

Prevalence and Characterization of Antibiotic Resistant *Pseudomonas* spp. in Poultry Meat from Different Districts of Karachi

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ABSTRACT

Food-borne pathogens pose a serious concern in relation to life threatening infections to global health, also lead to great impact on economy. Food producing animals being especially vulnerable to bacterial contamination due to the high protein content, poultry meat is no exception in this regard. *Pseudomonas* spp. is known to cause spoilage and foodborne illnesses with rising antibiotic resistance. Antimicrobials like essential oils and fixed oils have gained attention as natural alternatives. A total of 150 raw chicken breast samples from different local markets of 6 districts of Karachi were screened for *Pseudomonas* spp. via cultural and morphological appearance. Antibigram profiling and the antimicrobial activities of selected essential oils and fixed oils were examined using disc diffusion and agar well diffusion methods. The virulence potential of the isolates was further assessed by determining biofilm formation and hemolysin production assays. Twelve isolates (8%) were found positive for *Pseudomonas* spp. Virulence traits varied among isolates; 2 were biofilm formation, while haemolytic activity was observed in 3. Both biofilm producers exhibited multidrug resistance. Antibiotic susceptibility testing revealed that amikacin and ciprofloxacin showed comparatively higher activity. *Cinnamomum* demonstrated the strongest antibacterial activity, with inhibition zones ranging from 25–38 mm. Among fixed oil *Centratherum antihelminthicum* (CA) fractions, the chloroform fraction showed greater antimicrobial activity than hexane fraction. Overall, this *in vitro* study integrates antibiogram profiling, virulence characterization, and plant-based antimicrobial screening to provide region-specific, preliminary evidence suggesting the potential of essential and fixed oils against multidrug-resistant *Pseudomonas* isolates from poultry meat. Further studies are required to evaluate their applicability under food system conditions.

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Pseudomonas, Essential oil, Fixed oil, Antibiotic resistance, Poultry meat

INTRODUCTION

Food-borne pathogens have favorably led to posing a significant threat to public health and food security globally, with poultry meat recognized as one of the considerable sources of protein. Chicken (*Gallus gallus domesticus*) is considered the most vulnerable food commodities to microbial contamination in natura with a limited shelf life. Not only high protein content, but high nutrient and water content, favorable pH is also conducive

making an ideal medium for bacterial growth and causing spoilage (El-Wehedy *et al.*, 2025; Meshaal *et al.*, 2021). *Pseudomonas* spp. is one of those bacteria owing to their psychrotrophic nature, rapid growth under aerobic storage conditions, and strong proteolytic and lipolytic activities that significantly reduce product life and quality (Calhoun *et al.*, 2025). In addition to spoilage, several *Pseudomonas* species act as opportunistic pathogens, and their increasing antimicrobial resistance has further increased food safety concerns (Kačániová *et al.*, 2017). *Pseudomonas* species are isolated frequently from various foods and play a provital role in spoilage of food (Hanim *et al.*, 2025). This spoilage is mainly done through enzymatic degradation and metabolic activity. *Pseudomonas* spp. cause food spoilage primarily through the production of extracellular enzymes such as proteases and lipases (Snyder *et al.*, 2024). Each year, unsafe and harmful food causes 600 million cases of food-borne illnesses and 420,000 deaths worldwide (Prabuseenivasan *et al.*, 2006).

Pseudomonas is a genus of Gram-negative bacteria,

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ubiquitously present in the environment. Several species of this genus, collectively referred to as *Pseudomonas* spp., are opportunistic human pathogens. It is frequently recovered from raw/ poultry meat and is identified as one of the major players linked with meat spoilage. They are inherently resistant to various antimicrobial agents. Infections caused by *Pseudomonas* spp. can lead to severe clinical symptoms such as respiratory tract infections (pneumonia), UTIs, wound and burn infections and increase the risk of patient remission and mortality (Agarwal and Larson, 2018).

Microorganisms are sometimes enclosed within a structured matrix formed by prokaryotic organisms that have aggregated into a colony known as biofilms, which protect microorganisms (Mirghani *et al.*, 2022). Because of their role in many infectious diseases and their involvement in a range of infections, biofilms are extremely significant for public health. Bacterial biofilms serve as a source of cell dispersion in production processes, leading to contamination of final food products, economic losses, and posing a threat to human health (Tyfa *et al.*, 2025; Kavanaugh and Ribbeck, 2012).

Essential oils (EOs) have been recognized to contain diverse and complex volatile low molecular weight compounds such as aldehydes, terpenes, and phenols etc. which have been cited in the literature with potential antimicrobial activities (Stojković *et al.*, 2013; Nazzaro *et al.*, 2013; Sadgrove *et al.*, 2022). Plant derived natural products, particularly EOs can act as an effective inhibitor of pathogenic microflora in food. Since the Middle Ages, EOs have been used widely for the bactericidal, fungicidal, antiparasitic, virucidal, insecticidal, medicinal and cosmetic applications, especially nowadays in sanitary, cosmetics, agricultural, pharmaceutical, and food industries (Bakkali *et al.*, 2008). These oils have natural disinfecting properties such as they can help in relieving cold and flu symptoms (Chaves and Tadi, 2025). The flavonoid compounds within *cinnamomum* (cinnamon) essential oil serve a dual role; one is to preserve food and secondly it has a major role in bestowing antioxidant properties also endowing therapeutic benefits for metabolic disorders (Shu *et al.*, 2024). *Eucalyptus* (Gum trees) is extracted from leaves, fruits, buds and bark showing antibacterial, anti-inflammatory, antiseptic, antioxidant, anticancer activities and for this reason used in the treatment of respiratory diseases, common cold, influenza, and sinus congestion (Vecchio *et al.*, 2016; Oussalah *et al.*, 2007; Mehboob *et al.*, 2021). Moreover, *mentha* × *piperita* (Peppermint) oil has been extensively used for various diseases. However, its anesthetic properties remain unknown (Petluru *et al.*, 2024). *Syzygium aromaticum* (Clove) EO has been used as natural food preservative and colorant based on its

antibacterial and health promoting activities (Bai *et al.*, 2023). The strength of antimicrobial activity given by the EOs may influence their ability to penetrate through bacterial cell membranes and display the inhibitory activity on the different functional properties of the bacterial cell (Kačániová *et al.*, 2017).

Since past several years plants extracts have long been used in therapeutic medicine for the treatment of various diseases. Plant-derived compounds possess a well-documented history of clinical application and are generally associated with better patient tolerance and acceptance (Veeresham, 2012). Oils derived from plant and herbal sources, mainly fixed oils (Fos) from seeds gain remarkable interest due to their preventive and therapeutic actions, also they possess rich source of beneficial compounds with potential applications in different industries (Rahim *et al.*, 2023). FOs which are non-volatile oils (also called fats or lipids) that are typically extracted from seeds, nuts, or fruits. Unlike EOs (which are volatile and aromatic), FOs do not evaporate at room temperature and mainly consist of triglycerides of fatty acids. FOs have shown a strong tendency to change the action of antibiotics against various Gram-positive and Gram-negative isolates of standard and MDR bacteria (Dias *et al.*, 2022). *Centratherum antihelminthicum* (CA) commonly known as black cumin, a traditional medicine, has been used for centuries for curing common ailments such as fever, cough, and diarrhea, and it is also used as a general tonic (Amir and Chin, 2011; Bajpai *et al.*, 2007). Earlier, its phenolic compounds, antioxidant, and anti-hyperglycemic, antimicrobial activity have also been reported. EOs and FOs exert antimicrobial effects by disrupting bacterial cell membranes, increasing permeability, and causing leakage of cellular contents. Their bioactive compounds (e.g., phenolics, terpenes) interfere with enzyme activity, genetic material ultimately leading to cell death (Ambrosio *et al.*, 2020).

