EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
C
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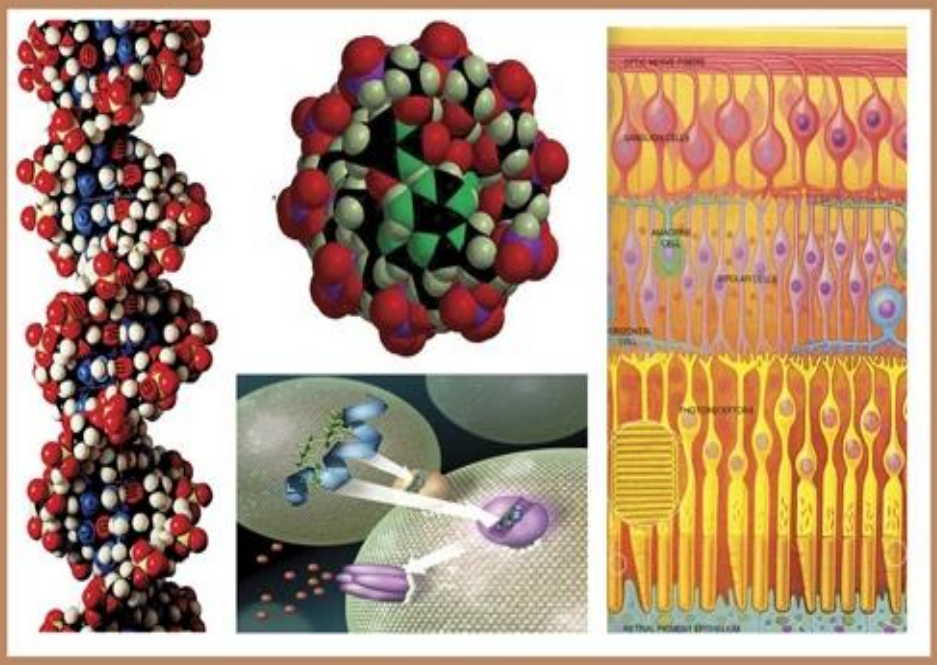

ISSN
2090-0767

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**Vol. 15 No. 1 (2023)**

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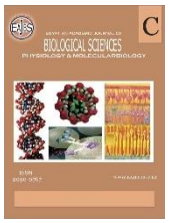
**Citation**: *Egypt.Acad.J.Biolog.Sci. ( C.Physiology and Molecular biology ) Vol. 15(1) pp55-61 (2023)*
**DOI: 10.21608/EAJBSC.2023.281336**

**Egypt. Acad. J. Biolog. Sci., 15(1):55-61 (2023)**

**Egyptian Academic Journal of Biological Sciences**
**C. Physiology & Molecular Biology**
**ISSN 2090-0767**
**www.eajbsc.journals.ekb.eg**



# Antibiotic Susceptibility and Plasmid Profiles of *Pseudomonas aeruginosa* from Humans, Animals, And Plants Sources

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**ARTICLE INFO**
**Article History**
Received:13/12/2022
Accepted:21/1/2023
Available:24/1/2023
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***Keywords***:
Antibiotics, *Pseudomonas aeruginosa*, Extended-spectrum beta-lactamase (ESBL), Plasmid.

**ABSTRACT**

The presence of multidrug-resistant organisms, often known as MDROs, is a significant risk to public health all over the world. Pseudomonas aeruginosa clinical isolates continue to be one of the most researched MDROs; nevertheless, there is a lack of information in Pakistan about the sensitivity of its animal and plant isolates to antipseudomonal drugs. *Pseudomonas aeruginosa* was isolated from 25 vegetable samples, 25 animal samples, and 50 clinical samples, for a total of 100 samples. Standard biochemical techniques were used to determine the identities of all the isolates. One hundred *P. aeruginosa* isolates were tested for their susceptibility to seven antipseudomonal drugs via disc diffusion AST, phenotypic detection of ESBL via double disc synergy test (DDST), and plasmid extraction on twenty isolates based on their resistance to two or more classes of antibiotics via alkaline lysis and analysis using Lambda DNA/Hind lll marker. In the overall assay, piperacillin-tazobactam and imipenem had the highest susceptibilities, whereas ceftazidime and carbenicillin had the highest resistances. 15 of 100 isolates 10 vegetable, 3 clinical, and 2 poultry—showed synergy with the beta-lactamase inhibitor, demonstrating ESBL generation by DDST. Plant, poultry, cow, and clinical isolates have plasmids. 6 strains contained 1 plasmid, 5 had 2–4, and 1 had 5. Plasmids are 1–25kbp. ESBL and Plasmids in the isolates reveal diverse resistance mechanisms. Multiple-resistance *P. aeruginosa* isolates in plants and animals are a public health risk. 6 strains contained 1 plasmid, 5 had 2–4, and 1 had 5. Plasmids are 1–25kbp. ESBL and Plasmids in the isolates reveal diverse resistance mechanisms. Multiple-resistance *P. aeruginosa* isolates in plants and animals are a public health risk.

