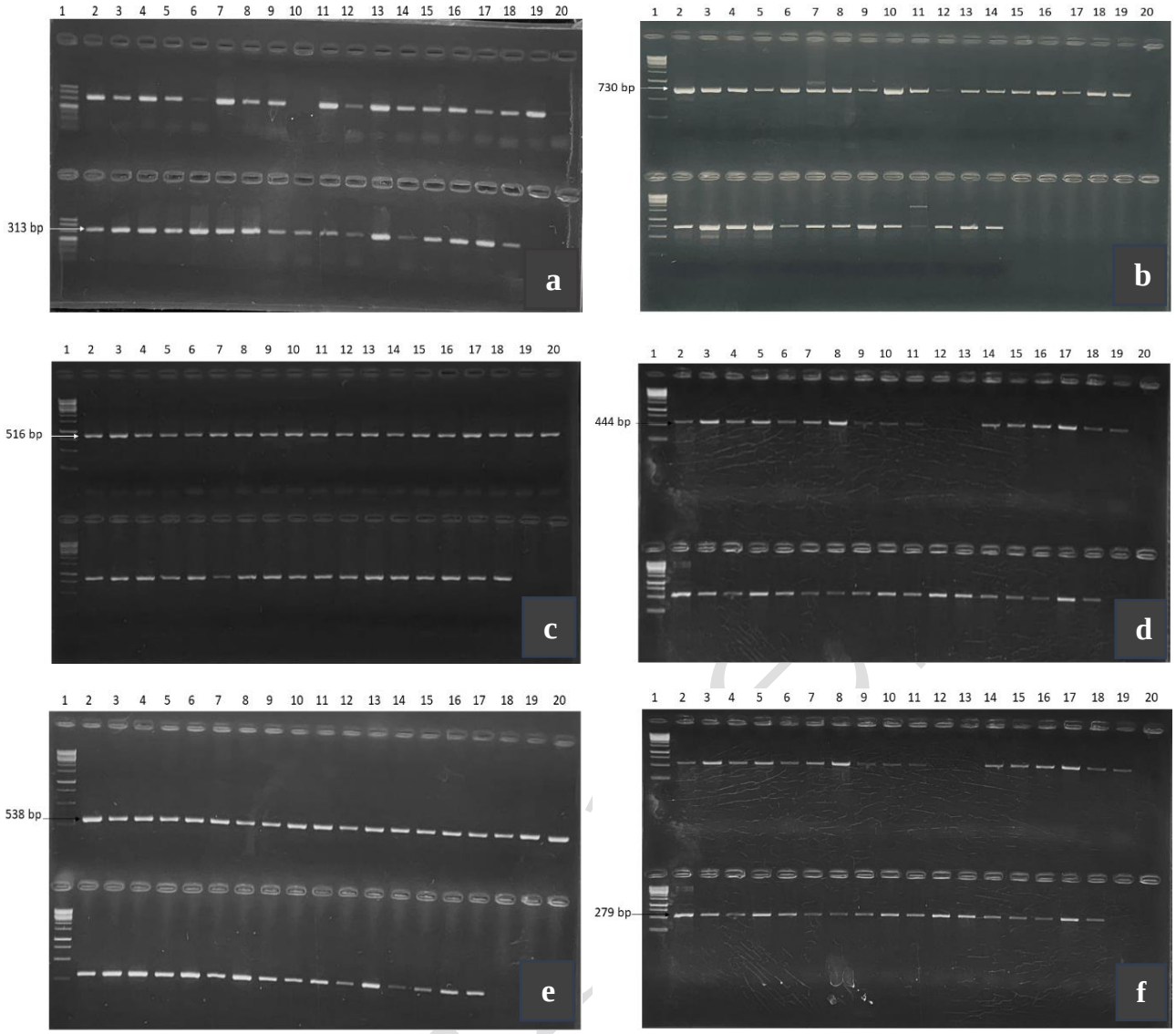

Fig. 3: (a) Gel electrophoresis image of *algD* gene obtained after gel electrophoresis; Lane 1 is the DNA ladder, lanes 2-18 are positive samples while 19 and 20 lanes are negative samples. **(b)** Gel electrophoresis diagram of *rhlR* gene and lasR obtained after PCR amplification; Lane 1 includes the DNA ladder in valve 1, Valves 2-19 are positive samples and Valve 20 is the negative sample for rhlR gene while in Lane 2 the first valve is Ladder while valve 2-13 are positive for lasR gene and 14-20 are negative for lasR gene. **(c)** Molecular detection of *bla*^{TEM} gene; Lane 1 is the DNA ladder, while other lanes (2-20) are positive samples. **(d)** Detection of *exoS* gene in positive *P. aeruginosa* samples; Lane 1 is the DNA ladder, lanes 2-11 and 14-19 are positive samples while other lanes (2-20) are positive samples. **(e)** Gel image of *exoU* gene obtained after PCR amplification; Lane 1 is the DNA ladder, while others (Lane 2-20) are positive samples of *P. aeruginosa* having *exoU* gene. **(f)** Molecular detection of *pslD* gene; Lane 1 is the DNA ladder, lanes 2-18 are positive samples while 19 and 20 lanes are negative samples.

Table 3: Prevalence of virulence genes in *P. aeruginosa* isolates of poultry meat and meat products (n=12)

Name of gene	No. of positive isolates	Prevalence (%)
bla^{TEM}	12	95
tetR	0	0
exoU	3	25
exoS	4	30
algD	10	80
pslD	11	85
lasR	11	85
rhlR	10	80

The positive isolates of *P. aeruginosa* from poultry meat and meat products (n=12) were further subjected to molecular detection of virulence genes (Fig. 3). The drug resistance genes detected were *exoU, exoS, lasR, rhlR, algD, pslD, TetR, and bla*^{TEM}. The results indicated that in the case of poultry meat samples, *bla*^{TEM} was reported in 95% of isolates, the prevalence of *pslD* was 85%, *exoS* was detected in only 30%, *exoU* as reported in 25%of the isolates, *algD* was 80%, and *lasR* genes were detected in 85% isolates, *rhlR* was detected in 80% of the isolates, while all the samples were negative for t*etR* gene as shown in Table 3. The bands observed in the Gel-Doc system for each specific gene are shown in Fig. 3.

DISCUSSION

