Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2022.074

RESEARCH ARTICLE

Molecular Detection of Biofilm Production among Multidrug Resistant Isolates of *Pseudomonas aeruginosa* from Meat Samples

Rasti Abbas[1], Zeeshan Nawaz[1*], Abu Baker Siddique[1], Rizwan Aslam[2], Azhar Rafique[3], Muhammad Asif Zahoor[1], Muhammad Usman Qamar[1], Muhammad Zishan Ahmad[4], Muhammad Moazam Jalees[5], Muhammad Qasim[1] and Abdullah F Alsayeqh[6]

[1]Department of Microbiology, Government College University Faisalabad, Pakistan
[2]Institute of Microbiology, University of Agriculture. Faisalabad, Pakistan
[3]Department of Zoology, Government College University Faisalabad, Pakistan
[4]Department of Veterinary Pathology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan
[5]Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan
[6]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah 51452, Kingdom of Saudi Arabia
*Corresponding author: zeeshannawaz@gcuf.edu.pk

ARTICLE HISTORY (22-167)

Received: May 19, 2022
Revised: October 05, 2022
Accepted: October 07, 2022
Published online: October 15, 2022

Key words:
Biofilm
MDR
Meat
Pseudomonas aeruginosa
*Psl*A gene

ABSTRACT

Multidrug resistant (MDR) *Pseudomonas aeruginosa* is a prominent bacterial pathogen conferring resistance to variety of antimicrobial agents. The ability to form biofilms further enhances the drug resistance phenomena of *P. aeruginosa*. The emergence of biofilm forming MDR strains of *P. aeruginosa* in meat and food products is a serious public health concern. In present study, total 100 meat samples were collected (50 each from chicken and mutton) from different butcher shops and supermarkets and *P. aeruginosa* was isolated by standard microbiological, biochemical and molecular techniques. The resistance profile against various antibiotics was detected by Kirby Bauer method while the biofilm production was observed by microtiter plate assay. The biofilm associated gene (*psl*A) and extended spectrum beta lactamase (ESBL) genes were detected by polymerase chain reaction (PCR). The study outcome revealed that *P. aeruginosa* was isolated from 24% meat samples comprising 14/50 (28%) from chicken and 10/50 (20%) from mutton samples. The highest resistance (100%) was observed against Amoxicillin-Clavulanic acid and Ceftriaxone followed by (95.83%) for Aztreonam, Ticracillin and (91.67%) to ciprofloxacin. Out of 24 isolated *P. aeruginosa,* 22 (91.66%) were detected as MDR. Furthermore, among the 22 MDR isolates, 19 (86.36%) were found biofilm producing *P. aeruginosa* and all of them detected positive for biofilm encoding gene (*psl*A). In addition, 9 (40.90%) MDR isolates showed presence of ESBL genes including 6 *bla*CTX-M and 03 *bla*TEM while none of the isolates was found positive for *bla*NDM and *bla*OXA genes. This study concludes that antibiotic resistance is more pronounced in biofilm producing *P. aeruginosa* and presence of these biofilm forming isolates in the food chain is a serious threat to public health.

To Cite This Article: Abbas R, Nawaz Z, Siddique AB, Aslam R, Rafique A, Zahoor MA, Qamar MU, Ahmad MZ, Jalees MM, Qasim M and Alsayeqh AF, 2022. Molecular detection of biofilm production among multidrug resistant isolates of *Pseudomonas aeruginosa* from meat samples. Pak Vet J, 42(4): 505-510. http://dx.doi.org/10.29261/pakvetj/2022.074

INTRODUCTION

Meat is considered the most nutritious source of proteins, fermentable carbohydrates, essential fatty acids, minerals and growth factors with high water contents consumed by humans that are suitable for microbial growth (Wickramasinghe *et al.,* 2019). This meat can be contaminated during slaughtering, handling, processing and transportation adopting unhygienic practices. The contaminated meat is a vital source of gastrointestinal infections and the microbial load represents the standard and hygiene quality of meat (Fakhkhari *et al*., 2022). *Pseudomonas aeruginosa* is prominently associated with food spoilage resulting in smelly, spoiled and off flavor food (Bantawa *et al*., 2018). The food borne *Pseudomonas* is recognized as chief spoilage microorganisms due to the

degrading activity of their extracellular enzymes, pigment secretion and biofilm formation (İnat *et al*., 2021). The spoilage of food renders it inedible and also ends in the severe and deadly illness (Nagarajan *et al*., 2018).

