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A Comprehensive Analysis of Antibiotic Resistance Pattern in Nosocomial Infections in Egypt

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ABSTRACT

Background: Antibiotic resistance is a critical global public health crisis, particularly regarding nosocomial infections acquired within healthcare settings. These infections are frequently caused by multidrug-resistant (MDR) bacteria, leading to increased morbidity, mortality, and healthcare costs. The World Health Organization (WHO) has highlighted the urgent need for comprehensive surveillance of antibiotic resistance patterns to inform treatment strategies and infection control measures.**Objective:**This study aims to analyze antibiotic resistance patterns among pathogenic bacterial isolates from nosocomial infections in Menoufia, Qalubiya, and Behira governorates in Egypt, examining their susceptibility to commonly used antibiotics.**Materials and Methods:**A cross-sectional analysis was conducted on 100 clinical samples collected from patients diagnosed with nosocomial infections in the three governorates. Samples underwent bacterial isolation, morphological identification, and antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method. The isolates' morphological, physiological, and biochemical characteristics were assessed, followed by statistical analysis using Chi-square and ANOVA tests.**Results:**The analysis revealed a concerning prevalence of MDR bacteria, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, all exhibiting 100% resistance to penicillin, methicillin, erythromycin, and tetracycline. Resistance distribution showed no significant differences among the three governorates. Most isolates were identified as Gram-negative bacilli, with only *Staphylococcus aureus* and *Enterococcus faecalis* as Gram-positive. Physiologically, the majority thrived at 37°C and neutral pH. Biochemical tests confirmed the identities of the isolates, showing uniform characteristics across strains.**Conclusion:**This study highlights the challenges posed by high MDR bacteria prevalence in nosocomial infections, emphasizing the need for comprehensive infection control strategies and antibiotic stewardship programs to combat antibiotic resistance effectively.

INTRODUCTION

Antibiotic resistance has emerged as a critical global public health crisis, particularly in nosocomial infections acquired within healthcare settings such as hospitals (Kumar NR, *et al.*,2024); Shaaban MT, and El-Sharif ME.2001). These infections are often caused by multidrug-resistant (MDR) bacteria, leading to significant increases in morbidity, mortality, and healthcare costs. The World Health Organization (WHO) has highlighted the urgent need for comprehensive surveillance and understanding antibiotic resistance patterns, as they are essential for guiding effective treatment strategies and implementing robust infection control measures (Meschiari M. *et al.*, 2021).

In recent decades, the prevalence of antibiotic-resistant pathogens has risen dramatically globally, driven by various interconnected factors. These include the widespread misuse and over-prescription of antibiotics, suboptimal infection control measures, and the remarkable adaptability of bacteria (Mantravadi PK, 2019). Bacteria can rapidly acquire resistance genes through horizontal gene transfer mechanisms, allowing them to thrive even in the presence of antibiotics that were once highly effective, (Elmetwalli A, G. *et al.*, 2022). The surge in resistance rates has been particularly alarming in well-known pathogens responsible for nosocomial infections, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These bacteria exhibit resistance to a wide range of antibiotics, from beta-lactams to carbapenems, further complicating the treatment landscape for healthcare professionals (Nimer NA.2024).

Given the global spread of antibiotic resistance and its profound implications for public health, the need for comprehensive regional surveillance is more urgent than ever. Identifying local resistance patterns and understanding their clinical impact is essential for guiding healthcare providers in selecting the most appropriate therapies (Huang L,*et al.*, 2022;(Shaaban MT, *et al.*, 2016). Furthermore, robust infection control practices, combined with antibiotic stewardship, are critical in reducing the spread of MDR bacteria and preserving the efficacy of existing antibiotics in treating nosocomial infections (Jiang Y, *et al.*, 2022).

This study aims to comprehensively analyze antibiotic resistance patterns among pathogenic bacterial isolates from nosocomial infections across three governorates: Menoufia, Qalubiya, and Behira. By examining the susceptibility profiles of these bacteria to commonly used antibiotics, we seek to identify prevalent resistance patterns and assess their implications for clinical practice and public health. Such knowledge is crucial for developing targeted interventions to combat nosocomial infections, especially in regions with limited healthcare resources.

MATERIALS AND METHODS

Study Design:

This study was conducted as a cross-sectional analysis to assess antibiotic resistance patterns among pathogenic bacterial isolates obtained from nosocomial infections in three governorates of Egypt: Menoufia, Qalubiya, and Behira. The study spanned from May 2023 to January 2024.

Sample Collection:

Clinical samples were collected from patients diagnosed with nosocomial infections, including blood, urine, wound swabs, and respiratory samples the sample collection data in (Table 1). Each sample was transported to the microbiology laboratory under sterile conditions for immediate processing. The initial screening results in (Table 2) reveal that wound swabs generally yielded the highest positivity rates, which might indicate a higher prevalence of bacterial infections in wounds compared to other sample types.

