Tackling food spoilage bacteria: How pomegranate peel extract can improve beef safety

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Abstract

Meat is among the most popular foods because of its high dietary component. However, it is contaminated with various food spoilage bacteria, including *Pseudomonas aeruginosa*, identified as a primary contributor to food spoilage and foodborne illnesses. This investigation evaluates the total aerobic and psychrotrophic counts, antibacterial resistance, and virulence features in *P. aeruginosa* bacteria discovered in 150 beef products. Thirty each of basterma, hot dogs, luncheon, frankfort, and burgers were obtained from numerous markets in El-Fayoum Governorate, Egypt, using culture and molecular techniques. Furthermore, the influence of pomegranate peel (PP) extract on marinated beef steak was evaluated in vitro during the storage period. Burger samples recorded the greatest total aerobic count (TAC) and psychrotrophic count (5.52 ± 2.5 and 3.88 ± 2.1 log CFU/g, respectively), while basterma samples had the lowest count (3.37 ± 2.1 and 1.89 ± 1.3 log CFU/g, respectively). Furthermore, *P. aeruginosa* contaminated 32.7% of the samples. Burger and hot dog samples had the greatest isolation percentage (46.7% and 36.7%, respectively), while basterma samples had the lowest proportions (20%). In addition, the mean *P. aeruginosa* count extended from 1.80 ± 1.1 log CFU/g (basterma) to 4.91 ± 1.3 log CFU/g (burger). PCR results of the *16S* rRNA gene at 150 bp exhibited that *P. aeruginosa* DNA was present in all of the suspected isolates. The virulence genes *pilB* (14.3%), *PslA* (18.4%), *toxR* (14.3%), and *exoS* (10.2%) were the most common ones found. High resistance rates were observed toward ampicillin and tetracycline (100%) and complete susceptibility to florfenicol and ciprofloxacin, making them the most significant antibiotics. Using 25%, 50%, 75%, and 100% PP extract solutions, respectively, reduced the *P. aeruginosa* count in the assessed samples over 48 h by 32.44%, 32.96%, 49.63%, and 52.11%. In addition, the investigation verified the group submerged in a 75% PP extract solution had better sensory criteria, which greatly varied from the control and other treatment groups.

Keywords: Beef product, food spoilage, pomegranate peel extract, *Pseudomonas aeruginosa*, natural preservatives

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Introduction

Meat products are in high demand and more appealing to consumers than fresh meat because of their high nutritional value, reasonable price, delicious flavor, ease of preparation, and convenient portion size. Despite their significance to consumers, handling, preparation, and storage practices can contaminate meat products with foodborne bacteria from altered sources (Al-Mutairi, 2011). Psychrotrophic microbes can develop in chilled environments, and temperature impacts many microbial growth factors, such as maximal development level and total bacterial counts (Caldera *et al.*, 2016).

Pseudomonas spp. are prominent psychrotrophic bacteria, and *Pseudomonas aeruginosa* is one of the main *Pseudomonas* strains included in foodstuff decomposition described by off-flavors, pigment excretion, and slime and malodor appearance (Rezaloo *et al*., 2022). This bacterium causes food spoilage worldwide, especially in developing nations because of inadequate handling and preservation technologies. This bacterium causes infections because of a variety of virulence factors, including exoenzymes (*exoS*), toxin A (*toxA*), and pilus genes (*pilA* and *pilB*). These factors often play a role in adhesions and attachments, inflammatory responses, and eventually, the attack of the host cell (Jurado-Martín *et al.,* 2021). Because *P. aeruginosa* so strongly resists a variety of antibacterials, it is more crucial compared to other spoilage microbes. It possesses a diverse array of virulence features that can result in serious and violent illnesses in people as well as animals. Shahrokhi *et al.* (2022) revealed that antibiotic resistance may arise from consuming contaminated meat products with encoding antibiotic-resistance genes.

Many researchers have been directed to find new techniques for extending the time that meat and its products can be preserved without the use of chemical ingredients. Nevertheless, recent developments have highlighted concerns regarding the use of various common substitutes to extend the shelf life and prolong the preservation of beef and its products. This is particularly evident in light of the significant increase in the production of beef products and their role in providing the desired flavor and taste (Sengun *et al.,* 2019).

Fruits, vegetables, and their leftovers are inexpensive, plentiful, and rich sources of natural polyphenolic and flavonoid constituents that may be useful as antibacterial substances by stopping bacterial development and several diseases, as well as antioxidants by avoiding lipid oxidation. Fruit peel is generally produced in great volume through the season (as a low-cost agri-waste) and is regarded as a potential natural antioxidant source throughout fruit processing and food industry operations (El-Hadary and Taha, 2020). Pomegranates (*Punica granatum L.*) are one of the oldest edible fruits, and they are grown in many tropical and subtropical locations, including Egypt (Qenawy *et al.,* 2024). Pomegranate peel (PP) is more beneficial than seeds, leaves, and flowers because it contains high levels of helpful substances like polyphenols and flavonoids, contributing to its antioxidant properties. This could be because peels contain a higher percentage of polyphenolic chemicals (~73%) than seeds do (~27%). PP extract (PPE) may include a greater level of polyphenols as well as antibacterial and antifungal properties, implying an essential role as a natural antioxidant and antimicrobial agent (Wang *et al.*, 2011). Recent research suggests that the high amounts of tannins, such as punicalagin, in PPE may be a big reason why they kill bacteria (Rosas-Burgos *et al.*, 2017). The cause could be the great concentration of polyphenols and tannins that can destroy microbial cell membranes and cause microbial loss (El-Hadary and Taha, 2020). Because of the hazard of microbial contamination in beef products and their relevance to human well-being, the present study thus focused on investigating the incidence, virulence gene detection, and antibacterial pattern of *P. aeruginosa* in sold beef products at El-Fayoum Governorate, Egypt. In addition, PPE's antibacterial efficacy against *P. aeruginosa* was investigated.

