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Effect of Natural Antimicrobials on the Reduction of *Pseudomonas aeruginosa* in Frozen Chicken Products

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Abstract

Pseudomonas is a food-poisoning microorganism that poses a threat to people's health. This study's goal was to assess the variety and occurrence of *Pseudomonas* species, with a focus on *Pseudomonas aeruginosa*, which was found to be contaminating frozen poultry products during storage in some markets. 200 frozen chicken product samples, including 40 of each type (breast, thigh, burger, pane, and kofta), were gathered from various stores in the Faiyoum government, Egypt, throughout 2022. *Pseudomonas* species were isolated from a total of 39.5% of all samples tested, according to the findings. Bacteriological and biochemical analyses revealed the main isolated *Pseudomonas* spp. to be *P. aeruginosa* (36.7%), followed by *P. fluorescens* (30.4%), *P. putida* (15.2%), and *P. diminuta* (6.3%). Antibiotic sensitivity affirmed the higher sensitivity of the isolates to various antibacterial drugs utilized in Egypt, comprising Tetracycline, Ampicillin, and Penicillin (100%) and being followed by Sulfamethoxazole (86.2%), Chloramphenicol (62%), and Streptomycin (51.15%). On the other hand, Amikacin (86.2%) and Norfloxacin (74.9%) were found to have the highest sensitivity. *P. aeruginosa* strains that were analyzed by polymerase chain reaction (PCR) were positive for the 16S rDNA unique to *P. aeruginosa* and carried the *toxR* (50%) and *exoS* (30%) virulence genes. Chicken fillets dipped in lemon juice and pomegranate peel extract (PPE) at 5% for 36 hours decreased the *P. aeruginosa* count by 62.4% and 56.4%, respectively. In conclusion, the current research confirms the contamination of frozen chicken products by *Pseudomonas* species. Immersion of chicken fillet in lemon juice (5% w/v) and PPE (5% w/v) can increase its quality and lengthen its shelf life by improving its sensual characteristics and implementing a successful approach for reducing *P. aeruginosa* in chicken products.

KEYWORDS
Antimicrobials, *P. aeruginosa*, Virulence genes, Lemon juice, Pomegranate peel extract

INTRODUCTION

Chicken meat is regarded as a highly nutritious diet with a reasonable cost and minimal levels of cholesterol and fat. However, due to its high perishability, even at refrigerator temperatures, its storing life is only short (Mantilla *et al*., 2011). Because psychrotrophic bacteria like *Pseudomonas* species result in taste changes or spoilage. Chicken meat has a short preservation period, even in cold storage settings (Carrizosa *et al*., 2017). *Pseudomonas* species are frequently present in both chilled and room-temperature foodstuffs and have a significant impact on the poultry business. Most isolates can grow at low temperatures and can release enzymes that may affect the overall quality of foodstuffs, including chilled food (Caldera *et al*., 2016). *Pseudomonas aeruginosa* (*P. aeruginosa*), a gram-negative, motile, non-spore-forming rod bacteria, is the main *Pseudomonas* strain inflicting a significant poultry infection and a zoonotic bacterial pathogen (Elsayed *et al*., 2016). It is distinguished by the production of a particular fruity flavor and a watery soluble green pigment. Additionally, *P. aeruginosa* can persist in a diversity of surroundings due to several virulence factors, including cell-associated (lipopolysaccharides, flocculum, and adhesins) and cell-free (protease, elastase, lipase, hemolysins, and exotoxin A) (Breidenstein *et al*., 2011). *P. aeruginosa* diagnosis is currently being pursued aggressively due to its significance for public health as well as its economic importance. Currently, People are susceptible to *Pseudomonas* spp., a foodborne illness, by ingesting or manipulating contaminated products (Gram *et al*., 2002). Since *P. aeruginosa* has one of the largest known resistance island genomes, it has a minor susceptibility to many different kinds of antibiotics, which makes it a very challenging pathogen to eradicate (Khattab *et al*., 2015). Marinades are mixtures of sugar, spices, oil, and acids that are used to improve the juiciness, tenderness, taste, and aroma of meat, poultry, and seafood (Cadun *et al*., 2005) also acetic acid and sodium work as preserving agents in marinades, which are semi-preserves. Concentrated forms of these substances have more potent inhibitory effects on enzymes and microbes. By tenderizing or altering the taste, textural, and structural properties of raw materials as well as delaying the action of bacteria and enzymes, a product with a distinctive flavor and extended shelf life is produced. The purpose of the current study was to investigate the presence of *P. aeruginosa* in some refrigerated chicken

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products and the impact that various marinades, such as lemon juice and pomegranate peel extract, on the quality of chicken fillet specimens that had been intentionally inoculated with *P. aeruginosa*.