## INTRODUCTION

There is presently a worldwide public health crisis due to the rise of antimicrobial resistance (AMR) among bacterial species. Different bacterial species often have unique combinations of antibiotic-resistance genes, transfer mechanisms, and antibiotic-resistance reservoirs that contribute to antimicrobial resistance (AMR). The indiscriminate use of antibiotics and prescriptions has been connected to the spread of antibiotic-resistant genes in the environment, as has the use of antibiotics in agricultural and animal management (Aibinu *et al.,* 2007). In agriculture, the management of livestock and crop production frequently involves the use of antibiotics in the form of consumables, which are generally excreted in the environment as biologically active metabolites. This can boost selection pressure and promote the emergence of antibiotic-resistant bacterium strains. Previous research on bacteria isolated from raw vegetables sold in various markets and restaurants that are resistant to commercially available antibiotics has been recorded (Akinjogunla *et al.,* 2010). Other research has focused on the prevalence of antibiotic-resistant bacteria in pets, food-processing, and agricultural animals. Documented findings of a high occurrence of AMR bacteria in chicken, cattle, and other food processing animals, all of which are often consumed, are noteworthy. It is also considered that health professionals and their families may act as a conduit for resistance genes to enter the community and hospital environments, where additional disease transmission is feasible (Alikhani *et al.,* 2014). *Pseudomonas aeruginosa*, a Gram-negative, rod-shaped, motile bacterium, has been linked to a number of human illnesses and may be found in a wide range of environments. *Pseudomonas aeruginosa* infections are notoriously difficult to treat because the bacteria have evolved strategies for resisting both naturally occurring and artificially introduced antibiotics (Ayeni *et al.,* 2016). *P. aeruginosa's* amazing antimicrobial drug resistance mechanisms and its growth assist the pathogen. Efflux pump, mobile genetic element, and hydrolysing enzyme antimicrobial resistance have all been identified in *P. aeruginosa* clinical isolates (Barber *et al.,* 2003). One (1) of the most important of these is the production of β-lactamases, which make them resistant to beta-lactam drugs. Over the past few decades, enzymes in this group have been found in *P. aeruginosa*, especially extended-spectrum beta-lactamase (ESBL) enzymes like OXA, VEB, PER, SHV, and TEM (Diarrassouba *et al.,* 2007). *Pseudomonas aeruginosa* is one of the main bacterial pathogens often isolated from a wide range of clinical and environmental samples, and it has been linked to as many as 10% of all human illnesses (Heil *et al.,* 2016).It is also one of the primary causes of illnesses in cattle as well as companion animals such as otitis, mastitis, endometritis, hemorrhagic pneumonia, and urinary tract infections. Variables that had a significant role in the development of multidrug-resistant organisms (MDROs) at the community hospital illnesses (Diarrassouba *et al.,* 2007). Unfortunately, the unchecked spread and proliferation of AMR genes among pathogenic bacteria is the cause of high morbidity and mortality among critically ill patients. Moreover, it has been discovered that this phenomenon contributes significantly to the rising cost of healthcare due to the need for longer hospitalisation and much more expensive prescription drugs for treatment methods annually. Because it is so prevalent in the natural world, there is a significant potential for the transmission of antimicrobial genes across plants, animals, and people. In despite of this, a reason for this study was formed since there is a scarcity of information in Pakistan about the susceptibility of anti-pseudomonas medications to organisms of animal and plant origin. This research was conducted with the intention of providing a snapshot of their resistance to anti-pseudomonas

medication and the generation of ESBL. Because of this, we will have a better understanding of how AMR is spread in our environment, which will allow us to develop effective countermeasures. Clinical isolates of *P. aeruginosa* taken from a variety of samples were also included in order to make a precise comparison.

## MATERIALS AND METHODS

Between the years 2021 and 2022, a total of 100 samples were taken, with 50 of them being clinical samples, 25 of them being vegetable samples, 10 of them being cow samples, and 15 of them being poultry samples. From those 100 samples, consecutive and non-duplicated *P. aeruginosa* strains were randomly isolated, with 50 of them coming from clinical isolates, 25 from vegetable samples, 10 from cow samples, and 15 from poultry samples. These samples were gathered from various farms, hospitals, and geographical areas across the kpk states in south-western Peshawar, Pakistan, which results in these isolates having distinct epidemiological associations with one another. All samples were obtained by informed consent of the patients in this study, proper ethical clearance approval was obtained from Abasyn University Health Research Ethics committee 10/09/2022.