The poor sanitary conditions of meat shops, unhygienic processing environment and lack of awareness of meat retailers about guidelines of meat shops invite *Pseudomonas* contamination (Bantawa *et al*., 2018). This contamination through antibiotic-resistant microbes might be most important risk to the public health by the spread of antibiotic resistance determinants to clinically significant bacteria of humans (Stellato *et al*., 2016). The unwanted and extreme use of antibiotics for the treatment and prophylaxis of food animals is developing multidrug resistance (MDR) in microorganisms. This resistance is further shifted to humans by using meat and its products and becoming one of the most daunting challenges of the 21st century (Uddin *et al*., 2021) leading to limited therapeutic options for treatment (Mouiche *et al*., 2019).

The biofilm formation is one of the key factors for the pathogenesis of *P. aeruginosa* in acute and chronic infections. The bacteria residing in biofilms are found much more resistant to antimicrobials, host immune functions and adverse environment as compared to their planktonic cells. This resistance creates difficulties in the clearance of biofilms, leads to chronic or prolonged infections which are difficult to treat and eradicate (Maurice *et al*., 2018). The main exopolysaccharides associated with *P. aeruginosa* biofilm formation includes Pel, Psl and alginate (Ghafoor *et al*., 2011). The Psl is a pentasaccharide which has a vital role in preliminary biofilm production. The binding of mannose present in Psl with lactin (LecB) enhances the bacterial aggregation and retention in biofilms (Passos da Silva *et al*., 2019). The biofilm formation retards the effect of antibiotics against bacteria because it develops a barricade which scrutinizes the drug penetration leading to treatment failure and also hurdles the microbial recognition by host immunity (Tuon *et al*., 2022). Additionally presence of biofilms sometimes requires nearly 1000 times greater dose of antibiotics for their clearance in contrast to their planktonic bacteria resulting in toxicity among the hosts (Goltermann and Tolker-Nielsen, 2017).

There was no previous detection report of biofilm producing *Pseudomonas aeruginosa* from meat samples in Pakistan, so keeping in consideration the health beneficial and nutritional properties of meat and its contamination by MDR pathogens, the present study was designed to detect the biofilm production and presence of *psl*A genes among MDR *Pseudomonas aeruginosa* from various meat samples.

MATERIALS AND METHODS

Samples collection: Fresh (n=50) and frozen (n=50) meat samples were collected from various butcher shops and super markets including chicken and mutton equally. The samples were packaged aseptically into polyethylene zip bags. The samples were labelled properly with date, sample type and collection site then transferred to food microbiology laboratory in icebox for further processing (Stellato *et al*., 2016).

Isolation and identification of *Pseudomonas aeruginosa*: A total of 25g of every meat sample was aseptically mixed in the 225 ml of sterile peptone water followed by overnight enrichment at 37°C. The enriched cultures were further inoculated on *Pseudomonas* Citramide agar (Oxoid, UK) and incubated aerobically for 24 hours at 37°C. The isolates were initially identified on the basis of their cultural and morphological characters while confirmation was done using biochemical testing. The isolates were preserved in peptone broth with 30% glycerol and stored at -80°C (Ijaz *et al*., 2019).

Molecular confirmation of *Pseudomonas aeruginosa:* Molecular confirmation of *P. aeruginosa* isolates was performed by polymerase chain reaction (PCR) after the DNA was extracted using commercial Kit (Thermo Scientific, USA). For the confirmation of *Pseudomonas aeruginosa* (*opr*L) gene was targeted with thermal profile setting as initial denaturation at 95°C for (5 mins) followed by 30 cycles of 95°C for (30 sec), 57°C for (30 sec), 72°C for (10 sec) and final extension at 72°C for (10 mins) in (BioRad, USA) thermal cycler. The *P. aeruginosa* strain (ATCC 27853) was used as positive control and distilled water was used as negative control. The visualization of products was carried out using 1% agarose gel stained with ethidium bromide under (Slite 200W, Taiwan) Gel documentation system as described by (Abdulhaq *et al*., 2020). The primers used and product size are mentioned in (Table 1).

Table 1: List of primers used for amplification of various genes of *Pseudomonas aeruginosa*

Gene Target	Primer Sequence	Size (bp)	References
*opr*L	F- ATGGAAATGCTGAAATTCGGC		(Abdulhaq *et*
	R- CTTCTTCAGCTCGACGCGACG	504	*al*., 2020)
*psl*A	F-TGGGTCTTCAAGTTCCGCTC		
	R-ATGCTGGTCTTGCGGATGAA	119	
bla CTX-M	F-CGTCACGCTGTTGTTAGGAA		(Nawaz *et al*.,
	R-ACGGCTTTCTGCCTTAGGTT	780	2021)
bla TEM	F-ATGAGTATTCAACATTTCCG		
	R-GACAGTTACCAATGCTTAATCA	862	
bla OXA	F-GCGTGGTTAAGGATGAACAC		
	R-CATCAAGTTCAACCCAACCG	438	
bla NDM	F-GGTTTGGCGATCTGGTTTTC		(Nordmann
	R-CGGAATGGCTCATCACGATC	621	*et al*., 2011)