MATERIALS AND METHODS

Samples Collection

Two hundred frozen chicken products, 40 of each type (breast, thigh, burger, pane, and kofta), were purchased from various stores in the Faiyoum government of Egypt throughout 2022. Before conducting a bacteriological examination in the lab, every specimen was transported immediately to the lab in a refrigerated ice box, preserved, and separated in a single plastic bag.

Samples preparation (FDA, 2001)

Under serious aseptic conditions, 25 g of each sample was weighed and added to 225 ml of sterilized peptone water (0.1%) in a sterilized flask. After being homogenized for 3 min at a pace of 14000 rpm, the contents of the flask were allowed to sit for 5 min at ambient temperature. For the following processes, 1ml of the mixture was transported to a different tube holding 9 ml of sterilized peptone water (0.1%) and used to create a tenfold serial dilution.

Isolation, enumeration, and identification of Pseudomonas spp.

Pseudomonas agar base media (HiMedia) improved with glycerol was distributed with 100 µL of each dilution and incubated at 25°C for 48 h and the regionalized colonies (greenish-yellow colonies) were counted up (Roberts and Greenwood, 2003) Colonies were also collected, purified, and propagated on nutrient agar before being kept for 24 hours at 37°C. Following Quinn *et al*., (2002) and Austin and Austin (2002), the purified colonies were submitted to biochemical tests and Gram stain identification.

Molecular characterization of P. aeruginosa virulence genes

P. aeruginosa-specific 16S rDNA primers and two sets of primers (*toxR* and *exoS*) were used from the Willowfort Company (United Kingdom), and biochemically identified colonies were genetically confirmed. DNA from *P. aeruginosa* was extracted following the recommendations of the QIAamp DNA mini kit (QIAGEN GmbH, Hilden, Germany, Catalogue no. 51304). Specific primers were used to finish the amplification of *P. aeruginosa* 16S rDNA, F- 5´GGGGGATCTTCGGACCTCA3´and R- 5´TCCTTAGAGTGCCCACCCG3´ (Spilker *et al*., 2004) which targeted fragment size 956 bp. The primer for the detection of the *toxR* gene was F-5´GACAACGCCCTCAGCATCACCAGC3´and R-5´CGCTGGCCCATTCGCTCCAGCGCT3´ (Matar *et al*., 2002) which targeted fragment size 396 bp while for *ExoS* gene was F- 5´ CTTGAAGGGACTCGACAAGG3´and R- 5´ TTCAGGTCCGCGTAGTGA AT3´ which targeted fragment size 504 bp (Strateva, 2008). These primers were made in a 25µl reaction with the following ingredients: 12.5 µl of Emerald Amp Max PCR Mastermix (Takara, Japan), 1 l of each primer at a concentration of 20 pmol, 4.5µl of water, and 6 µl of the template. The PCR reactions required a 5min preheating stage at 94°C, following 35 cycles of denaturation at 94°C for 30 sec., the annealing temperature was 52°C for 45 sec 16S rDNA, 72˚C for 45-sec *toxR* and 60 0C for 60 sec *ExoS*. Extension at 72 °C for one min, followed by a final extension at 72°C for 10 min. A reference sample of *P. aeruginosa* provided by the Animal Health Institute in Giza, Egypt, was used as a positive sample, and a PCR reaction deprived of any DNA was used as a negative sample. A documentation system supported the visualization of the 1.5% agarose gel electrophoresis by using Qiagen 100 bp DNA Ladder (USA).

Sensitivity to antibiotics

The distribution of antibiotic resistance was examined using a simple disk diffusion method (Kirby Bauer) following Amalina *et al*., (2019). *P. aeruginosa* isolates were cultivated in Tryptone Soya Broth (HiMedia) after a 24-hour incubation period at 37°C in an aerobic environment. Following the creation of 0.5 McFarland dilutions, isolates were cultivated on Mueller-Hinton agar (Hi-Media) in the presence of different antibiotic discs. The following medicines were used (g/disk): Ampicillin (10), Tetracycline (30), Penicillin (10), Nalidixic acid (30), Amoxicillin (25), Amikacin (30), Chloramphenicol (30), Streptomycin (10), Sulfamethoxazole (25), Cefoxitin (10), Ciprofloxacin (5), Norfloxacin (10), and Gentamicin (10) (Oxoid, UK). Cultures were kept at 37°C for 24 hours. To measure the resistance pattern, the diameter of the halo encircling the growing inhibition was recorded according to CLSI (2018), and the observed zone was interpreted.