**Antimicrobial Susceptibility Testing:**

Antimicrobial susceptibility testing was performed and interpreted according to the Clinical Laboratory Standard Guidelines 2010 (CLSI 2010) on Mueller–Hinton agar (Oxoid UK) for 7 antibiotics: piperacillin–tazobactam (100/10 μg), cefepime (35 μg), imipenem (20 μg), amikacin (30 μg), ciprofloxacin (50 μg), carbenicillin (100 μg), and ceftazidime (50 μ). Quality control employed *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 26733 (Faldynova *et al.,* 2013).

**Phenotypic Detection of Esbl by Double-Disk Synergy Testmethod (Ddst):**

All beta-lactam-resistant and intermediate-resistant strains were tested using a modified disc diffusion assay on Mueller–Hinton agar. A 0.5 Mac Farland standard suspension of the test microorganisms were equally inoculated on Mueller–Hinton agar by gently swabbing with sterile sticks. Disks containing 40 μg of aztreonam, ceftazidime, and cefepime were put 25 mm apart (centre to centre) successively from piperacillin/tazobactam (120 μg) and incubated for 18–24 h at 37 °C.

**Plasmid Isolation and Profiling:**

Plasmid DNA was extracted from 20 ESBL-positive and multidrug-resistant bacteria. Briefly, Mueller–Hinton broth bacterial cultures were suspended in microcentrifuge tubes with lysis buffer, boiled for 15 min at 70 °C, and mixed with an equivalent volume of phenol: (26:25:1) for plasmid extraction. Supernatants were put on a 1% agarose gel inTris–acetate–EDTA buffer and ran at 80 V for an hour. Representative plasmids were digested with EcoRI and compared to Lambda DNA/HindIII marker motilities on an agarose gel to estimate the molecular size. After staining with gel red and UV-transmitted light, the Gel Documentation system photographed Plasmid DNA (Gençer, *et al.,* 2002).

**Statistical Analysis:**

The Chi-square test was performed to assess the frequency of isolates retrieved from different sample groups at $\alpha = 5\%$ significance.

## RESULTS AND DISCUSSION

The overall distribution of *Pseudomonas aeruginosa* among the samples obtained is as follows: 25% of the bacteria were found in chicken, 25% of the bacteria were found in vegetables, 50% of the bacteria were found in clinical isolates, and 15% of the bacteria were found in cow dungs. The findings of the antimicrobial susceptibility tests for *P. aeruginosa* demonstrate a high degree of resistance (100%) to ceftazidime among clinical and animal isolates, but the results for vegetable isolated strains show a resistance rate of 12 (73.3%). (Tables 1, 2, 3, 4 & 5). The total susceptibility analyses revealed that 70% of the isolates were resistant to carbenicillin, while 91.1% of the isolates were resistant to

ceftazidime. The susceptibilities of the isolates were as follows: 91%, 94%, 95%, 96%, and 85% for imipenem, piperacillin–tazobactam, cefepime, and ciprofloxacin, respectively. Fifteen (15) out of a total of one hundred *P. aeruginosa* isolates were positive for the phenotypic detection of ESBL. As a consequence, a total percentage of 40% positive ESBL was found in the whole sample. This was constituted of 12% (10/17) from chicken isolates, 15% (4/25) from clinical, and 20% (15/25) from vegetables. In every single one of the isolates, ESBL was found at a distance of 26 mm with synergy towards cefepime and aztreonam. All the isolates harbouring plasmids were resistant to carbenicillin and ceftazidime but shows complete susceptibilities to cefepime, imipenem, and piperacillin-tazobactam antibiotics. Statistical analysis shows no significant difference in the prevalence of isolate recovered from the various categories at $\alpha$ = 5 % significant level.