Pseudomonas aeruginosa is a food bacterium that is commonly distributed in the environment and frequently acts as an opportunistic human pathogen (Islam *et al*., 2024) contributing significantly to the spoilage of many foods and food products. Thus, it is significant to demonstrate the epidemiological properties, virulence, and prevalence. The frozen samples had a greater rate of

bacterial contamination due to the psychrophilic nature of the bacterium (Farghaly *et al.*, 2022). The high contamination rate of imported meat samples might be because of the importation of low-quality meat or the meat's extended storage under unfavorable circumstances in customs. *P. aeruginosa* in these samples may have originated from improper functionality of the heat chain, improper cooking, preparation time, and contamination in samples of chicken products (Rezaloo *et al*., 2022).

P. aeruginosa has the potential to form biofilm and resistance to multiple drugs and these factors help it to survive under harsh environments. Moreover, the zoonotic nature and cross-contamination of this bacterium may have a key role in its colonization and infections (Hassan *et al.*, 2020). Drug resistance characteristics of *P. aeruginosa* have made it less susceptible to many antimicrobials, making it difficult to eliminate as the *P. aeruginosa* genome contains large known resistance island genes. The important reason for antimicrobial resistance was impermeability which belongs to the outer membrane lipoprotein (or gene) that is implicated in efflux transport systems and affects cell permeability (Miao *et al.*, 2022).

In the present study, a total of 110 samples were collected from poultry meat, and meat products, and based on phenotypic and biochemical characterization (green colonies on cetrimide agar and positive catalase and oxidase tests), the *P. aeruginosa* showed 10% overall positivity. The results indicated that *P. aeruginosa* was presented in 12 out of 110 poultry meat samples. Based on the morphological, cultural, biochemical, and molecular characteristics of the isolates, it was observed that the overall prevalence of *P. aeruginosa* (10%) was low which is a good surge in findings of MDR pathogen.