Effect of lemon juice and pomegranate peel extract on P. aeruginosa in fresh chicken fillets

With some minor modifications, *P. aeruginosa* strains used in the present study that was positive for both the *toxR* and *exoS* virulence genes were used in the experimental trial stated by Ünalan *et al*. (2011) and Morshdy *et al*. (2022). For each experiment, five groups of five raw chicken fillets (weighing 150 grams each) were used. Chicken fillets were cleaned by submerging them in 70% ethyl alcohol for 3-5 minutes, and then allowing them to dry. Each fillet was infected with about one ml of *P. aeruginosa* broth that had been adjusted to 0.5 McFarland. The inoculated steaks were kept at 25°C for 60 min after that, the chicken fillet that had been infected with *P. aeruginosa* was placed in solutions of lemon juice (3% and 5%) and pomegranate peel extract (PPE) (3% and 5%) which were provided by the National Research Center. Then, chicken fillet samples were divided into five groups: the untreated control group only got a microorganism inoculation; the second group was submerged in a solution of 3% lemon juice; the third group was submerged in a solution of 5% lemon juice; the fourth group was submerged in a solution of 3% PPE; and the fifth group was submerged in a solution of 5% PPE. To determine the antimicrobial effect of lemon juice and pomegranate peel extract against *P. aeruginosa*, sensory analysis (texture, color, and odor) following Hemin (2013) and total bacterial were calculated at zero, 12, 24, 48, and 36 h in triplicate. The findings were presented as mean values and standard error.

Statistical analysis

According to Feldman *et al*. (2003), ANOVA (analysis of variance) evaluation was utilized to statistically analyze all the findings that were obtained.

RESULTS

From all the samples that were examined, 39.5% of *Pseudomonas* spp. were isolated after a bacterial analysis of 200 frozen chicken product samples. Chicken burger and chicken kofta had the greatest isolation rates (both 45%), while chicken breast

had the lowest rates (30%). Additionally, the analyzed samples' mean *Pseudomonas* count (cfu/g) ranged from $1.44\times10^3\pm3.3$ for the breast to $1.7\times10^4\pm1.2$ for the thigh to $3.24\times10^4\pm3.2$ for the burger to $2.8\times10^4\pm1.02$ for the pane to $3.8\times10^4\pm2.42$ for kofta (Table 1). *P. aeruginosa* (36.7%), *P. fluorescens* (30.4%), *P. putida* (15.2%), and *P. diminuta* (6.3%) were the most prevalent separated *Pseudomonas* species. The biochemical instruments available, however, were unable to identify some species (Table 2). PCR results using 16S rDNA at 956 bp demonstrated the presence of *P. aeruginosa* DNA in all samples. (Fig. 1), while 50% and 30% of samples tested positive for the *toxR* and *exoS* virulence genes, respectively (Figs. 1, 2).

Table 3 showed that there was a clear indication of resistance to Tetracycline, Ampicillin, and Penicillin (100%) with Sulfamethoxazole (86.2%), Chloramphenicol (62%), and Streptomycin following closely behind. (51.15). Contrarily, Amikacin (86.2%) and

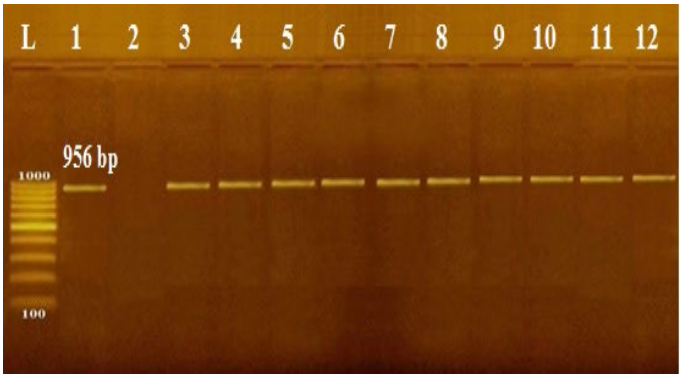

Fig. 1. Representative 1.5% agarose gel electrophoresis for PCR analysis of 16S rDNA (956 bp) gene in *P. aeruginosa* isolated from different frozen chicken product samples. L: 100 bp ladder as molecular size DNA marker. 1: positive control. 2: negative control. 3-12: Positive *P. aeruginosa* for 16S rDNA gene.