**Table 1** Antibiotic resistance patterns of the 25/100 *P. aeruginosa* isolates from ready-to-eat vegetables in percentage distribution.

| ype of antibiotic | No (%) of resistant | No (%) of intermediate | No (%) of susceptible |
|---|---|---|---|
| Carbenicillin (100 µg) | 11 (89.0) | 8 (15.18) | 14 (25.9) |
| Amikacin (30 µg) | 16 (70.9) | 10 (19.4) | 9 (90.7) |
| Ceftazidime (30 µg) | 10 (83.3) | — | 10 (16.7) |
| Cefepime (30 µg) | — | — | 19 (100) |
| Ciprofloxacin (5 µg) | — | — | 13 (100) |
| Imipenem (10 µg) | — | — | 15 (100) |
| Piperacillin–Tazobactam (110 µg) | — | — | 14 (100) |

**Table 2:** Antibiotic resistance patterns of the 15/25 *P. aeruginosa* isolates from cow

| Type of antibiotic | No (%) of resistant | No (%) of intermediate | No (%) of susceptible |
|---|---|---|---|
| Carbenicillin (100 µg) | 13 (42.9) | 1 (14.2) | 3 (42.9) |
| Amikacin (30 µg) | — | — | 7 (100) |
| Ceftazidime (30 µg) | 7 (100) | — | — |
| Cefepime (30 µg) | — | — | 7 (100) |
| Ciprofloxacin (5 µg) | — | — | 7 (100) |
| Imipenem (10 µg) | — | — | 7 (100) |
| Piperacillin–Tazobactam (110 µg) | — | — | 7 (100) |

**Table 3:** Percentage distribution of antibiotic resistance patterns of the 10/25 *P. aeruginosa* isolates from poultry**.**

| Type of antibiotic | No (%) of resistant | No (%) of intermediate | No (%) of susceptible |
|---|---|---|---|
| Carbenicillin (100 µg) | 8 (82.4) | — | 3 (17.6) |
| Amikacin (30 µg) | 3 (17.6) | 5 (11.8) | 8 (70.6) |
| Ceftazidime (30 µg) | 6 (100) | — | — |
| Cefepime (30 µg) | — | — | 7 (100) |
| Ciprofloxacin (5 µg) | — | — | 8(100) |
| Imipenem (10 µg) | — | — | 7 (100) |
| Piperacillin–Tazobactam (110 µg) | — | — | 9 (100) |

**Table 4:** Percentage distribution of antibiotic resistance patterns of 50/100 clinical *P. aeruginosa* isolates

| Type of antibiotic | No (%) of resistant | No (%) of intermediate | No (%) of susceptible |
|---|---|---|---|
| Carbenicillin (100 µg) | 18(18.2) | 5 (22.7) | 23 (59.1) |
| Amikacin (30 µg) | 9 (9.1) | — | 20 (90.9) |
| Ceftazidime (30 µg) | 22 (100) | — | — |
| Cefepime (30 µg) | 11 (4.55) | 1 (4.55) | 22 (90.9) |
| Ciprofloxacin (5 µg) | 41 (18.2) | — | 28 (81.8) |
| Imipenem (10 µg) | 11 (4.5) | — | 22 (95.5) |
| Piperacillin–Tazobactam (110 µg) | 19 (4.5) | — | 21 (95.5) |

**Table 5:** Antibiotic resistance patterns of the 100 *Pseudomonas aeruginosa* isolates in percentage distribution.

| Type of antibiotic | No (%) of resistant | No (%) of intermediate | No (%) of susceptible |
|---|---|---|---|
| Amikacin (30 µg) | 8 | 7 | 88 |
| Carbenicillin (100 µg) | 50 | 15 | 35 |
| Ceftazidime (30 µg) | 88 | — | 12 |
| Cefepime (30 µg) | 11 | 10 | 79 |
| Ciprofloxacin (5 µg) | 12 | 17 | 71 |
| Imipenem (10 µg) | 18 | 9 | 73 |
| Piperacillin–tazobactam (110 µg) | 10 | 19 | 71 |