This study was designed to screen the prevalence of *Pseudomonas* spp. in poultry meat samples collected from different districts of Karachi and to evaluate the distribution of positive samples across various poultry retail shop types. Also, to characterize their antimicrobial resistance and virulence traits (biofilm formation and hemolysin production), and to evaluate the *in vitro* antimicrobial activity of selected essential oils and fixed oil fractions. Although number of studies have reported the antimicrobial activity of EOs against *Pseudomonas* spp., most investigations have focused either on clinical isolates or on a single class of natural products. This work is novel in that it provides a multifaceted investigation regarding the comparative evaluation of natural antimicrobials i.e. EOs and fixed oil fractions against poultry-derived

Pseudomonas isolates, while simultaneously augmenting their AST patterns. Furthermore, data on *Pseudomonas* contamination in raw chicken meat from different retail shops across several districts of Karachi are scarce, making this study one of the few region-specific findings addressing this gap. By integrating prevalence, antibiogram profiling and natural oil-based antimicrobial assessment within a single framework, this study offers a more holistic understanding of food-borne *Pseudomonas* risks and potential non-antibiotic control strategies. The objective of this study was to determine the prevalence of *Pseudomonas* spp. in poultry meat samples, assess their antimicrobial resistance and virulence traits, and evaluate the antimicrobial potential of selected essential and fixed oils.

MATERIALS AND METHODS

Sample collection

A cross-sectional study was employed in which 150 raw chicken breast samples were collected for a stratified sampling design. Total six districts of Karachi were selected i.e. Karachi Central, South, East, West, Korangi and Malir). 25 samples from each district, including open poultry stalls, neighborhood butcher shops, frozen poultry wholesale markets, supermarkets, and branded chicken retail shops equally stratified by retail source (n = 5 per shop).

The sampling and the pre-enrichment protocols were done in accordance with ISO 6579 standard guidelines. Samples were placed in sterile zip-lock bags after collection and transported to the laboratory in insulated iceboxes maintained at approximately 0-8 °C to ensure sample integrity during transit. Within one hour, the samples were transported and processed immediately upon arrival.

Identification and isolation of *Pseudomonas* spp.

Each 25 g chicken sample was minced and homogenized in 225 mL of buffered peptone water (BPW) (w/v). 0.1 mL of the labelled homogenate sample was plated onto cetrimide agar (Peptone: 20 g, Magnesium chloride: 1.4 g, Potassium sulfate: 10 g, Cetrimide: 0.3 g, Agar 13.6 g, distilled water 1000 ml) and incubated overnight at 37 °C. In parallel, a positive control; *Pseudomonas* spp., (ATCC 27853) was run. Those isolates which appeared as rod-shaped colonies on an agar media, displaying off-white, pinkish, or bright green pigmentation were also further confirmed as *Pseudomonas* by different biochemical tests.

Antibiotic susceptibility testing (AST)

AST of confirmed *Pseudomonas* isolates was

assessed following CLSI guidelines by using disk diffusion method (CLSI, 2017). Revived cultures matched with 0.5 McFarland index (0.5 mL of 1.175% barium chloride (BaCl₂) with 99.5 mL of 1% sulfuric acid (H₂SO₄)), were lawned over Mueller–Hinton agar (MHA) (Beef extract 2 g, Acid hydrolysate of casein 17.5 g, Starch 1.5 g, Agar 17 g). Antibiotic discs; amikacin (30 µg), piperacillin (100 µg), cefepime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and gentamicin (10 µg), were applied aseptically on media plates with the help of sterile forceps. Plates were incubated at 37°C for approximately 24 h. The diameters of inhibition zones were measured in millimeters, including the disc, and interpreted as susceptible, intermediate, or resistant based on CLSI guidelines (CLSI, 2017). Data were presented as mean±SD of three independent experiments performed in triplicate. Quality control was ensured using *P. aeruginosa* ATCC 27853 under the same conditions.

Screening of virulence factors

Biofilm formation

Each isolated culture was inoculated in all labelled test tubes (P1 – P12) filled with 10 mL of enriched broth i.e. heart infusion broth (calf brain infusion 200 g, Beef heart infusion 250 g, proteose peptone 10 g, NaCl 5 g). Blank heart infusion (HI) broth tube was also run as a negative control. The tubes were incubated for 24 h at 37 °C. After incubation, the broth was aspirated and the tubes were washed thrice with PBS i.e. phosphate buffer saline. 0.1% crystal violet (CV) solution was used for the staining of washed tubes for 15 min. Subsequently after staining, the dye CV was decanted, and the tubes were placed in an upright position and were allowed to air dry. The cultures observed with visibility of the ring at solid-liquid interface along the circumference of the tube were considered as biofilm producers (BPs) whereas appearance of no ring indicated non-biofilm producers (NBPs).

Hemolysin activity

Nutrient agar (peptone 5 g, beef extract 3 g, NaCl 5 g, agar 15 g) plates supplemented with blood (5%) were used to evaluate the hemolytic ability of isolates. These plates were streaked with *Pseudomonas* isolates and incubated overnight at 37 °C. After 24 h, the plates were observed for hemolysis and stratified as β (complete), α (partial) and γ (no zone) of hemolysis.

Screening antimicrobial activity

The antimicrobial activity of EOs and FOs against *Pseudomonas* spp. was evaluated via agar well diffusion method.

Antimicrobial activity of EOs

The antimicrobial activity of four EOs *Cinnamomum* (Cinnamon), *Eucalyptus* (gum trees), *Syzygium aromaticum* (clove) and *Mentha × piperita* (peppermint) were studied along with control gentamicin. Briefly, a loopful of the bacterial suspension was uniformly spread over MHA plates and allowed to rest at room temperature for 10–15 min to facilitate surface absorption. Using a sterile cork borer, 6 mm wells were created in the agar, and each well was labeled and loaded with 20 µL of a specific essential oil along with 20 µL of 5% DMSO which was used as a negative control. After permitting diffusion at room temperature for one hour, the plates were incubated upright at 37°C for 24 h. Post-incubation, the zones of inhibition surrounding each well were measured in millimeters and recorded to evaluate antimicrobial efficacy. Results were reported as the mean zone of inhibition (ZOI) from three independent replicates.

Antimicrobial activity of FOs

The fixed oil from seeds of *CA* was obtained via cold-press extraction at temperatures below 50 °C to preserve its native phytochemical profile. Cold-press extraction involves mechanical pressing of seeds at low temperatures (<50°C) without chemical solvents. This preserves heat-sensitive bioactive compounds such as phenolics and fatty acids, maintaining the natural antimicrobial properties of the oil.

Sequentially, the crude oil was fractionated: firstly, defatted with hexane to isolate the hexane-soluble fraction (HF), and residual matter was extracted with chloroform to yield the chloroform-soluble fraction (CF) (Baig *et al.*, 2022). Bacterial suspensions were spread uniformly onto MHA plates by sterile cotton swabs. Wells (each 6 mm) were formed by a sterile borer, labeled and filled with 20 µL of the undiluted FO. All experiments were performed in triplicate, and the mean ±SD was calculated.