Materials and Methods

Samples collection

During February–June 2024, a total of 150 beef products—30 each of basterma, hot dogs, luncheon, frankfort, and burgers—were obtained from different markets in El-Fayoum Governate, Egypt, recognized, and promptly transported to the laboratory in an icebox for further analysis.

Samples preparation

Under strict aseptic conditions, 25 g of the analyzed material was mixed with 225 mL of sterile peptone water 0.1% (M028, HiMedia) in a sterile flask. The flask was left to settle for 5 min at room temperature after homogenizing in a Stomacher 400 (Colworth, England) for 3 min at a speed of 14,000 rpm. One milliliter of the suspension was moved to a distinct tube holding 9 mL of sterilized peptone water (0.1%) to produce a 10-fold serial dilution for the next procedures. For the subsequent analysis, 1 mL of each serial dilution that had been previously prepared was poured individually into three triplicate petri dishes that were appropriately marked.

TAC

For 48 h, the plate count agar (M091A, HiMedia) with 1 mL of every serial dilution was incubated at 35°C, as determined by the pour plating technique, and the outcome was stated as log CFU/g (Elbarbary *et al.,* 2024).

Psychrotrophic count

Applying the pour plating technique, for 48 h at 4°C, 1 mL of every serial dilution was cultured on plate count agar (M091A, HiMedia). The outcome was shown as log CFU/g (Wang *et al.,* 2023).

Isolation, enumeration, and identification of *P. aeruginosa*

An amount of 0.1 mL of each dilution was spread on *Pseudomonas* Cetrimide agar (MH024, HiMedia), and selectivity was made by the inclusion of cetyltrimethyl ammonium bromide (cetrimide; 200 mg/L) and nalidixic acid sodium salt (15 mg/L) and incubated at 25°C for 48 h. The number of developed colonies (greenish-yellow colonies) was counted (Khairy *et al.,* 2024). The recovered isolates were identified according to their morphological characteristics using Gram's stain, culture characters, pigment production, motility, and biochemical reactions using the following tests: oxidase, catalase, urease, indole, citrate utilization, H_2S production, mannitol fermentation, methyl red, and Voges-Proskauer tests, as earlier described by Quinn *et al. (*2002).

DNA verification of *P. aeruginosa* and their virulence factor recognition

Probable colonies were cultivated in Tryptic soy agar (HiMedia) for 12 h at 37°C. By Gene JET Genomic DNA purification reagent (Catalogue No. #K0721; Thermo Scientific, Waltham, MA, USA), DNA extraction was performed under the manufacturer's instructions. The concentration of DNA (25–100 ng/µL) was measured by a NanoDrop spectrophotometer (Thermo Scientific, ND8000). Using the primers shown in Table 1, the PCR analysis included a negative control (DNA-free) and reference strain of *P. aeruginosa*, positive for each tested gene supplied by Animal Health Research Institute (AHRI), Egypt, that was cultivated on Cetrimide agar and incubated at 25°C for 48 h. The reactions consisted of 12.5 µL deionized water, 1.25 µL of each 20 pmol/µL forward and transverse primer, 5 µL of DNA, and 5 µL of 5X master mix (TaQl/high yield; Jena Bioscience, Jena, Germany). The PCR processes needed a 5 min heating stage at 94°C, followed by 35 cycles of denaturation at 94°C for 30 sec. The annealing was done at 60°C/30 sec for *16S* rDNA, 55°C/60 sec for *pilB*, 60°C/40 sec for *PslA*, 72˚C/45 sec for *toxR*, and 60°C/60 sec for *ExoS*. After a 60 sec first extension at 72°C, there was another extension for 7 min at 72°C. The amplified PCR products were separated using an electrophoretic technique on a 1.5% agarose gel by DNA Ladder (Qiagen, USA) and captured using a UV LED (BioRad).

Antimicrobial susceptibility testing

Following the procedures set by the Clinical and Laboratory Standards Institute (CLSI, 2021), the discovered *P. aeruginosa* was verified by the disc diffusion technique. The antibacterials that were tested using 15 antibiotics (Oxoid, UK) included ampicillin (AM, 10 µg), erythromycin (E, 15 µg), amoxicillin (AX, 25 µg), gentamicin (G, 10 µg), florfenicol (FF, 30 µg), doxycycline (DO, 30 µg), tetracycline (T, 30 µg), penicillin (P, 10 µg), sulfamethoxazole (SL, 25 µg), norfloxacin (NF, 10 µg), ciprofloxacin (CF, 5 µg), amikacin (AK, 30 µg), kanamycin (K, 30 µg), nalidixic acid (NL, 30 µg), and cefoxitin (CX, 10 µg).

Tryptone soya broth (HiMedia) was used to cultivate pure colonies of the identified isolates for 12 h at 25°C.

Table 1. Oligonucleotide primers of gene targets for *P. aeruginosa*.

Target gene	Oligonucleotide sequence (5′ → 3′)	size (bp)	References
16S rRNA	CTACGGGAGGCAGCAGTGG TCGGTAACGTCAAAACAGCAAAGT	150	Purohit *et al.* (2003)
pilB	TCGCCATGACCGATACGCTC ACAACCTGAGCCAGCCTTCC	408	Shahrokhi *et al.* (2022)
PslA	TCCCTACCTCAGCAGCAAGC TGTTGTAGCCGTAGCGTTTCTG	656	Ghadaksaz *et al.* (2015)
toxR	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	396	Abdelmotilib and Elbarbary (2023)
ExoS	CTTGAAGGGACTCGACAAGG TTCAGGTCCGCGTAGTGAAT	504	

Then, 100 µL of culture broth was placed on Mueller-Hinton agar (Hi-Media) by sterile glass rods, and antibacterial discs were painstakingly spread and incubated at 25°C for 24 h before the inhibition zones were measured. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler, which was held on the back of the inverted Petri plate, and the isolates were categorized as resistant (R), intermediate (I), or susceptible (S) and were assigned using criteria outlined in CLSI (2021). The formula for calculating multiple antibiotic resistance (MAR) is MAR = A/B, where A represents the number of the antibiotic to which the isolate is resistant and B represents the number of the total antibacterials used in the analysis. The strain was derived from a low-risk source of pollution, as indicated by a MAR index of < 0.2. In contrast, isolates from high-risk sources of contamination have been identified by a MAR > 0.2 (Lozano *et al.,* 2020).