Antimicrobial susceptibility testing: After the confirmation of *P. aeruginosa*, antibiotic susceptibility profiling of isolates was established against various antimicrobials including Amikacin (30µg), Ciprofloxacin (5µg), Ticracillin (75µg), Piperacillin (100µg), Imipenem (10µg), Tobramycin (30µg), Meropenem (10µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefepime (30µg), Azteronam (30µg), Amoxicillin/Clavulanic acid (30µg) and Gentamicin (10µg). The results were analyzed according to the recommendations of Clinical Laboratory Standards Institute as described by Nawaz *et al*. (2021).

Detection of biofilm Production: The phenotype detection of biofilm production was performed by microtiter plate assay (MPA). The fresh cultures were diluted (100 folds) in tryptic soy broth. Aliquots (250µL) of isolates were then dispended into a 96-well (flat-bottom) plate and incubated overnight at 37°C. Washing of wells was performed by agitating and shaking to ensure

proper removal of poorly attached bacteria. The plates were heat dried to favour the fixation of biofilms. After that staining with 250µL of crystal violet (0.1%) was performed for 20 minutes. Washing and drying was repeated followed by treatment of wells with 250µL of 50% acetone. The un-inoculated wells containing sterile tryptic soy broth were used as negative controls. The optical density (OD) values of the biofilms were measured at 594nm with (BioRad, USA) ELISA reader.

Furthermore, a cut-off value (ODc) was determined as ODc = average OD of negative control + (3 × negative control SD). In the non-biofilm producers (OD<ODc), while weak producers showed (ODc<OD<2×ODc), moderate producer showed (2×ODc<OD<4×ODc) and strong producers of biofilm showed (4×ODc<OD) as described by Saxena *et al*. (2014).

Detection of biofilm encoding and ESBL genes: The PCR amplification of biofilm forming gene (*psl*A) was performed in a thermal cycler according to the thermal conditions as initial denaturation at 95°C for (5 mins) followed by 35 cycles of 94°C for (30 sec), 52°C for (40 sec), 72°C for (50 sec) and final extension at 72°C for (10 mins) in (BioRad, USA) thermal cycler. The (PAO1) strain of *P. aeruginosa* was used as control positive for the *pslA* gene and distilled water was used as control negative (Abdulhaq *et al*., 2020). The ESBLs genes (*bla*CTX-M, *bla*TEM, *bla*OXA and *bla*NDM) were also detected by PCR using the specific primers according to the conditions described by Nawaz *et al*. (2021) and Nordmann *et al*. (2011). The positive controls used were previously confirmed isolates of *Escherichia coli* (Nawaz *et al*., 2021).

Statistical analysis: The data was presented in percentages and Chi square test was applied. The value (P<0.05) was reflected as significant. All the statistical analyses were performed by Minitab 11 software (USA).

RESULTS

Frequency of *Pseudomonas aeruginosa* among Meat Samples: In current study, total 100 meat samples were collected including chicken and mutton. The results exhibited that *Pseudomonas aeruginosa* was detected from (n=24) samples on the basis of morphological, biochemical and molecular methods making the overall prevalence of 24% (Fig. 1A). The chicken samples were found more contaminated with the presence of *P. aeruginosa* 14/50 (28%) in contrast to mutton samples 10/50 (20%). Correspondingly, detection of *P. aeruginosa* contamination was more noticeable in fresh samples 16/50 (32%) than the frozen samples 8/50 (16%) as shown in (Table 2).

Antimicrobial Susceptibility Profiling of *Pseudomonas aeruginosa:* The isolates were subjected to antibiotic susceptibility profiling and the results revealed highest resistance pattern (100%) against Amoxicillin-Clavulanic acid and Ceftriaxone followed by (95.83%) to Aztreonam and Ticracillin, (91.67%) Ciprofloxacin, (83.33%) Gentamicin, (75%) Tobramycin and Amikacin, (58.83%) Piperacillin and Cefepime. The most effective drug was ceftazidime found 41.67% sensitive as shown in (Table 3). Among total 24 isolates, 22 (91.66%) were considered as multi drug resistant *Pseudomonas aeruginosa* (MDR-PA) due to their respective resistant nature for antimicrobials of more than 3 groups according to CLSI guidelines 2020.