Statistical analysis

Descriptive statistics were used to analyze data. Chi-square test was used to determine the associations between *Pseudomonas* positivity and districts, or shop types were assessed, with a $p < 0.05$ considered statistically significant. ZOIs among different EOs compared to the control were evaluated using the Kruskal-Wallis's test, followed by post hoc test i.e. Dunn's multiple comparison. The difference in antimicrobial activity between the HF and CF was assessed using the Wilcoxon signed-rank test (non-parametric paired t-test). All statistical analyses were done using GraphPad Prism version.9 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Colonial characteristics of *Pseudomonas* isolates

Out of 150 chicken meat samples (labeled 1–150), 12 samples yielding 8% of overall prevalence identified as *Pseudomonas* spp. District-wise analysis showed that *Pseudomonas*-positive samples were detected in Karachi Central (16.0%), Karachi East (8.0%), Karachi West (8.0%), Karachi South (8.0%), and Malir (8.0%), whereas no *Pseudomonas*-positive sample was detected from Korangi district (Table I).

Variation in prevalence was observed among different shop types. The factors include differences in hygiene practices, storage temperature and duration, environmental contamination. The highest prevalence of *Pseudomonas* was recorded in branded poultry retail shops (13.3%), followed by neighborhood butcher shops and frozen poultry outlets/wholesale (10.0% each). Wet markets/open poultry stalls showed a lower prevalence (6.7%), while no *Pseudomonas* was detected in samples obtained from supermarkets/fresh meat counters (Table I).

Table I. Distribution of *Pseudomonas* positive poultry samples across districts and shop types in Karachi.

District/ Shop type	Wet market / Open stall n (%)	Neighbourhood butcher n (%)	Supermarket/ fresh meat counter n (%)	Frozen poultry/ Wholesale n (%)	Branded poultry retail n (%)	Total positive/ 25 (%)
Karachi Central	1 (20%)	1 (20%)	0 (0%)	0 (0%)	2 (40%)	4 / 25 (16.0%)
Karachi East	0 (0%)	0 (0%)	0 (0%)	1 (20%)	1 (20%)	2 / 25 (8.0%)
Karachi West	0 (0%)	1 (20%)	0 (0%)	1 (20%)	0 (0%)	2 / 25 (8.0%)
Karachi South	0 (0%)	1 (20%)	0 (0%)	1 (20%)	0 (0%)	2 / 25 (8.0%)
Malir	1 (20%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	2 / 25 (8.0%)
Korangi	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 / 25 (0.0%)
Overall/ Prevalence	2 / 30 (6.7%)	3 / 30 (10.0%)	0 / 30 (0%)	3 / 30 (10.0%)	4 / 30 (13.3%)	12 / 150 (8.0%)

Six districts, i.e. Karachi Central, Karachi South, Karachi East, Karachi West, Korangi and Malir were selected and 25 samples were collected from each district. The collection points include open poultry stalls, neighborhood butcher shops, frozen poultry wholesale markets, supermarkets, and branded chicken retail shops, equally stratified by retail source (n = 5 per shop).

Table II. Antibiotic susceptibility test results of isolated *Pseudomonas* isolates (n = 12) showing zone of inhibition (mean $\pm$ SD, mm).

Isolate	Amikacin	Piperacillin	Cefepime	Ciprofloxacin	Levofloxacin	Gentamicin
P1	18 $\pm$ 0.08	0	12 $\pm$ 0.05	18 $\pm$ 0.08	0	14 $\pm$ 0.08
P2	20 $\pm$ 0.05	22 $\pm$ 0.08	14 $\pm$ 0.08	17 $\pm$ 0.09	15 $\pm$ 0.08	15 $\pm$ 0.08
P3	13 $\pm$ 0.05	18 $\pm$ 0.05	15 $\pm$ 0.08	22 $\pm$ 0.08	12 $\pm$ 0.08	24 $\pm$ 0.05
P4	20 $\pm$ 0.08	23 $\pm$ 0.05	11 $\pm$ 0.05	23 $\pm$ 0.08	18 $\pm$ 0.05	45 $\pm$ 0.08
P5	0	11 $\pm$ 0.08	10 $\pm$ 0.05	13 $\pm$ 0.08	0	14 $\pm$ 0.08
P6	20 $\pm$ 0.05	23 $\pm$ 0.08	0	23 $\pm$ 0.05	0	14 $\pm$ 0.08
P7	21 $\pm$ 0.05	21 $\pm$ 0.08	0	22 $\pm$ 0.08	26 $\pm$ 0.08	32 $\pm$ 0.05
P8	19 $\pm$ 0.08	22 $\pm$ 0.05	15 $\pm$ 0.08	24 $\pm$ 0.08	0	37 $\pm$ 0.05
P9	0	13 $\pm$ 0.08	0	19 $\pm$ 0.05	0	25 $\pm$ 0.08
P10	0	11 $\pm$ 0.05	0	18 $\pm$ 0.05	0	12 $\pm$ 0.05
P11	0	0	0	17 $\pm$ 0.08	9 $\pm$ 0.05	19 $\pm$ 0.08
P12	19 $\pm$ 0.08	24 $\pm$ 0.08	0	24 $\pm$ 0.05	0	40 $\pm$ 0.05

P1-P12 represent different poultry isolates tested against antibiotics given in table. Zero (0) indicates no zone of inhibition i.e. resistance.

The distribution of area-wise sampling across different districts of Karachi and retail shop types is presented in [Supplementary Table SI](#). Twelve samples (Sample IDs: 3, 8, 21, 25, 43, 50, 56, 72, 84, 93, 101, and 125) showed growth on cetrimide agar. The microscopic examination i.e. gram staining of *Pseudomonas* isolates showed gram-negative, rod-shaped bacteria ([Al-Bayati et al., 2021](#)) ([Supplementary Table SII](#)). Based on cultural characteristics, the isolates appeared as pink rods, with some off-white and bright green-colored colonies on cetrimide agar plate. For the sake of convenience, these isolates were labeled as P1–P12 corresponding to original sample IDs: P1 (5), P2 (8), P3 (21), P4 (25), P5 (43), P6 (50), P7 (56), P8 (72), P9 (84), P10 (93), P11 (101), and P12 (125). The *Pseudomonas* isolates (P1–P12) were further confirmed as *Pseudomonas* spp. by the biochemical analysis ([Supplementary Table SIII](#)). All numerical values, percentages, and isolate codes reported in the text correspond directly to the data presented in [Tables I–IV](#). These findings were compared with a positive control *P. aeruginosa* ATCC 27853.

Despite differences in prevalence across shop types and districts, the Chi-square test revealed no statistically significant association between *Pseudomonas* positivity and shop type or district ($\chi^2 = 4.87$, $df = 20$, $p > 0.05$).

Antimicrobial susceptibility testing (AST)

The antibiotic susceptibility patterns of *Pseudomonas* isolates are summarized in ([Table II](#)). Amikacin and gentamicin demonstrated relatively higher antibacterial activity, with several isolates exhibiting susceptible

responses. Piperacillin susceptibility was observed in a subset of isolates (6), whereas resistance or intermediate susceptibility was detected in others i.e. 4 and 2 isolates respectively. Notably, most of the isolates (9) categorized as resistant against cefepime based on CLSI criteria, indicating widespread resistance to fourth-generation cephalosporins.

Among fluoroquinolones, ciprofloxacin exhibited higher effectiveness compared to levofloxacin, with 7 isolates remaining susceptible. However, relatively less activity was demonstrated by levofloxacin exhibiting 6 isolates resistant ([Table III](#)).

Table III. Antibiogram Result of *Pseudomonas* Isolates (n=12).

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Amikacin	7 (58.3)	0 (0)	5 (41.7)
Piperacillin	6 (50.0)	2 (16.7)	4 (33.3)
Cefepime	0 (0)	3 (25.0)	9 (75.0)
Ciprofloxacin	7 (58.3)	5 (41.7)	0 (0)
Levofloxacin	2 (16.7)	4 (33.3)	6 (50.0)
Gentamicin	6 (50.0)	6 (50.0)	0 (0)

N, number of isolates; %, percentage of total isolates tested.