In vitro effects of PPE on marinated beef steak

PPE collecting and preparation

Pomegranate fruit weighing 7 kg was procured from the local market in El-Fayoum Governorate, Egypt. Seeds were manually detached from the peel for sample manufacture of PPE; then, the separated peels were chopped into small portions and dried in an air oven at 50°C for 16 h until continuous weight was obtained. After homogenizing dried peel on a coffee grinder, it was passed through a 40-mesh screen, packed in polyethene bags, and saved at −20°C until use. Peel powder (20 g) was employed for obtaining natural antioxidants (in triplicate) using organic solvent (petroleum ether) for 15 h using the Soxhlet apparatus. The compounds were mixed in a brown bottle, sieved with 45 µm sterilized filters, and vacuum-evaporated at 40°C by a mini-rotary evaporator (N-N series, EYELA) until almost all of the solvent was uninvolved. Every solvent has semi-dried extracts kept at −20°C (El-Hadary and Taha 2020).

The antimicrobial efficacy of PPE

The experimental trial used a positive pathogenic *P. aeruginosa* strain with some minor alterations, as described by Khairy *et al.* (2024). Thirty samples of beef steak from beef meat fillet, each weighing 200 g, were gathered from prominent meat stands in El Fayoum Governorate, Egypt. All samples were sent to the lab as soon as they were collected. The samples were collected in the early morning within 8 h of slaughter to reduce microbial contamination caused by the outside temperature. Each sample was sliced from the exterior cut on the surface and cut into smaller pieces of 100 g and 3 cm thick. For each trial, two groups of five beef steaks (100 g each) were employed, one for sensory assessment and the other to determine PPE's antibacterial action against *P. aeruginosa*. The present experiments employed the negative steak, which was tested for the presence of *P. aeruginosa* in beef steak. Each steak was inoculated with 1 mL *P. aeruginosa* broth (10^6 CFU/mL) equivalent to a 0.5 McFarland standard and incubated at 25°C for 60 min. The inoculated steak was immersed in PPE solution at various concentrations (*v/v*): 25% (G1), 50% (G2), 75% (G3), and 100% (G4), while the untreated control group (CG) received just a microbe infection. All groups incubated at 25°C. Under strict aseptic conditions at 0, 12, 24, and 48 h, 10 g of each group was mixed with 90 mL of sterile peptone water 0.1% (M028, HiMedia) in a sterile flask. The flask was left to settle for 5 min at room temperature after homogenizing in a Stomacher 400 (Colworth, England) for 2 min at 14,000 rpm. One milliliter of the suspension was serially diluted with 9 mL of sterile peptone water 0.1% and surface plated with 0.1 mL in duplicate on *Pseudomonas* Cetrimide agar (MH024, HiMedia). After 24 h of incubation at 25°C, the recovered isolates were identified according to their morphological characteristics and biochemical reactions to confirm their group Quinn *et al. (*2002). The petri dishes containing 30 to 300 greenish-yellow colonies were selected and counted to ascertain the antibacterial action of PPE. The outcomes were described as mean with standard error.

Natural PPE marinating's impact on organoleptic characteristics

A scoring test was implemented by 55 evaluators, including staff members and postgraduate students from the Faculty of Veterinary Medicine at Aswan University, to assess the sensory qualities of beef steak (100 g) samples marinated for 60 min at 4°C with varying concentrations of PPE. The beef steak samples received random codes, and the panels were not told how the research was conducted. They were asked to assess the general level of satisfaction, texture, flavor, smell, and appearance (Kilinc and Cakli, 2004).

Statistical analysis

Microsoft Excel 2007 was used to compile the data and create the tables. The outcomes with a significance value of $p < 0.05$ display a mean and standard error calculated by the software (SPSS, 2001).

Results

Incidence of bacterial contamination

The results presented in Table 2 demonstrate substantial variances between the samples ($p < 0.05$) concerning the TAC and psychrotrophic count. Burger samples recorded the greatest total aerobic and psychrotrophic

Table 2. Means ± standard error of the bacterial count (log CFU/g) of examined samples.

Product	No.	Total aerobic count	Psychrotrophic count
Basterma	30	3.37±2.1^{d}	1.89±1.3^{c}
Hot dog	30	4.88±2.4^{b}	3.62±1.8^{a}
Luncheon	30	3.81±1.8^{c}	3.79±1.5^{a}
Frankfort	30	4.46±2.1^{b}	2.74±2.0^{b}
Burger	30	5.52±2.5^{a}	3.88±2.1^{a}

$p < 0.005$ is considered to be significantly different. Mean values with the same letters in each column have no significant difference.

Table 3. Prevalence of *P. aeruginosa* in the examined products ($n = 30$ each).