Table 2: Frequency of *Pseudomonas aeruginosa* from meat samples

Meat Samples Type	Total Samples Collected	*Pseudomonas aeruginosa* Positive	*Pseudomonas aeruginosa* Positive (%)	P value
Fresh Chicken	25	09	36%	0.343
Frozen Chicken	25	05	20%	
Fresh Mutton	25	07	28%	0.247
Frozen Mutton	25	03	12%	
Total	100	24	24%	

Statistically significant at (P<0.05).

Prevalence of Biofilm producing *Pseudomonas aeruginosa:* Among the total (n=22) MDR *P. aeruginosa* isolated from meat samples, 19 (86.36%) showed variable amount of biofilm production phenotypically which includes 05 isolates as strong biofilm producers, 10 isolates as moderate and 04 as weak biofilm producers (Table 4).

Frequency of Biofilm encoding and ESBL genes in *Pseudomonas aeruginosa* isolates: The results of present study showed that all the phenotypic biofilm producers were found positive for (*psl*A) gene (Figure 1B). The MDR *P. aeruginosa* isolates from meat samples harboring ESBLs genes were found 09 (40.90%). The *bla*CTX-M gene was detected in 06 (27.27%) and *bla*TEM in 03 (13.63%) isolates while none of the isolates were detected positive for *bla*NDM and *bla*OXA genes (Fig. 2A & 2B).

Table 3: Resistance percentage of *Pseudomonas aeruginosa* against different antibiotics

Antibiotics Used	CLSI Breakpoints Diameters (mm)			Sensitive (%age)	Intermediate (%age)	Resistance (%age)
	S	I	R			
Amoxicillin/ Clavulanic acid	>18	14-17	<13	0 (0)	0 (0)	24 (100)
Gentamicin	>15	13-14	<12	2 (8.3)	2 (8.33)	20 (83.33)
Piperacillin	>21	15-20	<14	1(4.16)	9(37.50)	14(58.33)
Ticracillin	>24	16-23	<15	1(4.2)	0(0)	23(95.83)
Tobramycin	>15	13-14	<12	3(12.5)	3(12.50)	18(75)
Cefepime	>18	15-17	<14	6 (25)	4(16.67)	14 (58.33)
Ceftriaxone	>18	15-17	<14	0 (0)	0 (0)	24(100)
Ciprofloxacin	>25	19-24	<18	1 (4.16)	1 (4.16)	22 (91.67)
Ceftazidime	>18	15-17	<14	10 (41.67)	2 (8.33)	12(50)
Amikacin	>17	15-16	<14	5 (20.83)	1(4.16)	18 (75)
Aztreonam	>22	16-21	<15	0(0)	1(4.16)	23(95.83)
Imipenem	>19	16-18	<15	8(33.33)	3(12.50)	13(54.16)
Meropenem	>19	16-18	<15	3 (12.50)	0 (0)	21(87.50)