In a nutshell, the susceptibility patterns revealed that half of the *Pseudomonas* isolates showed multidrug-resistant *Pseudomonas* isolates (resistance to ≥ 3 antimicrobial classes) ([Table IV](#)).

Table IV. Distribution of *Pseudomonas* isolates according to resistance to number of different antimicrobial drug classes i.e. aminoglycosides, penicillin, cephalosporins, fluoroquinolones.

Resistance profile	Number of isolates (n = 12)	Percentage
Resistant to none of the classes	0	0
Resistant to one class	2	16.7
Resistant to two classes	4	33.3
Resistant to three or more classes (MDR)	6	50.0

MDR= Multidrug resistant isolates

Screening of virulence factors

Biofilm formation was detected in 16.67% (n= 2) of the isolates, specifically P5 and P10. Hemolysin production was observed in 33.33% (n= 4) of isolates, with β -hemolysis detected in P4, P5, and P10, and α -hemolysis in P8 (Table V).

Table V. Virulence factors production by *Pseudomonas* isolates.

<i>Pseudomonas</i> samples % (n)	Hemolysis % (n)			Biofilm formation % (n)
	α	β	γ	
100% (12)	8.33% (1)	25% (3)	66.67% (8)	16.67% (2)

N, number of samples

Notably, both the isolates i.e. P5 and P10 exhibited the coexistence of biofilm production and β -hemolytic activity also been observed to have the highest levels of multidrug resistance exhibited resistance to four to five antibiotics. In contrast, other isolates showed comparatively lower resistance levels. This indicates an association between biofilm formation and enhanced antimicrobial resistance in the isolated studies.

Antimicrobial activity of EOs and FOs

The antibacterial activity of four Eos-Cinnamomum (cinnamon), Syzygium aromaticum (clove), Eucalyptus, and Mentha $\times$ piperita (peppermint) against the *Pseudomonas* isolates, measured as zones of inhibition (ZOI). Variation in effectiveness was observed in the inhibition pattern of EOs (Owlia *et al.*, 2009). Cinnamon EO demonstrated the strongest antibacterial effect, producing comparatively larger inhibition zones across most isolates, with several showing statistically significant inhibition ($p < 0.05$ to $p < 0.001$) relative to gentamicin. Eucalyptus and clove EOs also showed notable activity, peppermint EO exhibited moderate and isolate-dependent

antibacterial effects (Fig. 1). Significant differences between essential oil treated isolates and the gentamicin control were revealed, confirming varied antibacterial activity among the EOs tested.

CF exhibited antibacterial efficacy against all isolates, inhibition zones ranging from 18.6 ± 0.47 mm to 30.6 ± 0.47 mm in diameter, with a mean ZOI of 24.63 mm (Table VI). Compared to this, HF showed less activity, with no inhibition against P1 and P2 isolates, and smaller ZOIs against the remaining *Pseudomonas* isolates (mean ZOI 16.33 mm). Statistically significant difference between the CF and HF ($p = 0.0036$) indicated the higher antibacterial activity of the CF.

Table VI. Inhibition zones of *Centratherum antihelminthicum* hexane and chloroform fractions against *Pseudomonas* isolates.

Isolates	Hexane fraction	Chloroform fraction	P-value
P1	ND	25.6 $\pm$ 0.47	0.0036
P2	ND	20.3 $\pm$ 0.47	
P3	15.6 $\pm$ 0.47	18.6 $\pm$ 0.47	0.0036
P4	18.3 $\pm$ 0.47	19.3 $\pm$ 0.47	
P5	22.3 $\pm$ 0.47	30.6 $\pm$ 0.47	
P6	26.3 $\pm$ 0.47	28.7 $\pm$ 0.47	
P7	19.6 $\pm$ 0.47	28.6 $\pm$ 0.47	
P8	18.3 $\pm$ 0.47	20.3 $\pm$ 0.47	
P9	14 $\pm$ 1.41	19.3 $\pm$ 0.47	
P10	16.6 $\pm$ 0.47	30.6 $\pm$ 0.47	
P11	24.3 $\pm$ 0.47	29.3 $\pm$ 0.94	
P12	20.6 $\pm$ 0.47	24.3 $\pm$ 0.47	
Mean ZOI	16.33 mm	24.63 mm	

*ND, no activity detected.

DISCUSSION

In recent years, Pakistan has experienced significant growth in poultry production, ranking as the 11th largest poultry-producing country worldwide (Okeke *et al.*, 1999). However, the advancement of hygienic raw food processing technologies has not kept pace with the rapid growth of poultry production. This issue is further exacerbated by the absence of an organized regulatory framework to control antimicrobial use within the poultry industry (Rahman *et al.*, 2020). It can be hypothesized that modern poultry production practices particularly the routine administration of antibiotics through feed and drinking water for disease prevention, control, and growth promotion have significantly contributed to the emergence and spread of AMR (Moreno *et al.*, 2008).