Product	Positive samples		Count (log CFU/g)		
	No.	%	Min	Max	Mean ± SE
Basterma	6	20	1.56	2.61	1.80±1.1^{d}
Hot dog	11	36.7	2.83	5.69	3.55±1.4^{b}
Luncheon	8	26.7	2.91	4.81	2.93±2.1^{c}
Frankfort	10	33.3	2.81	4.93	2.73±1.0^{c}
Burger	14	46.7	2.93	6.71	4.91±1.3^{a}
Total	49			32.7	

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

counts (5.52 ± 2.5 and 3.88 ± 2.1 log CFU/g), while basterma samples had the lowest count (3.37 ± 2.1 and 1.89 ± 1.3 log CFU/g). The results presented in Table 2 demonstrate substantial variances between the samples ($p < 0.05$) concerning the TAC and psychrotrophic count. Burger samples recorded the greatest total aerobic and psychrotrophic counts (5.52 ± 2.5 and 3.88 ± 2.1 log CFU/g), while basterma samples had the lowest count (3.37 ± 2.1 and 1.89 ± 1.3 log CFU/g). The bacteriological analysis demonstrated that the revealed *P. aeruginosa* isolates were Gram-negative, with motile rods arranged alone or in small chains. On Cetrimide agar, the *P. aeruginosa* colonies that were collected were large, irregular in shape, had a fruity smell, and produced a distinctive greenish-yellow pigment. The examined *P. aeruginosa* isolates showed positive results for oxidase, mannitol fermentation, gelatin hydrolysis, catalase, citrate utilization, and nitrate reduction assays but tested negative for urease, methyl red, H_2S generation, indole, and Voges-Proskauer.

Bacteriological analysis of the samples revealed that *P. aeruginosa* occurs with an overall percentage of 32.7% (Table 3). Burger and hot dog samples had the greatest isolation rates (46.7% and 36.7%), while basterma samples had the lowest proportions (20%). Furthermore, the evaluated samples had mean *P. aeruginosa* counts (CFU/g) extended from 1.80 ± 1.1 log CFU/g (basterma) to 4.91 ± 1.3 log CFU/g (burger). PCR results by *16S* rRNA gene at 150 bp verified the incidence of *P. aeruginosa* DNA in all presumptive isolates (Figure 1), harboring one or more virulence genes such as *pilB* (Figure 2), *PslA* (Figure 3), *toxR* (Figure 4), and *exoS* (Figure 5) with rates of 14.3%, 18.4%, 14.3%, and 10.2%, respectively.

Antimicrobial susceptibility profile

The antibiotic susceptibility presented in Table 4 demonstrated that *P. aeruginosa* strains (n = 49) were resistant to ampicillin and tetracycline (100%) while being completely susceptible to florfenicol and ciprofloxacin, making them the most significant antibiotics. Furthermore, *P. aeruginosa* showed resistance to a variety of tested antibacterials, for instance, penicillin (65.3%), gentamicin (71.4%), doxycycline (67.3%), sulfamethoxazole (61.2%), and cefoxitin (79.6%). In addition, the MAR of *P. aeruginosa* strains extended from 0.133 to 0.867, with an average of 0.511 (Table 5).

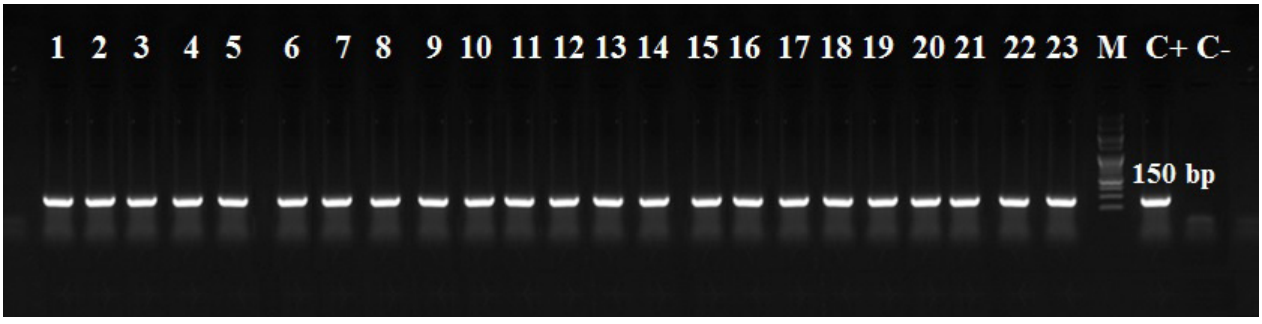

Figure 1. Electrophoretic profile of amplification products of *16S* rRNA *P. aeruginosa* gene at 150 bp, M: marker (50 bp), C+: positive control, and C–: negative control.

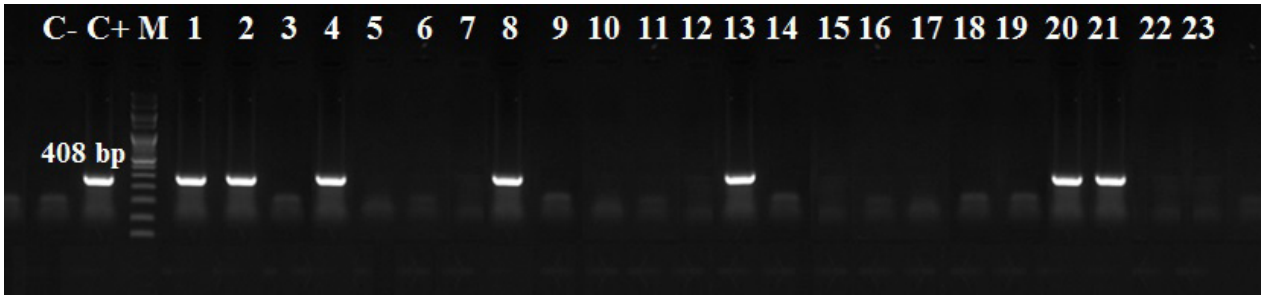

Figure 2. Electrophoretic profile of amplification products of *pilB P. aeruginosa* gene at 408 bp, M: marker (50 bp), C+: positive control, and C–: negative control.