Table 1. Prevalence total *Pseudomonas* count (CFU/g) of examined samples (n = 40, each).

Frozen Chicken product	Positive samples		Count CFU/g		
	No.	%	Min.	Max.	Mean ± SE
Breast	12	30	1.9×10^2	3.45×10^4	$1.44\times10^3\pm3.3^a$
Thigh	16	40	1.72×10^2	1.31×10^5	$1.7\times10^4\pm1.2^b$
Burger	18	45	3.19×10^2	3.25×10^5	$3.24\times10^4\pm3.2^{ab}$
Pane	15	37.5	1.27×10^2	5.53×10^4	$2.8\times10^4\pm1.02^c$
Kofta	18	45	4.23×10^2	3.64×10^5	$3.8\times10^4\pm2.42^b$
Total	79	39.5			

Values within the same column have different superscript letters are significantly different.

Table 2. Incidence of isolated *Pseudomonas* species in the examined samples.

Pseudomonas spp.	No	%	Frozen Chicken product									
			Breast		Thigh		Burger		Pane		Kofta	
			No	%	No	%	No	%	No	%	No	%
P. aeruginosa	29	36.7	4	14.8	7	8.9	7	8.9	5	6.3	6	7.6
P. fluorescens	24	30.4	3	3.8	8	10.1	5	6.3	2	2.5	6	7.6
P. putida	12	15.2	3	3.8	2	2.5	2	2.5	1	1.2	4	5
P. diminuta	5	6.3	0	0	1	1.2	2	2.5	1	1.2	1	1.2
Other spp.	9	11.4	0	0	3	3.8	3	3.8	1	1.2	2	2.5

Table 3. The interpretation of antimicrobial resistance of *P. aeruginosa* isolates according to CLSI (2018).

Antimicrobial agents	Conc. (µg)	Resistance		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
Tetracycline	30	29	100	-	-	-	-
Chloramphenicol	30	18	62	6	20.7	5	17.2
Sulfamethoxazole	25	25	86.2	4	13.8	-	-
Gentamicin	10	8	27.6	4	13.8	17	58.6
Ciprofloxacin	5	6	20.7	2	6.9	21	72.4
Norfloxacin	10	3	10.3	4	13.8	22	75.9
Streptomycin	10	16	55.1	3	10.3	10	34.4
Ampicillin	10	29	100	-	-	-	-
Penicillin	10	29	100	-	-	-	-
Amoxicillin	25	13	44.8	4	13.8	12	41.4
Cefoxitin	10	9	31	3	10.3	17	58.6
Amikacin	30	4	13.8	-	-	25	86.2
Nalidixic acid	30	9	31	7	24.1	13	44.8

Norfloxacin (75.9%) had the greatest levels of sensitivity, making them the most significant antibiotics. Table 4 displays how lemon and pomegranate peel extracts affected the general acceptability of chicken fillet samples that had been intentionally inoculated with *P. aeruginosa* during cold storage. Tables 5 and 6 evaluate the decontamination of *P. aeruginosa* following immersion in 3% and 5% lemon juice solution and pomegranate peel extract. The mean counts of *P. aeruginosa* in the analyzed samples over 36 hours decreased by 5.77 log cfu/g (62.4%) in the lemon juice solution and by 5.21 log cfu/g (56.4%) in the pomegranate peel extract.

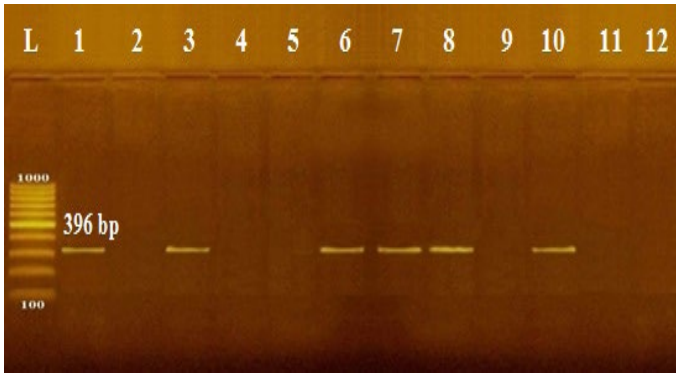

Fig. 2. Representative 1.5% agarose gel electrophoresis for PCR analysis of *toxR* (396 bp) gene in *P. aeruginosa* isolated from different frozen chicken product samples. L: 100 bp ladder as molecular size DNA marker. 1: positive control. 2: negative control. 3, 6, 7, 8 and 10: Positive *P. aeruginosa* for *toxR* gene.