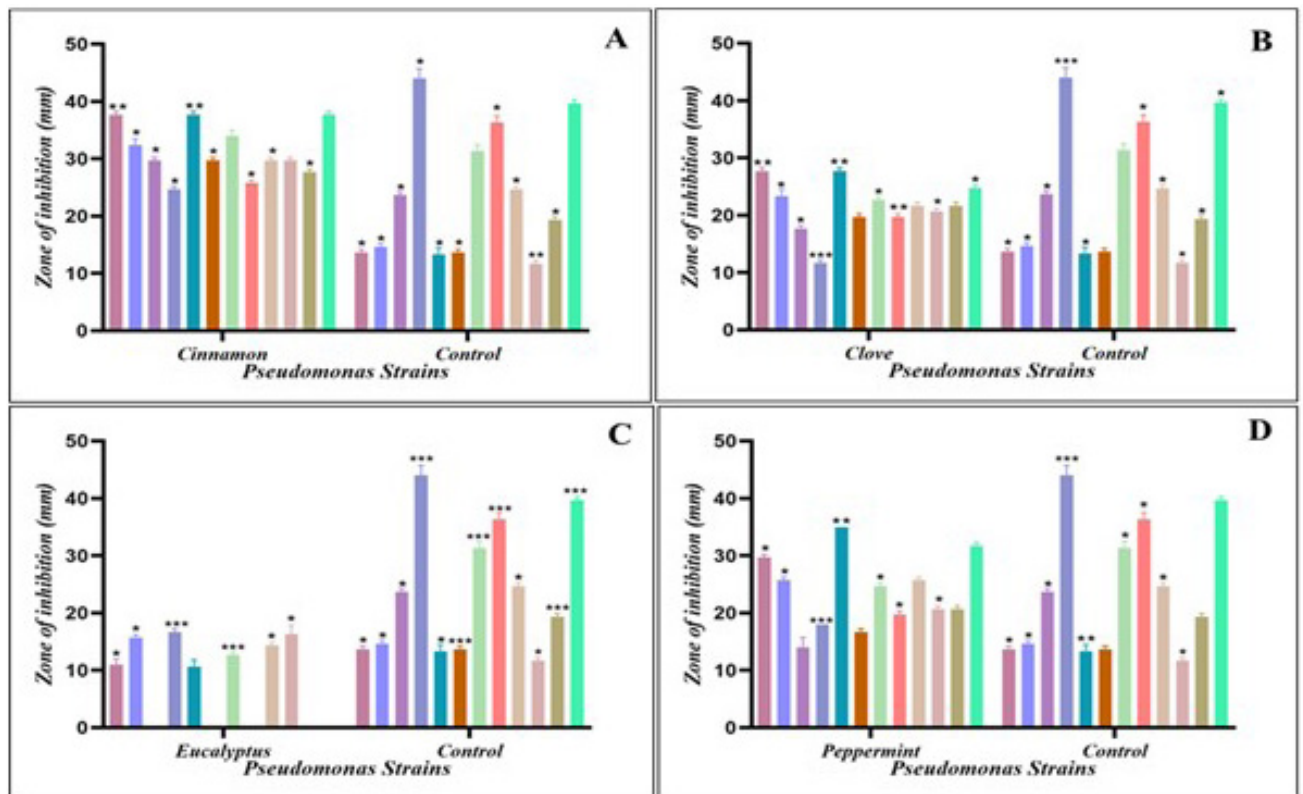


Fig. 1. Antibacterial activity of *Cinnamomum* (cinnamon) (a), *Syzygium aromaticum* (clove) (b), *Eucalyptus* (Gum trees) (c), and *Mentha X piperita* (peppermint) (d) E Os against isolated *Pseudomonas* isolates. Antibacterial activity of *Cinnamomum* (cinnamon) (a), *Syzygium aromaticum* (clove) (b), *Eucalyptus* (gum tree) (c), and *Mentha X piperita* (peppermint) (d) essential oils against *Pseudomonas* isolates with gentamicin used as the positive control. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test. Data are presented as mean $\pm$ SD. (* p < 0.05, ** p < 0.01, *** p < 0.001).

The low prevalence i.e. 8% (Table I) could be associated with effective temperature control, hygienic slaughtering, and improved storage practices, which are responsible for limiting the growth of psychotropic bacteria. Walid *et al.*, reported 12% of prevalence of *Pseudomonas* in chicken meat. Additionally, sampling from retail formats, including frozen and branded poultry outlets, may have reduced the overall contamination rate compared to studies focusing solely on wet markets. *Pseudomonas* spp. is commonly found in protein-rich and high-moisture foods such as raw poultry, meat, fish, milk, and dairy products, especially under refrigerated storage.

This low prevalence is in line with previous research done by Abdullah *et al.* (2024) reported 10% prevalence of *Pseudomonas* spp. in poultry environment. In contrast, research done in Egypt reported relatively higher prevalence i.e. 21.6% in chicken meat samples (Elbehiry *et al.*, 2022).

While not always directly associated with severe

disease in healthy individuals, *Pseudomonas* can pose significant risks in immunocompromised hosts and contributes substantially to food spoilage due to its metabolic versatility and ability to thrive under refrigeration conditions. The isolates observed in this study were consistent with classical *Pseudomonas* characteristics (Supplementary Table SII and SIII), both morphologically and biochemically, including their Gram-negative rod shape, motility, catalase and oxidase positivity, and in some cases, hemolytic activity further underscoring their pathogenic potential (Al-Bayati *et al.*, 2021).

The result of the study reveals a concerning level of AMR among poultry-associated *Pseudomonas* isolates against broad spectrum antibiotics. AST showed reduced susceptibility to cefepime which is increasingly being reported to food-associated *Pseudomonas* isolates (Rodrigues *et al.*, 2021). The higher resistance of fluoroquinolone among some isolates may be due to an extensive antibiotic usage in poultry production (Kousar

et al., 2021). These findings highlight the widespread nature of antibiotic resistance in chicken meat associated with *Pseudomonas* isolates. Also it underscores the need for alternative antimicrobial agents which are eco-friendly, such as the use of EOs and FOs to combat the MDR bacteria.

Pseudomonas spp. have been frequently studied, including both in spoilage-associated pathogens and their strong biofilm-forming ability (Calhoun *et al.*, 2025; Wang *et al.*, 2023). Virulence traits, including biofilm formation and hemolytic activity may enhance the pathogenic ability and AMR of bacterial isolates (Vani *et al.*, 2023). Isolates exhibiting both biofilm production and β -hemolysis (P5 and P10) showed markedly higher resistance (83.33% and 66.66% resistance, respectively) compared to isolate expressing hemolysis alone (P8) (resistance up to 16.66%) (Supplementary Table SIV). This suggests that biofilm formation plays a more important role in conferring antimicrobial tolerance while hemolysin production may contribute to tissue damage and pathogenicity. Similar findings have been reported where *Pseudomonas* isolates expressing multiple virulence determinants exhibited increased resistance and persistence against antibiotics (Hoiby *et al.*, 2010; Badr *et al.*, 2020). Unfortunately, due to the budget constraints we could not target other virulence genes like the extended spectrum beta-lactamases enzymes (ESBLs) which were also needed to be analyzed to screen their putative association in biofilm production in order to get more robust evidence-based conclusions. Additionally, biofilm formation was assessed using only the test tube method (qualitative) due to resource limitations, whereas a microplate reader-based assay (quantitative) would have provided more accurate results. Formation of biofilm food quality by promoting microbial persistence, increasing resistance to sanitizers, and causing spoilage through enzyme production, leading to slime formation, off-odors, and reduced shelf life.

The present study evaluated the antibacterial potential of selected EOs (Núñez-Núñez *et al.*, 2018; Ooi *et al.*, 2006) and plant-derived hexane and chloroform fractions against *Pseudomonas* isolates, using gentamicin (CN) as a positive control. Among the essential oils, cinnamon EO exhibited the highest activity, in some isolates overlapping with the ZOIs produced by gentamicin. This remarkable activity may be attributed to its composition especially cinnamaldehyde and related phenolic compounds that disrupt bacterial cell membranes (CM) and interfere with important metabolic pathways (Kim *et al.*, 2015; Elcocks *et al.*, 2020; Coşeriu *et al.*, 2023). Several studies have demonstrated that phytochemicals such as cinnamaldehyde, eugenol, and terpenes exhibit strong antibacterial activity against *Pseudomonas* spp. by disrupting cell membranes,

inhibiting quorum sensing, and reducing biofilm formation (Shariati *et al.*, 2024). Clove and eucalyptus EOs showed moderate activity, while peppermint EOs demonstrated comparatively weaker but still measurable inhibition.

The absence of activity for some isolates in the HF may indicate that non-polar constituents alone are insufficient to exert strong antibacterial effects (Khan *et al.*, 2024). This shows the increased potential of CFs for use in food preservative formulations. These findings align with Wijesinghe *et al.* (2021), who found that chloroform-extracted fractions caused damage to bacterial cell walls and biofilm matrices while maintaining low cytotoxicity to human cells. These fractions likely contain non-volatile, lipophilic compounds capable of integrating into the bacterial membrane, causing structural destabilization. Their demonstrated efficacy supports the idea that FO fractions could be utilized as safe and effective preservatives in food systems.

Collectively, these results highlight the potential of essential oils and plant-derived fractions as alternative or adjunct antimicrobial agents against *Pseudomonas* species. However, variability among isolates underscores the need for further studies focusing on chemical characterization of active constituents, determination of minimum inhibitory concentrations, and evaluation of synergistic effects with conventional antibiotics.

However, the interpretation of their antimicrobial activity could be more profound if the chemical composition of EOs and FOs (GC-MS profiling) was also reported in the study but due to budget constraints these were not done. Furthermore, the study could be improved by exploring the synergism between both the oils (clove and cinnamon) as it has been recently observed by de Almeida *et al.* (2023).