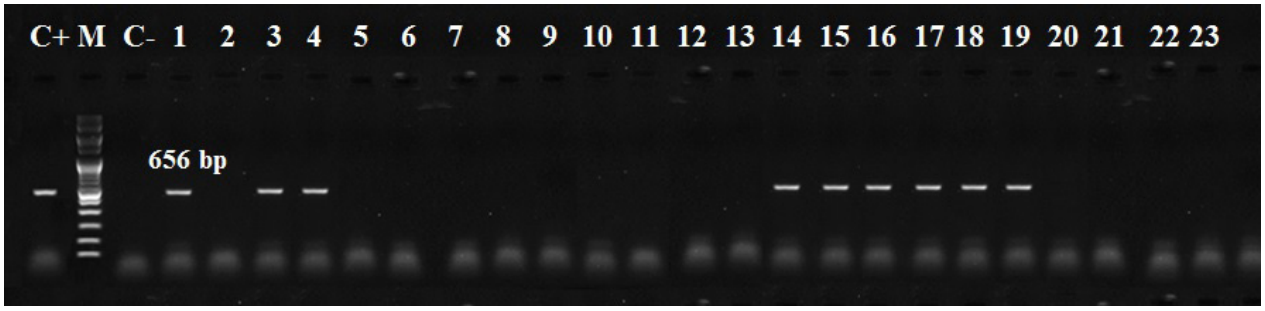

Figure 3. Electrophoretic profile of amplification products of *PslA P. aeruginosa* gene at 656 bp, M: marker (50 bp), C+: positive control, and C–: negative control.

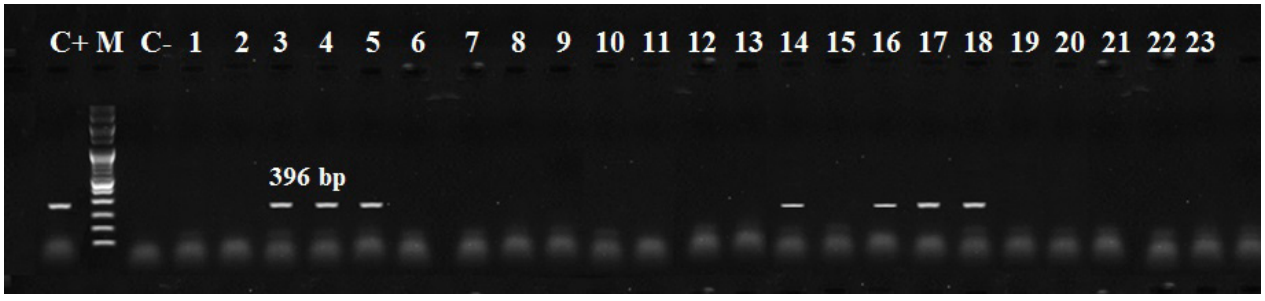

Figure 4. Electrophoretic profile of amplification products of *toxR P. aeruginosa* gene at 396 bp, M: marker (50 bp), C+: positive control, and C–: negative control.

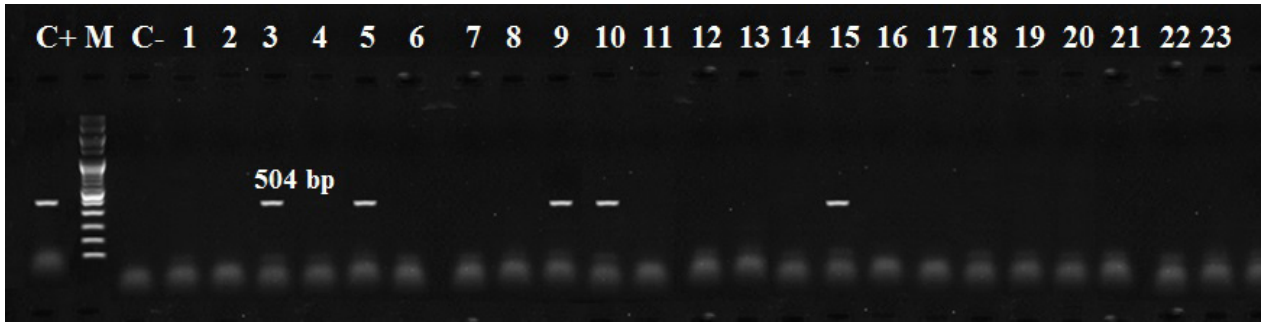

Figure 5. Electrophoretic profile of amplification products of *ExoS P. aeruginosa* gene at 504 bp, M: marker (50 bp), C+: positive control, and C–: negative control.

Table 4. The interpretation of antimicrobial resistance of *P. aeruginosa* isolates (*n* = 49).

Antimicrobial agents	Sensitive		Intermediate		Resistance	
	No.	%	No.	%	No.	%
Ampicillin (10µg)	0	0	0	0	49	100
Erythromycin (15µg)	12	24.5	17	34.7	20	40.1
Penicillin (10µg)	3	61.2	14	28.6	32	65.3
Amoxicillin (25µg)	17	34.7	11	22.4	21	42.3
Gentamicin (10 µg)	14	28.6	0	0	35	71.4
Florfenicol (30µg)	49	100	0	0	0	0
Doxycycline (30µg)	8	16.3	8	16.3	33	67.3
Tetracycline (30 µg)	0	0	0	0	49	100
Sulfamethoxazole (25µg)	12	24.5	7	14.3	30	61.2
Norfloxacin (10 µg)	34	69.3	3	61.2	12	24.5
Ciprofloxacin (5µg)	49	100	0	0	0	0
Cefoxitin (10 µg)	7	14.3	3	61.2	39	79.6
Amikacin (30 µg),	29	59.2	6	12.2	14	28.6
Kanamycin (30 µg)	33	67.3	6	12.2	10	20.4
Nalidixic acid (30 µg)	27	55.1	8	16.3	14	28.6

Table 5. Antimicrobial resistance profile of *P. aeruginosa* (*n*=49).