DISCUSSION

Because *Pseudomonas* spp. are indicators of meat quality and can cause foodborne illness, bacterial testing of chicken products for their presence has significant significance. In the current research, 39.5% of the samples of chicken products that were examined contained *Pseudomonas* spp. According to the findings, there were 12 (30%), 16 (40%), 18 (45%), 15 (37.5%), and 18 (45%) incidences of *Pseudomonas* spp. in chicken breast, chicken thigh, chicken burger, chicken Pane, and chicken kofta, respectively. The data in Table 1 demonstrate that chicken Kofta ($3.8\times10^4\pm2.42$), chicken burger ($3.24\times10^4\pm3.2$), chicken pane ($2.8\times10^4\pm1.02$), and chicken thighs ($1.7\times10^4\pm1.2$) had higher mean *Pseudomonas* counts than chicken breast samples ($1.44\times10^3\pm3.3$). According to these findings, the greatest *Pseudomonas* species contamination was found in the burger and kofta samples, which may have been caused by improper handling, poor raw material hygiene, in light of the additional spices, and unsanitary manufacturing and preservation practices. However, a low amount of $3.51\times10^3\pm 0.76\times10^3$, $6.29\times10^3\pm 1.12\times10^3$, and $1.71\times10^4\pm 0.36\times10^4$ was noted for the breast, thigh, and hamburger (Hassan *et al*., 2020). Additionally, poultry products contained *Pseudomonas* counts of 3.6×10^3 cfu/g (Morshdy *et al*., 2018), 2.6×10^4 cfu/g (Abd El-Aziz, 2015), and 2.7-3.8 (Bruckner *et al*., 2012). Even though psychrotrophic were not isolated from broiler carcasses just after washing with chlorinated water, *Pseudomonas* spices were the most commonly isolated psychrotroph from all carcasses kept for 7 to 14 days (Hinton *et al*., 2007). *Pseudomonas* spices are ubiquitous

Table 4. Effect of lemon and pomegranate peel extract on overall acceptability of artificially inoculated chicken fillet samples with *P. aeruginosa* during cold storage.

Treatment Time	Control	lemon juice		pomegranate peel extract	
		3%	5%	3%	5%
zero	5[a]	5[a]	5[a]	5[a]	5[a]
12h	4.12±0.13[b]	5[a]	5[a]	4.56±0.73[b]	5[a]
24h	3.51±0.3[a]	4.76±0.44[b]	4.86±0.28[a]	4.21±0.13[c]	4.52±0.17[c]
48h	2.71±0.34[a]	4.53±0.26[b]	4.64±0.33[b]	4.33±0.16[b]	4.15±0.13[c]
36h	-	4.31±0.14[a]	4.47±0.56[b]	3.83±0.46[c]	4.03±0.23[a]

5 = very acceptable, 4 = Acceptable, 3 =Middle, 2 = Unacceptable, 1 = Rejected, - =spoilage.
Values are presented as Mean ± SEM of three experiments. Values within the same raw have altered superscript letters are significantly different.

Table 5. Effect of Lemon juice on *P. aeruginosa* count log10cfu/g in chicken fillet after different exposure time.

Treatment Time	Control	lemon juice					
		3%			5%		
		Mean ± SEM	R. count	R. %	Mean ± SEM	R. count	R. %
zero	6.24±0.45[a]	6.13±0.2[a]	0.11	1.76	6.05±0.37[a]	0.19	3
12h	6.76±0.52[a]	5.83±0.42[a]	0.93	13.8	5.63±0.62[b]	1.13	16.7
24h	7.62±0.04[a]	5.31±0.26[b]	2.31	30.3	4.73±0.08[c]	2.89	37.9
48h	9.24±0.35[a]	4.63±0.15[b]	4.61	49.9	4.04±0.45[c]	5.2	56.3
36h	spoiled	4.12±0.1[a]	5.12	55.4	3.47±0.35[b]	5.77	62.4

Values are presented as Mean ± SEM. R.: Reduction. Values within the same raw have different superscript letters are significantly different.

Table 6. Effect of pomegranate peel extract on *P. aeruginosa* count log10cfu/g in chicken fillet after different exposure time.