The fixed oil fractions, i.e. CFs and HF, also exhibited significant antimicrobial activity. CFs appeared to be more effective (Table VI), producing satisfactory ZOIs against the isolates compared to HF ($p < 0.05$). This shows potential of CFs for use in food preservative formulations. These results align with (Wijesinghe *et al.*, 2021), who found that chloroform-extracted fractions caused damage to bacterial cell walls and biofilm matrices while maintaining low cytotoxicity to human cells. These fractions likely contain non-volatile, lipophilic compounds capable of integrating into the bacterial membrane, causing structural destabilization. Their demonstrated efficacy supports the idea that fixed oil fractions could be utilized as safe and effective preservatives in food systems.

Recent research highlights that EOs are effective against both free-floating and biofilm-associated bacterial cells. They can limit bacterial adhesion, break down established biofilms, and influence the expression of

key virulence genes in *Pseudomonas* spp. These findings show that EOs work through multiple mechanisms at once, disrupting cell structures, communication systems, and genetic regulation of pathogenic traits. Moreover, EOs have demonstrated their potential as anti-microbial and anti-biofilm agents against MDR bacteria such as *Pseudomonas* spp, most often targets planktonic cells, motility, mature biofilms, and pathogenic factors such as pyocyanin production and quorum sensing (Maravić-Vlahoviček *et al.*, 2025).

Additionally, emerging research suggests that combining EOs with conventional antibiotics can result in synergistic effects. For example, Utcharyakiat *et al.* (2016) showed that *Cinnamomum* bark oil combined with antibiotics like ceftazidime, imipenem, and ciprofloxacin reduced the MICs required to inhibit multidrug resistant *Pseudomonas* isolates. This synergistic effect not only enhances the efficacy of existing antibiotics but also holds potential for reviving drugs whose effectiveness has been compromised by resistance. Such combinatory approaches can be particularly valuable in both therapeutic settings and food safety, where mitigating antibiotic-resistant bacteria is essential. From an applied perspective, the demonstrated antimicrobial activity of EOs and FOs against resistant *Pseudomonas* isolates indicates their potential incorporation into poultry decontamination strategies. Such applications could help extend shelf life, reduce spoilage, and minimize reliance on synthetic preservatives and antibiotics, aligning with global efforts toward safer and more sustainable food production systems.

Although the prevalence of *Pseudomonas* spp. in poultry was relatively low, its presence still poses a potential risk to consumers, particularly immunocompromised individuals. The findings highlight the need for improved hygiene practices in poultry processing and retail. Moreover, the antimicrobial activity demonstrated of the tested EOs suggests their potential application as natural preservatives or hygiene interventions in poultry production and processing. Future studies should explore optimized formulations and concentrations of these oils to reduce microbial contamination and enhance food safety.

Taken together, the findings of this study strongly support the role of natural compounds, particularly EOs and their fixed oil fractions as promising candidates in the fight against multidrug-resistant and biofilm-forming *Pseudomonas* spp. Their multifaceted mechanisms of action, relatively low toxicity, and broad-spectrum efficacy make them suitable for both clinical and food industry applications. This study was limited by the absence of molecular identification techniques, lack of GC-MS analysis of oils, and reliance on qualitative biofilm detection methods due to resource constraints.

Nevertheless, further studies are necessary to evaluate their safety *in vivo*, to optimize their formulations for stability and bioavailability, and to understand potential sensory impacts when used in food systems. Additionally, the standardization of extraction and characterization protocols for EOs remains a key challenge that must be addressed to ensure consistent antimicrobial performance. Future studies should focus on molecular characterization, detailed phytochemical analysis, evaluation of synergistic effects with antibiotics, and application of these oils in real food systems.

CONCLUSIONS

In conclusion, the data presented here underscores the need for alternative antimicrobial strategies due to the rising resistance of *Pseudomonas* spp. to conventional antibiotics. EOs, particularly *Cinnamomum* (Cinnamon) oil, along with fixed oil chloroform extracts, have demonstrated substantial potential in inhibiting both planktonic and biofilm-associated *Pseudomonas* isolates. Their ability to target bacterial membranes, virulence factors, and gene expression pathways highlights their promise as effective, natural antimicrobial agents suitable for both food safety interventions and possibly therapeutic applications in the era of escalating antimicrobial resistance.

The power of the presented study could be strengthened by exploring and identifying the isolates at molecular level. In addition, it is also important to not only screen/evaluate the prevalence pathogenic and commensal bacteria rather than evaluate the occurrence of antimicrobial resistance in other food commodities.

DECLARATIONS

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Ethical approval statement

Ethical approval was not required for this study as the samples were obtained from retail shops and did not involve human participants, animals, or identifiable personal data.

Supplementary material

There is supplementary material associated with

this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20260312070808>

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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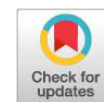
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Online First Article



Supplementary Material

Prevalence and Characterization of Antibiotic Resistant *Pseudomonas* spp. in Poultry Meat from Different Districts of Karachi

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Supplementary Table SI. Distribution of sample collection areas.

Sample_ID	Area	Stratum	Shop_Code	Sample_No. (District wise)
Karachi central				
KC-01-01-01	Liaquatabad	Wet market / open poultry stall	Positive	1
KC-02-01-02	Nazimabad	Wet market / open poultry stall		2
KC-03-01-03	North Nazimabad	Wet market / open poultry stall		3
KC-04-01-04	Federal B Area	Wet market / open poultry stall		4
KC-05-01-05	Karimabad	Wet market / open poultry stall		5
KC-06-02-01	Liaquatabad	Neighborhood butcher shop	Positive	6
KC-07-02-02	Nazimabad	Neighborhood butcher shop		7
KC-08-02-03	North Nazimabad	Neighborhood butcher shop		8
KC-09-02-04	Federal B Area	Neighborhood butcher shop		9
KC-10-02-05	Karimabad	Neighborhood butcher shop		10
KC-11-03-01	Liaquatabad	Supermarket / fresh meat counter		11
KC-12-03-02	Nazimabad	Supermarket / fresh meat counter		12
KC-13-03-03	North Nazimabad	Supermarket / fresh meat counter		13
KC-14-03-04	Federal B Area	Supermarket / fresh meat counter		14
KC-15-03-05	Karimabad	Supermarket / fresh meat counter		15
KC-16-04-01	Liaquatabad	Frozen poultry outlet / wholesale		16
KC-17-04-02	Nazimabad	Frozen poultry outlet / wholesale		17