Species	Isolates No.	Antimicrobial resistance profile	No. of antibiotics	MAR index
P. aeruginosa	17	AM, E, AX, DO, T, P, SL, G, NF, AK, NL, K, CX	13	0.867
	11	AM, E, AX, DO, T, P, SL, G, AK, CX	10	0.667
	9	AM, E, AX, DO, T, P, SL, G	8	0.533
	7	AM, DO, T, P, SL, G, CX	7	0.467
	3	AM, T, P, SL, NL, CX	6	0.40
	2	AM, T	2	0.133
Average				0.511

MAR: multiple antibiotic resistant indexes. AM: ampicillin, E: erythromycin, AX: amoxicillin, G: gentamicin, FF: florfenicol, DO: doxycycline, T: tetracycline, P: penicillin, SL: sulfamethoxazole, NF: norfloxacin, CF: ciprofloxacin, AK: amikacin, NL: nalidixic acid, K: kanamycin, and CX: cefoxitin.

Antimicrobial efficacy and sensory evaluation of PPE

Table 6 assesses the decontamination of infected beef steak with *P. aeruginosa* after altered exposure time following dipping in different concentrations of PPE. *P. aeruginosa* count in the assessed samples over 48 h was reduced by 32.44%, 32.96%, 49.63%, and 52.11% by using 25%, 50%, 75%, and 100% PPE solutions, respectively. There were no significant alterations between the samples at the initial stages of the treatments, except for using 100% PPE solution, but over time it improved substantially ($p < 0.05$). Furthermore, the sensory assessment of the beef steak processed with a different application of PPE is presented in Table 7. The research indicated the group submerged in 75% PPE solution possessed superior sensory criteria, which were significantly different in comparison with the control and other treated groups.

Discussion

Beef products are the chief appreciated foods for human consumption because of their high nutritious value, excellent flavor, ease of processing, and convenience of portion. Nevertheless, they are polluted by a variety of food-spoilage pathogens, including *P. aeruginosa*, from a variety of sources during the handling, processing, and preparation processes, thereby presenting a significant risk to public health (Khairy *et al.*, 2023). TAC is the main

Table 6. Effect of PPE on *P. aeruginosa* count (log CFU/g) at different marinating periods.

Exposure time (h)		Treatment				
		CG	G1	G2	G3	G4
Zero	Count	7.68 ± 0.47[a]	7.67 ± 0.66[a]	7.66 ± 0.93[a]	7.63 ± 0.29[a]	6.61 ± 0.54[b]
	R. count	–	0.01	0.02	0.05	1.07
	R. %	–	0.13	0.26	0.65	13.9
12	Count	7.93± 0.29[a]	7.71± 0.29[b]	7.64± 0.29[b]	7.62± 0.29[b]	6.62± 0.29[c]
	R. count	–	0.22	0.29	0.31	1.31
	R. %	–	2.77	3.65	3.91	16.52
24	Count	8.76± 0.29[a]	6.61± 0.29[b]	6.58± 0.29[c]	6.55± 0.29[c]	5.51± 0.29[d]
	R. count	–	2.15	2.18	2.21	3.25
	R. %	–	24.54	24.89	25.23	37.10
48	Count	9.71± 0.29[a]	6.56± 0.29[b]	6.51± 0.29[b]	4.89± 0.29[c]	4.65± 0.29[c]
	R. count	–	3.15	3.20	4.82	5.06
	R. %	–	32.44	32.96	49.63	52.11

R.: reduction, CG: control group without any treatment, G1: group submerged in 25% PPE solution, G2: group was submerged in 50% PPE solution, G3: group was submerged in 75% PPE solution, G4: group was submerged in 100% PPE solution.[a–d] Values within the same raw have different superscript letters that are significantly different at $p < 0.05$. Values are expressed as mean± standard error (SE) of three determinations.

Table 7. Effect of PPE marination on organoleptic properties of beef steak.

Treatment	Sensory criteria					
	Appearance (5)	Odor (5)	Texture (5)	Flavor (5)	Overall (20)*	Grade
CG	4.0	3.0	4.0	4.0	17.0[c]	Good
G1	4.6	4.7	4.2	4.5	18.0[b]	Very good
G2	4.5	5.0	4.4	5.0	18.9[b]	Very good
G3	5.0	5.0	5.0	5.0	20.0[a]	Excellent
G4	4.6	3.7	4.8	4.7	17.8[c]	Good

*>5 spoiled, 5–10 poor, 10–15 middle, 15–18 good, 18–19 very good, and 20 excellent. CG: the control group only got a microorganism inoculation; G1: group submerged in 25% PPE solution, G2: group was submerged in 50% PPE solution, G3: group was submerged in 75% PPE solution, G4: group was submerged in 100% PPE solution.[a–d] Values within the same raw have different superscript letters that are significantly different at $p < 0.05$. Values are expressed as mean± standard error (SE) of three determinations.

quantitative microbiological indicator of cleanliness of production process, safety assessment, and raw meat deterioration (Younes *et al.*, 2019). The current research found that there was an important variant ($p < 0.05$) in the total aerobic and psychrotrophic levels across the samples. Burger samples recorded the greatest TAC and psychrotrophic count, while basterma samples had the lowest count. According to the Egyptian Organization for Standardization and Quality (EOS) No. 1042/2005, 3492/2005, 1114/2005, and 1688/2005 for basterma, hot dog and frankfort, luncheon, and beef burger, respectively, TAC in the examined samples must not exceed 10^4 CFU/g. Consequently, in the current examination, certain products (such as burgers and hotdogs) did not comply with EOS and were unacceptable from a hygienic viewpoint and revealed lowly hygienic practices. Similar findings were verified by Shaltout *et al.* (2016), Ragab *et al.* (2022), and Nady *et al.* (2024), but the counts were lower than those identified by Hamed *et al.* (2015) and Younes *et al.* (2019). The several contaminated raw materials and components utilized in beef products, as well as the processing techniques, most likely contribute to the greater microbial load in them (Elbarbary *et al.*, 2024).