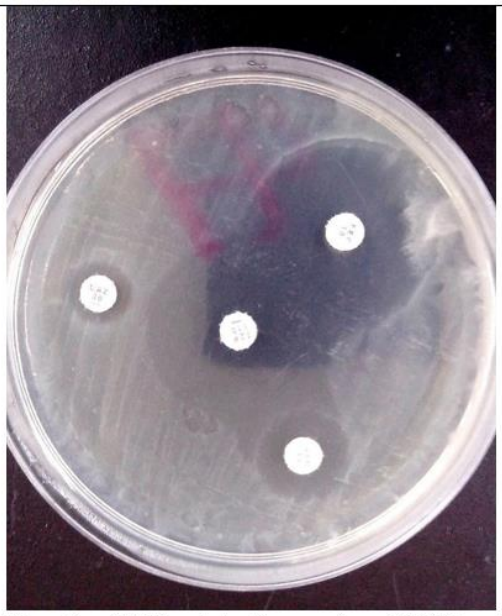

**Fig. 1:** ESBL detection using DDST with piperacillin/tazobactam as beta-lactamase inhibitor

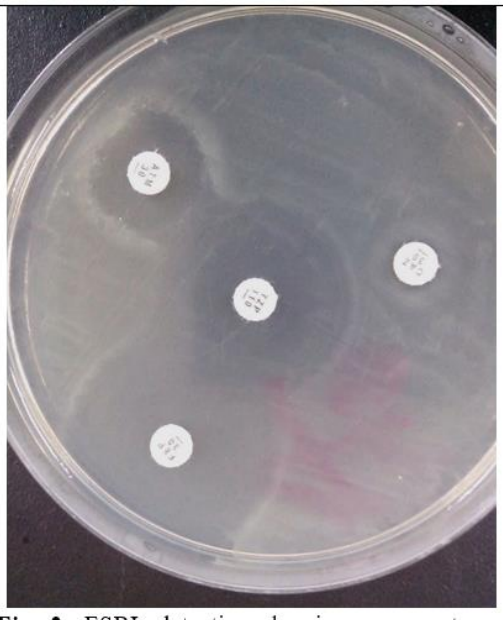

**Fig. 2:** ESBL detection showing synergy towards cefepime and aztre- onam antibiotics disk

As a conclusion of the examination that was conducted for this study, the diverse distribution of *P. aeruginosa* throughout the samples has been shown. This distribution is suggestive of the ubiquity of the pathogen, particularly in the environment. The count of *P. aeruginosa* discovered in ready-to-eat vegetables was much higher than the counts observed in animal and human sources of isolation. There have been prior reports of isolating a similar strain of *P. aeruginosa* from vegetables that had a high incidence (Guardabassi, *et al.*, 2004). The presence of *P. aeruginosa* in animal faeces that are either purposefully utilised as fertilisers or unavoidably present in the soil in the form of droppings from free-range animals shows that there is a potential source of contamination of raw vegetables as well as other foods. The phenotypic identification of

extended-spectrum beta-lactamase-producing bacteria (ESBL) among the isolates used in this research was accomplished using a technique that had been modified by the addition of a fourth-generation cephalosporin (cefotaxime) and tazobactam as the β – inhibitor. Cefepime resists *P. aeruginosa's* AmpC beta-lactamase, which typically masks ESBL phenotypes (Huhulescu, *et al.*,2011). Tazobactam inhibits ESBL and AmpC beta-lactamase almost times better than clavulanic acid, according to research. A total of 42 % of the 100 isolates tested positive for ESBL phenotypically, showing synergy with carbapenems. This is a better outcome than the stated frequencies of 6.0% in India and 9.1% in Iran. However, this contrasts with the observations of the previous two Pakistani studies. Which found higher frequencies of ESBL in *P. aeruginosa.* However, percentages can sometimes reflect the population of the study sample, so absolute differences may not be reflected (Figs. 1 & 2). It is concerning because the current research found a significant level of resistance to antibiotics belonging to the beta-lactam class, specifically the third-generation cephalosporin (ceftazidime) and carboxypenicillins (carbenicillin). Ceftazidime resistance was found in ninety (90%) percent of the *P. aeruginosa* samples taken from clinical, animal, and plant sources, whereas carbenicillin resistance was found in sixty-five (65%) percent of the *P. aeruginosa* samples. Previous research suggests that the majority of *P. aeruginosa's* resistance to ceftazidime and carbenicillin is caused by the presence of extended-spectrum beta-lactamases, such as OXA types and PER. These extended-spectrum beta-lactamases are frequently found in *P. aeruginosa* and are typically encoded on mobile genetic elements, such as plasmids and integrons. Previous research also suggests that these extended-spectrum beta- (Kidd e*t al.,* 2012).

## Conclusion

This study examines the antimicrobial susceptibility profile of *P. aeruginosa* isolated from plants, animals, and people to anti-pseudomonas antibiotics. The prevalence of antibiotic resistance, the discovery of ESBLs, and the presence of plasmids among the isolates in this study are concerning since it shows their potential contribution toward the spread of antimicrobial resistance. The presence of MDROs from both animals and plants in this study shows that there is a reasonable possibility of human vulnerability to AMR bacteria from these sources. Although this study did not give clear proof of their connection and the extent to which these sources contribute to antimicrobial resistance in contrast to the determination of trace and hospital settings, these sources may contribute to AMR. Urgent measures are required to curb the persistent misuse of antibiotics, particularly in cattle production. Further research will be conducted to fill in the gaps in this study, such as identifying the link between AMR bacteria found in animals and people (Haenni, *et al.,* 2015)

**Authors' Contributions:**All authors read and approved the final manuscript.

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