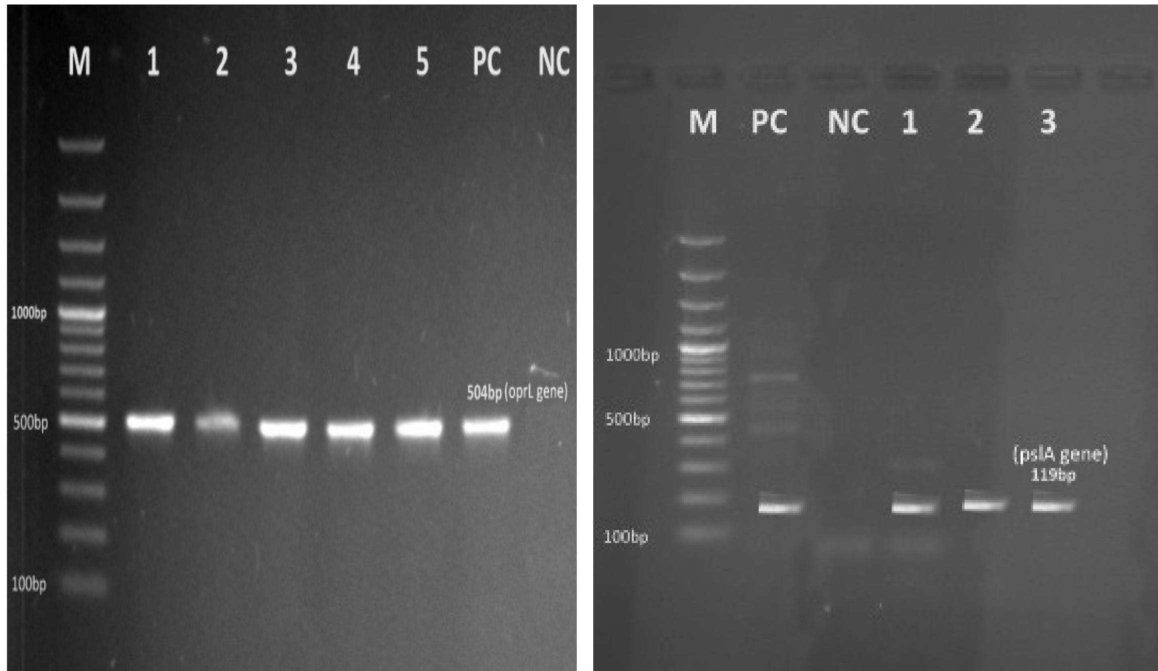

Fig. 1A: Agarose gel electrophoresis showing PCR product of *oprL* gene of *Pseudomonas aeruginosa.* Lane M: Marker (100bp), Lane 1-5: Positive samples showing 504bp band, Lane PC: Positive control (ATCC 27853), Lane NC: Negative control. B: Agarose gel electrophoresis showing PCR product of *psl*A gene of *Pseudomonas aeruginosa.* Lane M: Marker (100bp), Lane 1-3: Positive samples showing 119bp band, Lane PC: Positive control (PAO1), Lane NC: Negative control.

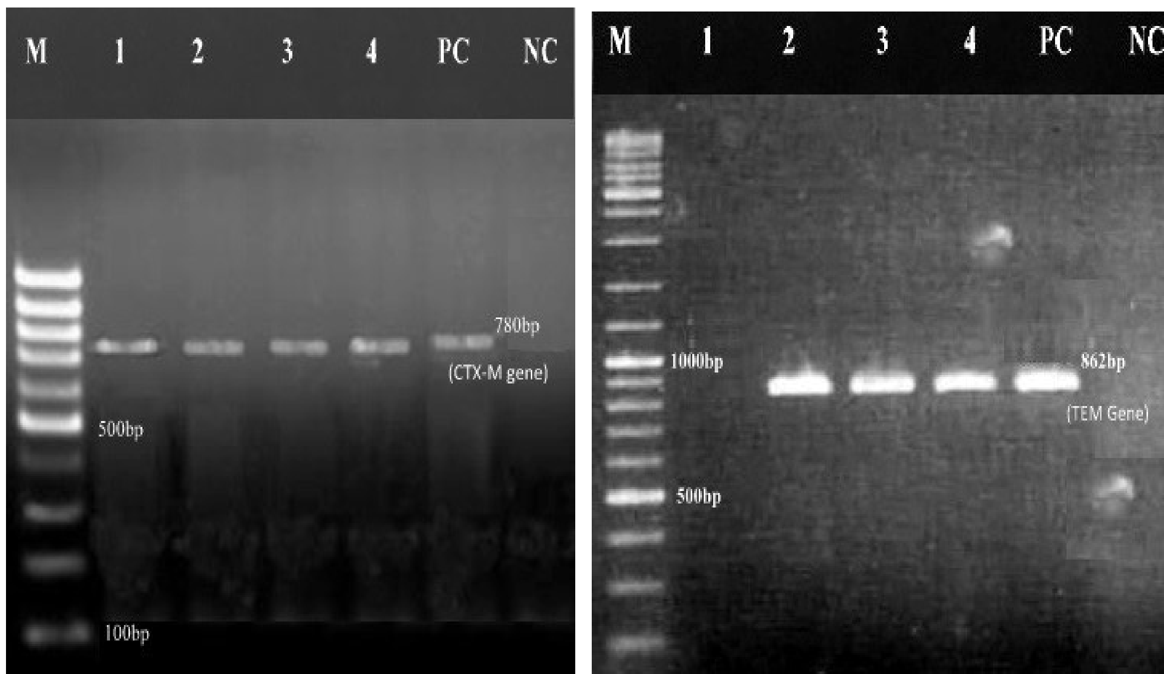

Fig. 2A: Agarose gel electrophoresis showing PCR product of blaCTX-M gene of *Pseudomonas aeruginosa.* Lane M: Marker (100bp), Lane 1-4: Positive samples showing 780bp band, Lane PC: Positive control (*E. coli*), Lane NC: Negative control. B: Agarose gel electrophoresis showing PCR product of *bla*TEM gene of *Pseudomonas aeruginosa.* Lane M: Marker (100bp), Lane 2-4: Positive samples showing 862bp band, Lane PC: Positive control (*E. coli*), Lane NC: Negative control.