Treatment Time	Control	PPE					
		3%			5%		
		Mean ± SEM	R. count	R. %	Mean ± SEM	R. count	R. %
zero	6.24±0.45[a]	6.2±0.32[b]	0.04	0.64	6.16±0.17[b]	0.08	1.3
12h	6.76±0.52[a]	5.88±0.51[b]	0.88	13	5.72±0.37[c]	1.04	15.4
24h	7.62±0.04[a]	5.53±0.5[b]	2.09	27.4	5.22±0.26[c]	2.4	31.5
48h	9.24±0.35[a]	4.84±0.15[b]	4.4	47.6	4.19±0.73[b]	5.05	54.7
36h	spoiled	4.62±0.1[a]	4.62	50	4.03±0.72[b]	5.21	56.4

Values are presented as Mean±SEM. R.: Reduction. Values within the same raw have different superscript letters are significantly different.

and distinct from several sources, including drinking water, vegetation, people, and various foods. To achieve the best storing period and sensory qualities, the preliminary count of *Pseudomonas* species on chicken products under aerobic circumstances shouldn't surpass 100 cfu/g (Mead, 2005).

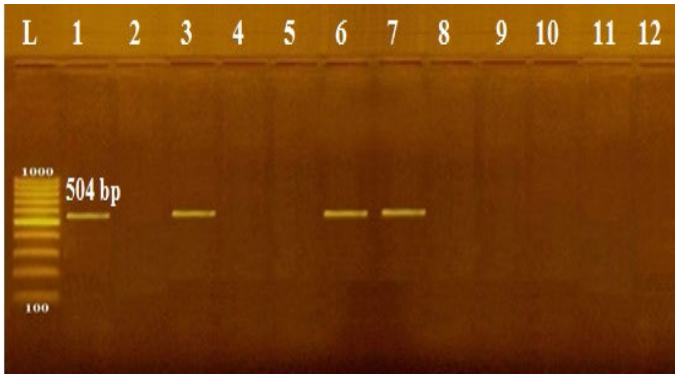

Fig. 3. Representative 1.5% agarose gel electrophoresis for PCR analysis of *ExoS* (504 bp) gene in *P. aeruginosa* isolated from different frozen chicken product samples. L: 100 bp ladder as molecular size DNA marker. 1: positive control. 2: negative control. 3, 6, and 7: Positive *P. aeruginosa* for *ExoS* gene.

Pseudomonas can be eliminated from poultry during the scaling process, but further processing stages could recontaminate the final product. Numerous studies have shown a clear correlation between the preliminary *Pseudomonas* count and the storage time of the product at chilling temperatures, as well as food spoilage that will occur once the *Pseudomonas* count ranges from 10^7 to 10^8 cfu/g. According to the current research, *P. aeruginosa* (36.7%), *P. fluorescens* (30.4%), *P. putida* (15.2%), and *P. diminuta* (6.3%) were the most common *Pseudomonas* species isolated. Nearly identical results showed that the chicken burger was the most contaminated product, with 56/166 (32.5%) *Pseudomonas* isolates present, and that the most prevalent isolates were *P. aeruginosa, P. cepacia, P. acidovorans, P. putida, P. fluorescens, P. fragi, P. proteolytica, P. putrefaciens,* and *P. stutzeri* (Hassan *et al*., 2020). Additionally, similar findings were verified by Franzetti and Scarpellini (2007) and lee *et al*. (2017) who isolated various types of *Pseudomonas* species. In comparison, none of the 100 samples of chicken meat contain *P. aeruginosa* (Iroha *et al*., 2011). Usually, *P. aeruginosa*, P. lundensis, P. fragi, and *P. fluorescens* are linked to food deterioration (Caldera *et al*., 2016). Additionally, because these bacteria can survive in cooling temperatures, storing food becomes difficult (Bellés *et al*., 2017). The occurrence of *Pseudomonas* spp. in foodstuff is significant because the organism is regarded as a bacterium that can cause disease in humans and as an indication of the quality of food (Yagoub, 2009). The findings of this research support the notion that only a combination of cultural, biochemical, and molecular tests can successfully identify and characterize *Pseudomonas* species. In the current research, PCR showed the occurrence of *P. aeruginosa* DNA in all isolates identified by conventional methods by using specific primers for *P. aeruginosa* (16S rDNA) at 956 bp (Fig. 1). Both Spilker *et al*. (2004) and Shahat *et al*. (2019) confirmed these results. Numerous cellular and extracellular virulence factors produced by *P. aeruginosa* play a role in illness (Habeeb *et al*., 2012). Due to these factors, this research was created to use PCR to identify the virulence genes *toxR* and *exoS* in *P. aeruginosa* isolates. Concerning the findings of the virulence factors, it was discovered that the *toxR* and *exoS* genes were identified in 50% and 30% of *P. aeruginosa* isolates, respectively (Figure 2, 3). Qin *et al*. (2003); Lavenir *et al*. (2007), and Shahat *et al*. (2019) all reported essentially similar findings of toxA. The *toxR* occurred in 96% of the analyzed *P. aeruginosa* isolates, according to Khan and Cerniglia (1994). In addition, Feltman *et al*. (2001) and Shahat *et al*. (2019) found a greater percentage (71.42% for each of them), compared to Tartor and El-naenaeey (2016) and Rezaloo *et al*. (2022), who found *exoS* in 78.6% and 75.86% of *P. aeruginosa* isolates, respectively. Additionally, *P. aeruginosa* possesses several virulence genes, which gives it a cause for variable effects of virulence and pathogenicity, according to Nikbin *et al*. (2012).