Among poultry meat samples, *P. aeruginosa* was more prevalent (50%) in fresh meat samples than others [frozen meat (40%) and meat products (8%)]. Farghaly *et al.* (2022) conducted a study on the identification and prevalence of Pseudomonas species, particularly *P. aeruginosa*, in various processed and ready-to-eat meat products and it was observed that using the colony morphology on Cetrimide agar, Pseudomonas spp. was present in 15% of the examined samples of meat and meat products. *P. aeruginosa* was present in 12 isolates (37.5%) using morphological and biochemical techniques, representing an incidence rate of 6% after the analysis of all samples (Edris *et al.*, 2023). We also performed a similar experiment to determine the prevalence of *Pseudomonas* species isolated from various meat products. It was observed that among species of Pseudomonas, the highest prevalence was of *P. aeruginosa* (4.1%) followed by other species (2%).

In our study, *P. aeruginosa* isolates were found as non-lactose fermenting, Gram-negative rods, catalase positive, oxidase positive, citrate positive, and indole negative. The *P. aeruginosa* isolates were able to produce a green pigment called pyocyanin attributing green color colonies on cetrimide agar (Algammal *et al*., 2023). In another study, non-lactose fermenting characteristics on MacConkey agar and green color colonies on cetrimide agar of *P. aeruginosa* (Adesoji *et al.*, 2023) were reported. Similar findings of *P. aeruginosa* isolates (positive oxidase, and catalase tests while negative for indole and VP tests) have been reported (Poursina *et al*., 2023). We reported positive isolates of *P. aeruginosa* as oxidase-positive, catalase-positive, and green color growth on selective agar (cetrimide agar). Alam *et al.* (2023) identified *P. aeruginosa* isolates based on morphological characteristics and reported similar results for culture identification (colorless colonies on MacConkey agar and green color colonies on cetrimide agar) and Gram's staining (Gram-negative rods). Dabbousi *et al*. (2022) used biochemical tests like oxidase, catalase, and citrate utilization for the identification of *P. aeruginosa* and reported the same results as our findings. These findings depict that although these cultural methods are laborious and time-consuming, still are useful for the isolation and identification of *P. aeruginosa*.

Apart from the increasing bacterial prevalence among the meat and meat product samples, *P. aeruginosa* showed high resistance to the majority of the antibiotics, including ampicillin, gentamicin, aztreonam, meropenem, enrofloxacin, azithromycin, amikacin, penicillin, tetracycline, cefoxitin, gentamicin, clindamycin, and sulfamethoxazole. The significant distribution of antibiotic resistance genes, particularly *bla*^{TEM}, *tetR, algD, pslD. lasR. rhlR, exoS*, and e*xoU* contributed to the high prevalence of antibiotic resistance among the microorganisms under investigation. As a result, MDR infections have become prevalent. *P. aeruginosa* has become resistant to antibiotics that are used commercially (Lianou *et al.*, 2017).

Our findings demonstrated that all the tested isolates all *P. aeruginosa* isolates were highly resistant to azithromycin (83%). High resistance rate was also observed for aztreonam (67%), gentamicin (58%) and enrofloxacin (50%) and all *P. aeruginosa* isolates were highly sensitive to meropenem (84%) followed amikacin (75%). In overall comparison, all *P. aeruginosa* isolates were highly sensitive to meropenem (84%), followed by amikacin (75%) and enrofloxacin (50%). The least sensitivity was observed for azithromycin (17%), aztreonam (33%), and gentamicin (42%). The highest resistance rate was for azithromycin (83%) and the highest sensitivity for meropenem (84%). The present study suggests a high proportion, potentially 90% of *P. aeruginosa* isolates from poultry meat and meat products exhibit multidrug resistance. In a study performed by Miao *et al.* (2022), it was reported that *P. aeruginosa* isolates were resistant to azithromycin (70%) and amikacin (65%). A study in China revealed the prevalence of *P. aeruginosa* in drinking water and foods of animal origin with presence of virulence and antibiotic resistance genes (Wei *et al.*, 2020). Most antibiotics (enrofloxacin, gentamicin, amikacin, meropenem, aztreonam, and azithromycin) were resistant against the *P. aeruginosa* isolates except enrofloxacin. Enrofloxacin was reported to be the most effective drug against *P. aeruginosa*. In a study, 30% of isolates of *P. aeruginosa* were reported as multidrug-resistant (Shahrokhi *et al.*, 2022). The prevalence of multidrug-resistant *P. aeruginosa* is very low, compared to our study which may be due to the unprescribed use of antibiotics in our study area.