DISCUSSION

P. aeruginosa plays an important role in the spoilage of variety of food items especially high value food as meat and its products. It is renowned for producing biofilms that confer an extreme ability to persist against phagocytosis, oxidative stresses, restriction of nutrient/ oxygen, accumulation of metabolic waste, competition between species, and conventional antimicrobial agents (Olsen, 2015). Due to its multidrug resistant nature and ability to produce biofilms, the infections caused by *P. aeruginosa* are becoming a challenge for physicians to treat.

Table 4: Frequency of biofilm production among MDR *P. aeruginosa* isolates

Mean OD Value	Biofilm Formation	No. of MDR Isolates (%)	No. of ESBL Isolates (%)
< 0.062	None	03 (13.63)	02 (22.22)
0.062-0.124	Weak	04 (18.18)	01 (11.11)
0.124-0.248	Moderate	10 (45.45)	04 (44.44)
> 0.248	Strong	05 (22.72)	02 (22.22)
	Total	22 (100)	09 (100)

In this study 100 meat samples from different sources were collected and it was found that *Pseudomonas aeruginosa* was detected among 24% of meat samples which was relatable to findings of Elhariri *et al*. (2017) with 22.5% and Elsayed *et al*. (2016) with 22.9%. Slightly higher prevalence 33.3% was reported by Chika *et al*. (2016) in Nigeria. The chicken samples were found to be more contaminated with the presence of *P. aeruginosa* 14/50 (28%) in contrast to mutton samples 10/50 (20%). A similar result was observed by Bantawa *et al*. (2018) which showed higher percentage of *Pseudomonas* in chicken 46.6% in contrast to mutton 40%. *P. aeruginosa* contamination was more noticeable in fresh samples 16/50 (32%) instead of frozen samples 8/50 (16%) and it is in accordance with the results of Elnawawi *et al*. (2012) who reported, 16% *Pseudomonas* in frozen samples. The main reason behind these findings is that poultry is a cheap source of protein and widely sold at roadside stalls which are dirty/polluted places leading to its contamination. In addition, the hygienic status of meat handlers also plays an important role in contamination and the trend of preserving meat in chillers and freezers is not common among the meat sellers in Pakistan.

The result of antimicrobial susceptibility profiling showed that highest resistance pattern (100%) was detected against Amoxicillin-clavulanic acid and Ceftriaxone followed by Aztreonam, Ticracillin (95.83%). The results of previous studies (Elhariri *et al*., 2017), (Qureshi *et al*., 2018) and (Ijaz *et al*., 2019) showed 100% resistance to ceftriaxone and 90% to Amoxicillin-clavulanic acid while Abdulhaq *et al*. (2020) reported 95% resistance to ceftriaxone in Pakistan. Benie *et al*. (2017) conducted a similar research on different samples and reported 98.4% resistance to aztreonam. Majority of isolates were found MDR which is in agreement with the results outcome of Benie *et al*. (2017) with 36.2% MDR and Elhariri *et al*. (2017) with 46% MDR strains. A higher MDR pattern was also reported previously by Elsayed *et al*. (2016). The presence of high antibiotic resistance in meat samples is linked with the irrational use of antibiotics for the management and control of various bacterial infections. For intensive poultry farming various antibiotics are used as growth promoters which serve as a potential risk to human beings.

Pseudomonas aeruginosa is one of the prominently studied microbes in the context of biofilms and biofilm formation is an important weapon of *P. aeruginosa* which contributes towards pathogenesis, antimicrobial resistance and escape from host immune system. In this study the biofilm production was detected using microtiter plate assay and it was observed that 86.36% of MDR isolates of *P. aeruginosa* were biofilm producers. Previously not a single study regarding biofilm producing *Pseudomonas aeruginosa* from Pakistan was reported in meat samples, but in clinical samples, similar type of findings were reported in previous studies (Abidi *et al*., 2013; Awan *et al*., 2019; Abdulhaq *et al*., 2020) showing nearly 90% prevalence of biofilms among MDR isolates. However (İnat *et al*., 2021) recorded 84% *P. aeruginosa* isolates as biofilm producers from chicken. In addition, the biofilm forming gene (*psl*A) was present in all the biofilm producing isolates which is very close to the observations of Nader *et al*. (2017) in Iraq, Cho *et al*. (2018) in Korea, Abdulhaq *et al*. (2020) in Pakistan and Divyashree *et al*. (2022) in India. The presence of *psl*A gene proved itself very superior identification marker for biofilm formation among *P. aeruginosa* isolates as described by Hou *et al*. (2012) because it plays an important role in initial biofilm formation and cellular attachment (Colvin *et al*., 2011).