The rise of antibiotic resistance is a significant issue the globe is currently facing, especially in underdeveloped nations. Therefore, it's critical to accurately and quickly discover *P. aeruginosa* and find the pattern of vulnerability to prevent unnecessary antibiotic use that could result in pathogens with drug resistance (Hamisi *et al*., 2012). Antibacterial residues in chicken products pose significant risks to the public's health. Both the direct transmission of bacterially acquired resistance and the indirect transmission of resistance genes via horizontal gene transfer from aquatic to terrestrial habitats are to blame for this health risk (Sun *et al*., 2015). Using the disc diffusion method, 13 antimicrobials were used to investigate *P. aeruginosa*'s antimicrobial sensitivity. The outcomes, as shown in Table 3, demonstrated that Tetracycline, Ampicillin, and Penicillin showed the highest levels of resistance (100%) followed by Sulfamethoxazole (86.2%), Chloramphenicol (62%), and Streptomycin (55.1%). Ampicillin, Amoxicillin, Sulphamethazone, Erythromycin, and Tetracycline showed the highest level of resistance (100%) for *P. aeruginosa*, next was Nalidixic acid (57.1%) and Streptomycin (42.9%) (Shahat *et al*., 2019). Additionally, these findings corroborated those of Walker *et al*. (2002), Ahmed (2016), and Tartor and Elnaenaeey (2016), who noted that Tetracycline, Erythromycin, and Ampicillin had a high rate of resistance. In contrast, Abd El-Gawad *et al*. (1998) found that chicken *P. aeruginosa* isolates were tetracycline-sensitive. According to Abdel-Tawab *et al*. (2016), 80% of P.aeruginosa strains were Nalidixic acid resistant. In the present study, Amikacin (86.2%), Norfloxacin (75.9%), and Ciprofloxacin (72.4%) were found to have high sensitivity. These results concur with those from Hebat-Allah (2004), Mohammad (2013), and Shahat *et al*. (2019), who found *P. aeruginosa* to be highly sensitive to Ciprofloxacin and Norfloxacin. In contrast, Abd El-Tawab *et al*. (2014) found *P. aeruginosa* to be less sensitive to Ciprofloxacin and Norfloxacin while Kurkure *et al*. (2001) demonstrated a strong sensitivity to Gentamycin (100%). These variations in findings may be attributed to differences in a variety of factors surrounding farms, sample sources, methodology, or hyper-mutation, which frequently present in *P. aeruginosa* strains and resulted in the rising of multiple antibacterial resistances (Maciá *et al*., 2005). Antimicrobial-resistant bacteria are the primary source of public health risks, which are easily spread throughout the food chain (Price *et al*., 2012; Da Costa *et al*., 2013; FAO, 2015; WHO, 2015).