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Sample_ID	Area	Stratum	Shop_Code	Sample_No. (District wise)
KC-18-04-03	North Nazimabad	Frozen poultry outlet / wholesale		18
KC-19-04-04	Federal B Area	Frozen poultry outlet / wholesale		19
KC-20-04-05	Karimabad	Frozen poultry outlet / wholesale		20
KC-21-05-01	Liaquatabad	Branded poultry retail shop	Positive	21
KC-22-05-02	Nazimabad	Branded poultry retail shop		22
KC-23-05-03	North Nazimabad	Branded poultry retail shop		23
KC-24-05-04	Federal B Area	Branded poultry retail shop		24
KC-25-05-05	Karimabad	Branded poultry retail shop	Positive	25
Karachi East				
KE-01-01-01	Gulshan-e-Iqbal	Wet market / open poultry stall		1
KE-02-01-02	Gulistan-e-Jauhar	Wet market / open poultry stall		2
KE-03-01-03	Bahadurabad	Wet market / open poultry stall		3
KE-04-01-04	PECHS	Wet market / open poultry stall		4
KE-05-01-05	Karsaz	Wet market / open poultry stall		5
KE-06-02-01	Gulshan-e-Iqbal	Neighborhood butcher shop		6
KE-07-02-02	Gulistan-e-Jauhar	Neighborhood butcher shop		7
KE-08-02-03	Bahadurabad	Neighborhood butcher shop		8
KE-09-02-04	PECHS	Neighborhood butcher shop		9
KE-10-02-05	Karsaz	Neighborhood butcher shop		10
KE-11-03-01	Gulshan-e-Iqbal	Supermarket / fresh meat counter		11
KE-12-03-02	Gulistan-e-Jauhar	Supermarket / fresh meat counter		12
KE-13-03-03	Bahadurabad	Supermarket / fresh meat counter		13
KE-14-03-04	PECHS	Supermarket / fresh meat counter		14
KE-15-03-05	Karsaz	Supermarket / fresh meat counter		15
KE-16-04-01	Gulshan-e-Iqbal	Frozen poultry outlet / wholesale		16
KE-17-04-02	Gulistan-e-Jauhar	Frozen poultry outlet / wholesale		17
KE-18-04-03	Bahadurabad	Frozen poultry outlet / wholesale	Positive	18
KE-19-04-04	PECHS	Frozen poultry outlet / wholesale		19
KE-20-04-05	Karsaz	Frozen poultry outlet / wholesale		20
KE-21-05-01	Gulshan-e-Iqbal	Branded poultry retail shop		21
KE-22-05-02	Gulistan-e-Jauhar	Branded poultry retail shop		22
KE-23-05-03	Bahadurabad	Branded poultry retail shop		23
KE-24-05-04	PECHS	Branded poultry retail shop		24
KE-25-05-05	Karsaz	Branded poultry retail shop	Positive	25
Karachi West				
KW-01-01-01	Orangi Town	Wet market / open poultry stall		1
KW-02-01-02	Baldia Town	Wet market / open poultry stall		2
KW-03-01-03	SITE Area	Wet market / open poultry stall		3
KW-04-01-04	Manghopir	Wet market / open poultry stall		4

Table continues on next page.....

Sample_ID	Area	Stratum	Shop_Code	Sample_No. (District wise)
KW-05-01-05	Qasba Colony	Wet market / open poultry stall		5
KW-06-02-01	Orangi Town	Neighborhood butcher shop	Positive	6
KW-07-02-02	Baldia Town	Neighborhood butcher shop		7
KW-08-02-03	SITE Area	Neighborhood butcher shop		8
KW-09-02-04	Manghopir	Neighborhood butcher shop		9
KW-10-02-05	Qasba Colony	Neighborhood butcher shop		10
KW-11-03-01	Orangi Town	Supermarket / fresh meat counter		11
KW-12-03-02	Baldia Town	Supermarket / fresh meat counter		12
KW-13-03-03	SITE Area	Supermarket / fresh meat counter		13
KW-14-03-04	Manghopir	Supermarket / fresh meat counter		14
KW-15-03-05	Qasba Colony	Supermarket / fresh meat counter		15
KW-16-04-01	Orangi Town	Frozen poultry outlet / wholesale		16
KW-17-04-02	Baldia Town	Frozen poultry outlet / wholesale		17
KW-18-04-03	SITE Area	Frozen poultry outlet / wholesale		18
KW-19-04-04	Manghopir	Frozen poultry outlet / wholesale		19
KW-20-04-05	Qasba Colony	Frozen poultry outlet / wholesale		20
KW-21-05-01	Orangi Town	Branded poultry retail shop		21
KW-22-05-02	Baldia Town	Branded poultry retail shop	Positive	22
KW-23-05-03	SITE Area	Branded poultry retail shop		23
KW-24-05-04	Manghopir	Branded poultry retail shop		24
KW-25-05-05	Qasba Colony	Branded poultry retail shop		25
Karachi South				
KS-01-01-01	Saddar	Wet market / open poultry stall		1
KS-02-01-02	Clifton	Wet market / open poultry stall		2
KS-03-01-03	DHA	Wet market / open poultry stall		3
KS-04-01-04	Burns Road	Wet market / open poultry stall		4
KS-05-01-05	Lyari	Wet market / open poultry stall		5
KS-06-02-01	Saddar	Neighborhood butcher shop		6
KS-07-02-02	Clifton	Neighborhood butcher shop		7
KS-08-02-03	DHA	Neighborhood butcher shop		8
KS-09-02-04	Burns Road	Neighborhood butcher shop	Positive	9
KS-10-02-05	Lyari	Neighborhood butcher shop		10
KS-11-03-01	Saddar	Supermarket / fresh meat counter		11
KS-12-03-02	Clifton	Supermarket / fresh meat counter		12
KS-13-03-03	DHA	Supermarket / fresh meat counter		13
KS-14-03-04	Burns Road	Supermarket / fresh meat counter		14
KS-15-03-05	Lyari	Supermarket / fresh meat counter		15
KS-16-04-01	Saddar	Frozen poultry outlet / wholesale		16
KS-17-04-02	Clifton	Frozen poultry outlet / wholesale		17

Table continues on next page.....

Sample_ID	Area	Stratum	Shop_Code	Sample_No. (District wise)
KS-18-04-03	DHA	Frozen poultry outlet / wholesale	Positive	18
KS-19-04-04	Burns Road	Frozen poultry outlet / wholesale		19
KS-20-04-05	Lyari	Frozen poultry outlet / wholesale		20
KS-21-05-01	Saddar	Branded poultry retail shop		21
KS-22-05-02	Clifton	Branded poultry retail shop		22
KS-23-05-03	DHA	Branded poultry retail shop		23
KS-24-05-04	Burns Road	Branded poultry retail shop		24
KS-25-05-05	Lyari	Branded poultry retail shop		25
Malir				
M-01-01-01	Malir City	Wet market / open poultry stall	Positive	1
M-02-01-02	Malir Halt	Wet market / open poultry stall		2
M-03-01-03	Model Colony	Wet market / open poultry stall		3
M-04-01-04	Saudabad	Wet market / open poultry stall		4
M-05-01-05	Shah Faisal Colony	Wet market / open poultry stall		5
M-06-02-01	Malir City	Neighborhood butcher shop		6
M-07-02-02	Malir Halt	Neighborhood butcher shop		7
M-08-02-03	Model Colony	Neighborhood butcher shop		8
M-09-02-04	Saudabad	Neighborhood butcher shop		9
M-10-02-05	Shah Faisal Colony	Neighborhood butcher shop		10
M-11-03-01	Malir City	Supermarket / fresh meat counter		11
M-12-03-02	Malir Halt	Supermarket / fresh meat counter		12
M-13-03-03	Model Colony	Supermarket / fresh meat counter		13
M-14-03-04	Saudabad	Supermarket / fresh meat counter		14
M-15-03-05	Shah Faisal Colony	Supermarket / fresh meat counter		15
M-16-04-01	Malir City	Frozen poultry outlet / wholesale		16
M-17-04-02	Malir Halt	Frozen poultry outlet / wholesale		17
M-18-04-03	Model Colony	Frozen poultry outlet / wholesale		18
M-19-04-04	Saudabad	Frozen poultry outlet / wholesale		19
M-20-04-05	Shah Faisal Colony	Frozen poultry outlet / wholesale		20
M-21-05-01	Malir City	Branded poultry retail shop		21
M-22-05-02	Malir Halt	Branded poultry retail shop		22
M-23-05-03	Model Colony	Branded poultry retail shop		23
M-24-05-04	Saudabad	Branded poultry retail shop		24
M-25-05-05	Shah Faisal Colony	Branded poultry retail shop		25
Korangi				
K-01-01-01	Korangi No.1-6	Wet market / open poultry stall		1
K-02-01-02	Zaman Town	Wet market / open poultry stall		2
K-03-01-03	Landhi	Wet market / open poultry stall		3
K-04-01-04	Mehran Town	Wet market / open poultry stall		4

Table continues on next page.....