Furthermore, among the total MDR isolates detected in this study (n=22), ESBL genes were found in 09 (40.90%) isolates. The detected genes were *bla* CTX-M (27.27%) and *bla* TEM (13.63%). A close finding to our study was reported by Elhariri *et al*. (2017) with *bla* CTX-M 38% and *bla* TEM 23.8% in camel meat of Egypt. A higher prevalence was detected by in Lebanon with 53% *bla* CTX-M and 83% *bla* TEM genes in chicken (Dandachi *et al*., 2018). These results are also in accordance with outcomes of Chen *et al*. (2015), Rezai *et al*. (2015) and Chakraborty *et al*. (2020). The variation in the results might be due to variation in the type of samples, class of antibiotics used and irrational use of antimicrobials in the study area.

Conclusions: The study results concluded that biofilm producing *P. aeruginosa* strains are more resistant to multiple antibiotics and a distinguishing role of *psl*A gene was found with the biofilm production of *P. aeruginosa*. So, the combination of drug resistance and biofilm production in *P. aeruginosa* found in meat samples is a shocking concern leading to serious public health complications.

Acknowledgments: This study was funded by National Research Program for Universities (NRPU) of Higher Education Commission (HEC), Pakistan (6949/Punjab/ NRPU/R&D/HEC/2017).

Disclosure: The authors declare that they have no conflict of interest.

Authors contribution: ZN and ABS conceived and designed the study; RA, MQ and MUQ performed the experiments; MAZ, MZA and RA compiled the data and results; MMJ, AR and AFA wrote and critically reviewed the manuscript.

REFERENCES

Abdulhaq N, Nawaz Z, Zahoor MA, *et al*., 2020. Association of biofilm formation with multidrug resistance in clinical isolates of *Pseudomonas aeruginosa*. Excli J 19:201-8.

Abidi SH, Sherwani SK, Siddiqui TR, *et al*., 2013. Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan. BMC Ophthalmol 13:57.

Awan AB, Schiebel J, Böhm A, Nitschke J, Sarwar Y, Schierack P and Ali A, 2019. Association of biofilm formation and cytotoxic potential with multidrug resistance in clinical isolates of *Pseudomonas aeruginosa*. Excli J 18:79-90.

Bantawa K, Rai K, Limbu DS, *et al*., 2018. Food-borne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. BMC Res Notes 11:1-5.

Benie CKD, Dadié A, Guessennd N, *et al*., 2017. Characterization of virulence potential of *Pseudomonas aeruginosa* isolated from bovine meat, fresh fish, and smoked fish. Eur J Microbiol Immunol 7:55-64.

Chakraborty S, Dutta TK, Roychoudhury P, *et al*., 2020. Molecular characterization of biofilm-producing *Pseudomonas aeruginosa* isolated from healthy pigs and chicken in India. Indian J Anim Res 54:1400-7.

Chen Z, Niu H, Chen G, *et al*., 2015. Prevalence of ESBLs-producing *Pseudomonas aeruginosa* isolates from different wards in a Chinese teaching hospital. Int J Clin Exp Med 8:19400-5.

Chika E, Charles E, Ifeanyichukwu I, *et al*., 2016. Phenotypic detection of AmpC Beta-Lactamase among anal *Pseudomonas aeruginosa* isolates in a Nigerian Abattoir. Arch Clin Microbiol 7:2-5.

Cho HH, Kwon KC, Kim S, *et al*., 2018. Association between biofilm formation and antimicrobial resistance in carbapenem-resistant *Pseudomonas aeruginosa.* Annals Clin Lab Sci 48:363-8.

Colvin KM, Gordon VD, Murakami K, *et al*., 2011. The pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. PLoS Pathog 7:e1001264.

Dandachi I, Sokhn ES, Dahdouh EA, *et al*., 2018. Prevalence and characterization of multi-drug-resistant Gram-negative bacilli isolated from Lebanese poultry: A nationwide study. Front Microbiol 9:550.

Divyashree M, Mani MK and Karunasagar I, 2022. Association of exopolysaccharide genes in biofilm developing antibiotic-resistant *Pseudomonas aeruginosa* from hospital wastewater. J Water Health 20:176-84.

Elhariri M, Hamza D, Elhelw R, *et al*., 2017. Extended-spectrum beta-lactamase-producing *Pseudomonas aeruginosa* in camel in Egypt: Potential human hazard. Ann Clin Microbiol Antimicrob 16:1-6.

Elnawawi FA, Attala OA and Saleh S, 2012. Enteropathogens of public health importance in imported frozen meat and chicken. Int J Microbiol Res 3:59-63.