Because beef products are present in a variety of habitats, have basic nutritional needs, and can withstand a variety of temperatures, *P. aeruginosa* can readily pollute and spoil them; moreover, consuming contaminated meat products can transmit the bacterium to humans and cause gastrointestinal infections (Poursina *et al.*, 2023). The attendance of *P. aeruginosa* in the samples under examination is noteworthy since this bacterium is

frequently used as a food quality indicator and can result in food-borne illnesses (Shahrokhi *et al.*, 2022). The current outcomes indicated that 32.7% of the samples under examination contained *P. aeruginosa* (Table 3), and along with the findings, burger and hotdog samples recorded the greatest prevalence (46.7% and 36.7%) and had higher mean counts (4.91 ± 1.3 and 3.55 ± 1.4 log CFU/g, respectively). The significant spreading of *P. aeruginosa* in analyzed products may be attributable to high *P. aeruginosa* adaptability to a dissimilar habitat, room temperatures (4–42°C), and minor water activity (72%–97%) (Sofy *et al.,* 2017); consequently, it is critical to reflect this bacterium in frozen beef samples. Moreover, Rezaloo *et al.* (2022) and Sheir *et al.* (2020) confirmed that *P. aeruginosa* was identified in 7.83% and 4% of frozen meat product samples, respectively. Furthermore, Khairy *et al.* (2023) documented higher results. According to Salem *et al.* (2018), the capability of these spoilage microbes to live under cold temperatures could provide challenges during the preservation of foods, and the presence of *P. aeruginosa* is of pronounced importance as the microbe is regarded as a pathogenic microbe for humans and as an indication of food assessment (Wang *et al.,* 2017). Given the bacterium's psychrophilic nature, the frozen products presumably contained increased bacterial contamination. The use of inferior meat or its extended storage in hazardous conditions during customs procedures could be the cause of the high bacterial count of the examined products. Also, the presence of *P. aeruginosa* in these samples could be because of the incorrect use of the heat chain and cooking duration, as well as pollution of meat samples after production (Rezaloo *et al.,* 2022).

The molecular detection of *P. aeruginosa* is critical for overcoming the restrictions of traditional tests. Moreover, the rapid, dependable, and precise recognition of *P. aeruginosa* is facilitated by the amplification of species-specific primers, such as the *16S* rRNA gene (Algammal *et al.,* 2020). Using particular primers for *P. aeruginosa* (*16S* rRNA) at 150 bp, PCR revealed the appearance of *P. aeruginosa* DNA in entire isolates detected by standard techniques in the existing study (Figure 1). *P. aeruginosa* produces a variation of cellular and extracellular virulence features that contribute to the development of infection (Habeeb *et al.,* 2012). Owing to these considerations, this study was designed to apply PCR to detect the virulence factors in *P. aeruginosa* isolates. Concerning the outcomes of the virulence features, it was revealed that the *pilB* (Figure 2), *PslA* (Figure 3), *toxR* (Figure 4), and *exoS* (Figure 5) genes were identified in 14.3%, 18.4%, 14.3%, and 10.3% of *P. aeruginosa* isolates, respectively.

The categories of samples and the distribution of virulence characteristics were revealed to be notably different ($p < 0.05$) through data analysis. Mostly, these genes are in charge of the attachment and penetration of microbes into host cells. Furthermore, the *pilB* gene is accountable for pili formation and additional motility, while the *exoS* is in charge of tissue damage and bacterial spreading (Rezaloo *et al*., 2022). Accordingly, eating food that contains virulent strains of *P. aeruginosa* can result in a serious foodborne illness (Rezaloo *et al*., 2022). These outcomes accentuate the public health influence of *P. aeruginosa*. Recently, the studies by Abdelmotilib and Elbarbary (2023), Algammal *et al*. (2022), Benie *et al.* (2017), and Rezaloo *et al*. (2022) reported essentially similar findings of detected virulence genes. Furthermore, Nikbin *et al.* (2012) have observed that *P. aeruginosa* has a variation of virulence genes, which contributes to its variable virulence and pathogenesis.

Antibiotic resistance is a serious problem that the world is now dealing with, mainly in developing nations. Consequently, it is imperative to promptly and accurately recognize *P. aeruginosa* and identify the outline of susceptibility to avoid the superfluous use of antibiotics, which could lead to the emergence of drug-resistant bacteria (Hamisi *et al.,* 2012). *P. aeruginosa* was not only abundant in the samples tested, but it also showed remarkable resistance to the majority of the antibiotics tested, including tetracycline, ampicillin, penicillin, tetracycline, gentamicin, sulfamethoxazole, and cefoxitin. The susceptibility of *P. aeruginosa* strains to various antibiotics shows statistically a notable variation ($p < 0.05$). Likewise, a pronounced occurrence of resistance to such antibacterials was informed by Abdelmotilib and Elbarbary (2023) and Algammal *et al*. (2022) in Egypt, Meng *et al.* (2020) in China, Khan *et al.* (2020) in Australia, and Rezaloo *et al*. (2022) in Iran. Antibiotic resistance in bacteria most likely results from irregular and illogical antibiotic treatment in veterinary medicine.

Furthermore, it may be spread by plasmids and transposons, and strains of *P. aeruginosa* may acquire resistance mechanisms and produce hydrolytic enzymes as a result of this multiresistance (Rostamzadeh *et al.,* 2016). High MAR (0.511) in the current investigation could be the result of the common, unregulated application of antibacterials in the disease therapy (Qenawy *et al.,* 2024), increasing the possibility for resistance factors found on plasmids to be exchanged between ecological isolates using horizontal gene transmission. Similarly, previous studies (Algammal *et al.,* 2022; Makharita *et al.,* 2020; Tartor and Elnaenaeey, 2016) found that *P. aeruginosa* isolates exhibited a high MAR index. Variations in geographic distribution, sample supply, and technique used could help explain the variation in the MAR index. Higher MAR in the present study indicated that the *P. aeruginosa* isolates were from a high-risk source; consequently, antibiotic resistance research is necessary. Examining the medicine resistance patterns is therefore essential to prevent the spread of antibacterial-resistant

microbes and to find more sensible substitutes (Qenawy *et al.,* 2024).