Sample_ID	Area	Stratum	Shop_Code	Sample_No. (District wise)
K-05-01-05	Bilal Colony	Wet market / open poultry stall		5
K-06-02-01	Korangi No.1-6	Neighborhood butcher shop		6
K-07-02-02	Zaman Town	Neighborhood butcher shop		7
K-08-02-03	Landhi	Neighborhood butcher shop		8
K-09-02-04	Mehran Town	Neighborhood butcher shop		9
K-10-02-05	Bilal Colony	Neighborhood butcher shop		10
K-11-03-01	Korangi No.1-6	Supermarket / fresh meat counter		11
K-12-03-02	Zaman Town	Supermarket / fresh meat counter		12
K-13-03-03	Landhi	Supermarket / fresh meat counter		13
K-14-03-04	Mehran Town	Supermarket / fresh meat counter		14
K-15-03-05	Bilal Colony	Supermarket / fresh meat counter		15
K-16-04-01	Korangi No.1-6	Frozen poultry outlet / wholesale		16
K-17-04-02	Zaman Town	Frozen poultry outlet / wholesale		17
K-18-04-03	Landhi	Frozen poultry outlet / wholesale		18
K-19-04-04	Mehran Town	Frozen poultry outlet / wholesale		19
K-20-04-05	Bilal Colony	Frozen poultry outlet / wholesale		20
K-21-05-01	Korangi No.1-6	Branded poultry retail shop		21
K-22-05-02	Zaman Town	Branded poultry retail shop		22
K-23-05-03	Landhi	Branded poultry retail shop		23
K-24-05-04	Mehran Town	Branded poultry retail shop		24
K-25-05-05	Bilal Colony	Branded poultry retail shop		25

Supplementary Table SII. Sample ID, cetrinide agar result, gram reaction.

Sample ID	Cetrinide Agar	Gram Reaction
1	Negative	N/A
2	Negative	N/A
3	Negative	N/A
4	Negative	N/A
5	Positive	Gram-negative bacilli
6	Negative	N/A
7	Negative	N/A
8	Positive	Gram-negative bacilli
9	Negative	N/A
10	Negative	N/A
11	Negative	N/A
12	Negative	N/A
13	Negative	N/A
14	Negative	N/A

Table continues on next column.....

Sample ID	Cetrinide Agar	Gram Reaction
15	Negative	N/A
16	Negative	N/A
17	Negative	N/A
18	Negative	N/A
19	Negative	N/A
20	Negative	N/A
21	Positive	Gram-negative bacilli
22	Negative	N/A
23	Negative	N/A
24	Negative	N/A
25	Positive	Gram-negative bacilli
26	Negative	N/A
27	Negative	N/A
28	Negative	N/A
29	Negative	N/A
30	Negative	N/A
31	Negative	N/A

Table continues on next page.....

Sample ID	Cetrimide Agar	Gram Reaction
32	Negative	N/A
33	Negative	N/A
34	Negative	N/A
35	Negative	N/A
36	Negative	N/A
37	Negative	N/A
38	Negative	N/A
39	Negative	N/A
40	Negative	N/A
41	Negative	N/A
42	Negative	N/A
43	Positive	Gram-negative bacilli
44	Negative	N/A
45	Negative	N/A
46	Negative	N/A
47	Negative	N/A
48	Negative	N/A
49	Negative	N/A
50	Positive	Gram-negative bacilli
51	Negative	N/A
52	Negative	N/A
53	Negative	N/A
54	Negative	N/A
55	Negative	N/A
56	Positive	Gram-negative bacilli
57	Negative	N/A
58	Negative	N/A
59	Negative	N/A
60	Negative	N/A
61	Negative	N/A
62	Negative	N/A
63	Negative	N/A
64	Negative	N/A
65	Negative	N/A
66	Negative	N/A
67	Negative	N/A
68	Negative	N/A
69	Negative	N/A
70	Negative	N/A
71	Negative	N/A
72	Positive	Gram-negative bacilli

Table continues on next column.....

Sample ID	Cetrimide Agar	Gram Reaction
73	Negative	N/A
74	Negative	N/A
75	Negative	N/A
76	Negative	N/A
77	Negative	N/A
78	Negative	N/A
79	Negative	N/A
80	Negative	N/A
81	Negative	N/A
82	Negative	N/A
83	Negative	N/A
84	Positive	Gram-negative bacilli
85	Negative	N/A
86	Negative	N/A
87	Negative	N/A
88	Negative	N/A
89	Negative	N/A
90	Negative	N/A
91	Negative	N/A
92	Negative	N/A
93	Positive	Gram-negative bacilli
94	Negative	N/A
95	Negative	N/A
96	Negative	N/A
97	Negative	N/A
98	Negative	N/A
99	Negative	N/A
100	Negative	N/A
101	Positive	Gram-negative bacilli
102	Negative	N/A
103	Negative	N/A
104	Negative	N/A
105	Negative	N/A
106	Negative	N/A
107	Negative	N/A
108	Negative	N/A
109	Negative	N/A
110	Negative	N/A
111	Negative	N/A
112	Negative	N/A
113	Negative	N/A

Table continues on next page.....

Sample ID	Cetrimide Agar	Gram Reaction
114	Negative	N/A
115	Negative	N/A
116	Negative	N/A
117	Negative	N/A
118	Negative	N/A
119	Negative	N/A
120	Negative	N/A
121	Negative	N/A
122	Negative	N/A
123	Negative	N/A
124	Negative	N/A
125	Positive	Gram-negative bacilli
126	Negative	N/A
127	Negative	N/A
128	Negative	N/A
129	Negative	N/A
130	Negative	N/A
131	Negative	N/A
132	Negative	N/A
133	Negative	N/A
134	Negative	N/A
135	Negative	N/A
136	Negative	N/A
137	Negative	N/A
138	Negative	N/A
139	Negative	N/A
140	Negative	N/A
141	Negative	N/A
142	Negative	N/A
143	Negative	N/A
144	Negative	N/A
145	Negative	N/A
146	Negative	N/A
147	Negative	N/A
148	Negative	N/A
149	Negative	N/A
150	Negative	N/A
<i>Pseudomonas</i> spp., Positive (ATCC 27853)		Gram-negative bacilli

Supplementary Table SIII. Identification and confirmation of *Pseudomonas* spp. by biochemical tests.

Isolates	Catalase	Citrate	Motility	Oxidase	H ₂ S	Indole
P1	+	+	+	+	+	–
P2	+	+	+	+	+	–
P3	+	+	+	+	+	–
P4	+	+	+	+	+	–
P5	+	+	+	+	+	–
P6	+	+	+	+	+	–
P7	+	+	+	+	+	–
P8	+	+	+	+	+	–
P9	+	+	+	+	+	–
P10	+	+	+	+	–	+
P11	+	+	+	+	–	+
P12	+	+	+	+	+	+
<i>Pseudomonas</i> spp., (ATCC 27853)	+	+	+	+	+	–

Supplementary Table SIV. CLSI-based susceptibility interpretation (S / I / R) of *Pseudomonas* isolates (n=12).

Iso-late	Amik-acin	Pipera-cillin	Cefepime	Cipro-floxacin	Levo-floxacin	Gen-tamicin
P1	S	R	R	I	R	I
P2	S	S	R	I	I	I
P3	R	I	I	S	I	S
P4	S	S	R	S	I	S
P5	R	R	R	R	R	I
P6	S	S	R	S	R	I
P7	S	S	R	S	S	S
P8	S	S	I	S	R	S
P9	R	R	R	I	R	S
P10	R	R	R	I	R	I
P11	R	R	R	I	I	I
P12	S	S	R	S	R	S