Elsayed MSA, Ammar AM, Al Shehri ZS, *et al*., 2016. Virulence repertoire of *Pseudomonas aeruginosa* from some poultry farms with detection of resistance to various antimicrobials and plant extracts. Cell Mol Biol 62:124.

Fakhkhari P, Tajeddin E, Azimirad M, *et al*., 2022. Involvement of *Pseudomonas aeruginosa* in the occurrence of community and hospital acquired diarrhea and its virulence diversity among the stool and the environmental samples. Int J Environ Health Res 32:61-71.

Ghafoor A, Hay ID and Rehm BH, 2011. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. Appl Environ Microbiol 77:5238-46.

Goltermann L and Tolker-Nielsen T, 2017. Importance of the exopolysaccharide matrix in antimicrobial tolerance of *Pseudomonas aeruginosa* aggregates. Antimicrob Agents Chemother 61:e02696-16.

Hou W, Sun X, Wang Z, *et al*., 2012. Biofilm-forming capacity of *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* from ocular infections. Invest Ophthalmol Vis Sci 53:5624-31.

Ijaz M, Siddique AB, Rasool MH, *et al*., 2019. Frequency of multi drug resistant *Pseudomonas aeruginosa* in different wound types of hospitalized patients. Pak J Pharm Sci 32:865-70.

İnat G, Sırıken B, Başkan C. *et al*., 2021. Quorum sensing systems and related virulence factors in *Pseudomonas aeruginosa* isolated from chicken meat and ground beef. Sci Rep 11:15639.

Maurice NM, Bedi B and Sadikot RT, 2018. *Pseudomonas aeruginosa* Biofilms: host response and clinical implications in lung infections. Am J Respir Cell Mol Biol 58:428-39.

Mouiche MM, Moffo F, Akoachere JF, *et al*., 2019. Antimicrobial resistance from a one health perspective in Cameroon: A systematic review and meta-analysis. BMC Public Health 19: 1-20.

Nader MI, Kareem AAA, Rasheed MN, *et al*., 2017. Biofilm formation and detection of pslÁ gene in multidrug resistant *Pseudomonas aeruginosa* isolated from Thi-Qar, Iraq. Iraqi J Biotechnol 16:89-103.

Nagarajan V, Wahab A, Shivraj S, *et al*., 2018. Study of bacterial contamination of raw meat in Hyderabad. MOJ Proteomics Bioinform 7:46-51.

Nawaz Z, Aslam B, Zahoor MA, *et al*., 2021. Frequency of extended spectrum beta lactamase producing *Escherichia coli* in fresh and frozen meat. Pak Vet J 41:102-6.

Nordmann P, Poirel L, Walsh TR, *et al*., 2011. The emerging NDM carbapenemases. Trends Microbiol 19:588-95.

Olsen I, 2015. Biofilm-specific antibiotic tolerance and resistance. Eur J Clin Microbiol Infect Dis 34:877-86.

Passos da Silva D, Matwichuk ML, Townsend DO, *et al*., 2019. The *Pseudomonas aeruginosa* lectin LecB binds to the exopolysaccharide Psl and stabilizes the biofilm matrix. Nat Commun 10:2183.

Qureshi R, Shafique M, Shahid M, *et al*., 2018. Molecular detection of *bla*VIM Metallo-β-lactamase producing clinically isolated *Pseudomonas aeruginosa* from tertiary care hospital, Faisalabad. Pak J Pharm Sci 31:2673-7.

Rezai MS, Salehifar E, Rafiei A, *et al*., 2015. Characterization of multidrug resistant extended-spectrum beta-lactamase-producing *Escherichia coli* among uropathogens of pediatrics in North of Iran. Biomed Res Int: 309478.

Saxena S, Banerjee G, Garg R and Singh M, 2014. Comparative study of biofilm formation in *Pseudomonas aeruginosa* isolates from patients of lower respiratory tract infection. J Clin Diagn Res 8:9-11.

Stellato G, La Storia A, De Filippis F, *et al*., 2016. Overlap of spoilage-associated microbiota between meat and the meat processing environment in small-scale and large-scale retail distributions. Appl Environ Microbiol 82:4045-54.

Tuon FF, Dantas LR, Suss PH and Tasca Ribeiro VS, 2022. Pathogenesis of the *Pseudomonas aeruginosa* biofilm: A review. Pathogens 11:300.

Uddin TM, Chakraborty AJ, Khusro A, Zidan, *et al*., 2021. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. J Infect Pub Health 14:1750-66.

Wickramasinghe NN, Ravensdale J, Coorey R, Chandry SP and Dykes GA, 2019. The predominance of psychrotrophic *Pseudomonads* on aerobically stored chilled red meat. Compr Rev Food Sci Food Saf 18:1622-35.