Food contamination is a big concern because of health issues posed by the contaminants contained in daily consumables (Rather *et al.*, 2017). The World Health Organization (WHO) calculated that, each year, eating unhealthy food results in the loss of 33 million individuals worldwide (WHO, 2022). Food safety eventually applies to the consumption stage, as the existence and severity of the risks posed by foods at that point are of primary importance. Given that hazards to food safety might emerge at any point along the chain, strict control procedures must be followed across the entire food chain. Ensuring food safety is a shared duty of all partners in the food chain. Successful food safety improvements will enable increased productivity and more secure livelihoods, as well as render better human health. The need to find chemical-free methods to extend the shelf life of meat products has prompted experts to seek out novel approaches. Recently, there has been a lot of interest in using natural alternatives to keep meat fresh longer and store it for longer. This is because they have therapeutic properties because of their chemical component of active elements like phenolic and organic acids (Al-Hadidy *et al.,* 2023). Because of its antimicrobial characteristics, organic acid treatments, like PP, are frequently applied as ingredients and preservatives to lengthen the shelf life of fresh products (Wang *et al.,* 2015). Furthermore, PP is a naturally preserved component that possesses potent antimicrobial and antioxidant effects (Rasuli *et al.,* 2021). PP is the most useful portion, as likened to the seeds, leaves, and flowers, because of its high concentration of bioactive elements such as polyphenolic and flavonoid chemicals, which contribute to the antimicrobial and antioxidant index (El-Hadary and Taha, 2020).

Flavonoids, a significant component of PP, have an antibacterial mechanism that involves blocking nucleic acid creation, disrupting cytoplasmic membrane functions, influencing energy metabolism, and affecting biofilm formation and membrane penetrability. According to Sattar *et al.* (2024), flavonols and phenolic acids in particular have significant antibacterial properties that inhibit microbial virulence agents, including toxins and enzymes, and increase the efficacy of antibiotics. Many studies have been conducted on the application of natural antioxidants to increase beef products' storage stability (Akuru *et al.,* 2020). One common method for reducing bacterial contamination and extending food's shelf life is marinating it with natural ingredients (Rasuli *et al.,* 2021). The current investigation successfully reduced *P. aeruginosa* counts after 48 h of dipping in various concentrations of PPE.

In addition, the reduction of *P. aeruginosa* increases with exposure time. Furthermore, the samples did not exhibit any significant variations at the outset of the treatments, except for the 100% PPE solution; however, this variation increased substantially over time ($p < 0.05$). PP has lately revealed that a substantial level of tannins, such as punicalagin, could be a main component involved in their antibacterial action, which may damage bacterial cellular membranes and lead to bacterial mortality. Nonetheless, many investigations have shown that there is a significant ($p < 0.05$) correlation between PPE level and preservation time (El-Hadary and Taha, 2020; Hayrapetyan *et al.,* 2012; Ibrahim *et al.,* 2018; Rasuli *et al.,* 2021; Qenawy *et al.,* 2024). The sensory assessment of beef steak produced with various ratios of PPE during cold preservation revealed that 75% PPE had the greatest sensory criteria, which differed considerably from the control and other treated groups. Previous studies supporting the conclusions also abound (Abdelmotilib and Elbarbary, 2023; Rasuli *et al.,* 2021; Qenawy *et al.,* 2024). Given the variations among the panel members for every assessment, we hypothesize that variations in average overall scores between assessments could potentially be explained by personal score disparities. Furthermore, the notable variations in the samples that the assessors examined following each storage period might have had a substantial impact on their evaluations. Still, the research had some restrictions; the scope was limited to in vitro circumstances, which may not be replicated in vivo settings. Therefore, upcoming studies should include in vivo experiments to confirm these findings. In addition, more research is necessary to determine the precise processes by which these extracts, primarily in combination treatments with other natural extracts, produce their antibacterial properties.

Conclusion

This investigation demonstrated that a diverse array of beef products are contaminated with antibiotic-resistant *P. aeruginosa*, thereby increasing the health hazard associated with food degradation and foodborne illness. Consequently, it is essential to establish more stringent sanitation policies at all stages of the processing and storage procedure. Furthermore, the marinating process with polyphenolic compounds—such as PPE—can reduce the virulence capacity of *P. aeruginosa*, enhance sensory qualities, extend the shelf life, and be a safe approach to beef product preservation without compromising the organoleptic qualities. Further research is needed to find additional characteristics of *P. aeruginosa* in beef products, look at other natural antibacterial agents, and ascertain the ideal marinating conditions for the best product shelf life.

Ethics Statement

The procedures employed for this investigation were examined and authorized by the Aswan University

Faculty of Veterinary Medicine's Scientific Research Committee and Bioethics Board (01/2024).

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Data Availability Statement

The entire data are offered in the publication.

Authors Contributions

Sohaila El-Hawary, Nady Elbarbary, and Maha Abdelhaseib performed conceptualization, data curation, validation, and methodology. Neveen Abdelmotilib did formal analysis. Ali Ahmed, Mounir Bekhit, and Ehab Taha were responsible for investigation, visualization, and supervision. Nady Elbarbary, Neveen Abdelmotilib, and Mohamed Salem were concerned with writing the original draft, revising and editing the paper. The authors approved the manuscript for publication.

Conflicts of Interest

No conflicts of interest among the authors.

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