The positive isolates of *P. aeruginosa* (poultry meat and meat products) were further subjected to molecular

detection of virulence genes. The virulence genes; *exoU*, *exoS*, *lasR*, *rhlR*, *algD*, *pslD*, and *bla*^{TEM} were detected in all the tested isolates. The *tetR* gene was not detected in any of the *P. aeruginosa* isolates. The results indicated that *bla*^{TEM} was present in 95% of *P. aeruginosa* isolates, e*xoU* in 25%, *exoS* in 30%, *lasR* in 85%, *rhlR* in 80%, *algD* in 80%, and *pslD* in 85% of isolates. A comprehensive study by Poursina *et al*. (2023), in which swab samples from various poultry meat and meat products and meat carcasses were collected and the prevalence of virulence genes in MDR *P. aeruginosa* isolates was determined in line with our study. The most frequently reported antibiotic resistance genes were bla^{CTXM} (53.19%), bla^{DHA} (42.55%), and *bla*^{TEM} (27.65%) (İnat *et al*., 2021). The virulence factors exoS (42.55%), algD (31.91%), lasA (31.91%), plcH (31.91%), and exon (25.53%) were the most commonly found. The distribution of antibiotic resistance genes *bla*^{SHV}, *bla*^{TEM}, and *bla*$^{CTX-M}$ was found in 93.3, 40.0, and 20.0% of *P. aeruginosa* isolates from non-clinical samples, respectively (Dawadi *et al*., 2022). The distribution of MDR *P. aeruginosa* strains is significantly influenced by the source of sampling, as demonstrated by the high distribution of virulence genes in *P. aeruginosa* strains (Elbehiry *et al*., 2022).

In another study (Heir *et al*., 2021), *P. aeruginosa* strains obtained from meat and poultry farms were found to have virulence genes. Virulence-related genes oprL, toxA, aprA, phzM, and exoS were found to be present in 100, 100, 42.5, 33.3, and 25.9% of the collected *P. aeruginosa* strains, according to PCR evidence. Jawher and Hassan (2022) found that the recovered *P. aeruginosa* strains had additional genes, lasI, lasR, rhlI, and rhlR, with the relative prevalence of 85.2, 85.2, 81.5, and 81.5%. Additionally, bla^{TEM}, tetA, bla^{CTX-M}, bla^{OXA-1}, and aadA1 genes were present in 40.7% of the isolated *P. aeruginosa* (Algammal *et al*., 2023). While in our study, the *TetR* gene was not detected in any MDR isolate of *P. aeruginosa*. The pathogenesis of *P. aeruginosa* could be explained by an understanding of virulence genes and their role in pathogenicity. Virulence genes provide resistance against drugs and antiseptic materials (Tesauro *et al*., 2022). To prevent menace of drug-resistant bacterial spread via food-chain, alternative to antibiotics can be used in poultry production system such as probiotics and bacteriophages (Rashid *et al.*, 2023).

Conclusion: We found a significant occurrence of *P. aeruginosa* in samples of poultry meat and meat products. These poultry origin samples also showed the distribution of antibiotic resistance and virulence genes in *P. aeruginosa*. Resistance to meropenem and aztreonam antibiotic agents was found in the current study with existence of antibiotic resistance genes *bla*^{TEM}, *algD*, and *pslD*. *exS*, *exoU*, *lasR*, and *rhlR* (mainly included in the adhesion and invasion to host cell). These findings indicate that *P. aeruginosa* showed resistance to antibiotics and meat and meat products, it may contribute in its spread from food chain to humans.

Authors Contribution: RMA: performed experiments, lab analysis and article writing; SA: data analysis and proof-reading; BA: Article proof-reading and formatting; MIA: designed study, data analysis and article writing.

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