Organic compounds are used to reduce foodborne pathogens and limit microbial contamination, thereby prolonging the shelf life of food (Lingham *et al*., 2012). Treatment with organic acids is one of the modern food processing methods that rely on non-thermal processing to produce foodstuffs that are nutritious and microbial-safe. Organic compounds are commonly used as food ingredients and preservatives to extend the expiration date of perishable foods due to their antimicrobial qualities (Mathur and Schaffner, 2013; Wang *et al*., 2015). Additionally, the findings in Table (4) display that only marinating by lemon juice at a concentration of 5% prevented the growth of *P. aeruginosa* until 36 h into the experiment and showed the greatest enhancement in sensual characteristics. Subsequently, lemons contain organic acids that have antioxidant properties, produce an acidic taste, reduce the amount of protein in the food, and increase the amount of acidity and free amino acids, which provide the marinated fillets with a distinct flavor whereas the PPE 5%-treated chicken fillet group had slightly improved sensory characteristics and a prolonged shelf life of 36 hours when compared to the control one during storage. In light of the results, sensory characteristics started to deteriorate after the first day of storage at 4°C, and the control samples' overall acceptability values decreased while at 36 hours, the control samples completely spoiled. Morshdy *et al*. (2021) looked into the impact of lemon essential oil and discovered that it had the highest acceptable sense evaluation and microbial qualities. Additionally, The total acceptability ratings for the use of lemon juice were 5; 4.66±0.33; 4.33±0.33; 3.66 ±0.33; 3.33± 0.33; 3.16±0.16 and 2.66±0.33 at zero-day, 1st day, 2nd day,

3rd day, 4th day, 5th day and 6th day of the duration of storing at 4°C, correspondingly, while PPE, the score was 5; 3.66±0.33; 3.33±0.33; 2.66±0.33; 1.66±0.33 and 1.33±0.33, accordingly, at the same storage time (Ibrahim *et al*., 2018). In comparison to the control samples during the same storing time, various concentrations of lemon juice and PPE were used to typically improve the sensorial qualities of treated chicken fillet samples during cold storage (4°C). Lemon juice showed a decreasing trend in the reduction percentages of *P. aeruginosa* from 3, 16.7, 37.9, and 56.3 after zero, 12 h, 24 h, and 48 h, respectively, to reach 62.4% reduction after 36 h of dipping at 5% lemon juice concentration. Additionally, there is a significantly different between the examined samples at $P < 0.05$ (Table 5). *P. aeruginosa* reduction percentages ranged from 1.3, 15.4, 31.5, and 54.7 after zero, 12 h, 24 h, and 48 h, respectively, to achieve 56.4% reduction after 36 h (Table 6), with significant differences between the examined samples at $p < 0.05$. These results were remarkably related to those published by Prateek and Donald (2013), who verified that lemon juice applied for 30 or 120 min has an antimicrobial effect and lowers levels from >7 log cfu/g to 5 log cfu/g. Additionally, PPE and lemon juice are the most powerful antibacterial agents, eliminating all germs within four days of incubation (Ibrahim *et al*., 2018). The antibacterial activity of lemon juice as a natural decontaminant was demonstrated by Nawi *et al*. (2017); Morshdy *et al*. (2021); Tsai *et al*. (2021); Morshdy *et al*. (2022), and Sushmita (2022). Furthermore, Daniela *et al*. (2011) also found that immersing in lemon juice increased lifespan at 4°C by reversing proteolysis and postponing the microbe's putrefaction. Lemon juice is primarily used because it is readily available, inexpensive, and has few to no adverse effects. Lemon juice's powerful antiviral, antibacterial, and immune-boosting properties are enhanced by its high concentration of citric acid, bioflavonoids, limonene, pectin, calcium, magnesium, and vitamins (Alsaraf *et al*., 2016). Therefore, the current findings demonstrated that, at 36 h of refrigerated storage at 4°C, marinating with lemon juice and PPE at a rate of 5% demonstrated maximal activity against *P. aeruginosa* in comparison to the control. The findings of the research indicate that marinating chicken fillets in lemon juice and PPE can increase both the safety and shelf life of the food by improving the flavor and by having antimicrobial activities on Gram-negative bacteria, especially *Pseudomonas* species.

CONCLUSION

The current research demonstrated that morphological, biochemical, and molecular tests must be combined to accurately identify and characterize the *Pseudomonas* species, which is widespread in frozen chicken products, as demonstrated by the current research. It was determined that a higher incidence level of antibiotic-resistant *P. aeruginosa* harboring *toxR* and *exoS* genes in marketed frozen chicken products presents a risk to human and public health. The application of appropriate food safety measures is advised for ensuring the quality of chicken products during consumption and the food manufacturing cycle. The usage of antibacterials on poultry farms should only be permitted after the veterinary examination. The findings obtained point to lemon juice and PPE as potential decontaminants for reducing *P. aeruginosa* loads and enhancing the quality and safety of chicken fillets for human consumption.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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