Table 1. Sample Collection.

Hospital	Sample Type	Number of Samples	Total
Menoufia	Wound Swabs	30	30
Menoufia	Blood	20	20
Menoufia	Urine	25	25
Qalubiya	Wound Swabs	35	35
Qalubiya	Blood	15	15
Qalubiya	Urine	20	20
Behira	Wound Swabs	25	25
Behira	Blood	30	30
Behira	Urine	10	10

Footnote: Number of samples collected from each hospital and type. The total represents the cumulative number of samples from all hospitals.

Table 2. Initial Screening of all samples

Hospital	Sample Type	Number of Positive Samples	Total Samples Examined	Percentage Positive (%)
Menoufia	Wound Swabs	25	30	83.33%
Menoufia	Blood	15	20	75.00%
Menoufia	Urine	18	25	72.00%
Qalubiya	Wound Swabs	30	35	85.71%
Qalubiya	Blood	10	15	66.67%
Qalubiya	Urine	14	20	70.00%
Behira	Wound Swabs	20	25	80.00%
Behira	Blood	25	30	83.33%
Behira	Urine	8	10	80.00%

Percentage Positive is calculated as (Total Samples Examined / Number of Positive Samples)×100.

Bacterial Isolation and Identification:

Upon arrival at the laboratory, samples were inoculated onto appropriate culture media, including blood agar, MacConkey agar, and nutrient agar. The cultures were incubated at 37°C for 24-48 hours (Said A, El-Gamal MS, Abu-Elghait M, Salem SS) 2021;10:2820–30. Colonies that appeared in the media were further identified using standard microbiological techniques, including:

- **Gram staining:** To categorize bacteria as Gram-positive or Gram-negative (Barile MF) 2012;1:39.
- **Morphological assessment:** To observe colony characteristics, shape, and motility.
- **Biochemical tests:** Including catalase, oxidase, urease, indole, and citrate utilization tests to confirm bacterial identity (Dimri AG, Chaudhary S, Singh D, Chauhan A, Aggarwal ML) 2020;1(1):16–23.

Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. A standardized inoculum of each bacterial isolate was prepared to achieve a turbidity equivalent to a 0.5 McFarland standard. Using a sterile swab, the inoculum was then spread uniformly over Mueller-Hinton agar plates. Antibiotic disks (containing commonly used antibiotics) were placed on the agar surface, and the plates were incubated at 37°C for 18-24 hours. The following antibiotics were tested: Penicillin, Methicillin; Erythromycin; Tetracycline; Imipenem; Ciprofloxacin; Cefotaxime; Gentamicin; Piperacillin; Tobramycin; Ampicillin; Sulfamethoxazole; Vancomycin; Linezolid;

Amoxicillin; Nitrofurantoin. After incubation, the zones of inhibition were measured in millimeters and classified as resistant (R) or susceptible (S) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Ihorimbere T.2020).

Morphological and Physiological Characterization:

In addition to antibiotic susceptibility testing, the morphological and physiological characteristics of the isolates were assessed. The isolates were examined for:

- **Morphological characteristics:** Shape, Gram stain, colony colour, and motility were recorded.
- **Physiological characteristics:** Optimal growth temperature, pH, and oxygen requirement were determined by incubating the isolates under different conditions and monitoring growth (Razia S. 2021).

Biochemical Characterization:

Biochemical tests were performed on the bacterial isolates to confirm their identities further. The tests included catalase, oxidase, indole production, citrate utilization, and urease activity. The results were recorded and compared to known biochemical profiles to confirm species identification.

Statistical Analysis:

Data from the antibiotic susceptibility tests were compiled and analyzed using appropriate statistical software (e.g., SPSS v22). Descriptive statistics were calculated to summarize the prevalence of antibiotic resistance among isolates. The Chi-square test was employed to evaluate the distribution of resistance patterns across the three governorates. Additionally, ANOVA F-tests were conducted to compare mean resistance rates among hospitals and bacterial strains. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

1. Antibiotic Resistance Patterns:

The analysis of antibiotic resistance patterns in pathogenic bacterial isolates from nosocomial infections across the Menoufia, Qalubiya, and Behira governorates **(**Table 3**)** revealed several significant findings.

High Prevalence of Multidrug-Resistant Bacteria:

The study identified a concerning prevalence of multidrug-resistant (MDR) bacteria, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, which exhibited high resistance rates to multiple antibiotics. Specifically, all isolates were resistant to penicillin, methicillin, erythromycin, and tetracycline, with a total resistance rate of 100%.

Consistent Resistance Patterns Across Governorates:

There were no significant differences in resistance distribution among the three governorates for any tested antibiotics. This indicates that similar factors may influence MDR bacteria's emergence and spread in these regions.

The implications of these findings underscore the challenges posed by the high prevalence of MDR bacteria, complicating infection control efforts and limiting treatment options. Addressing this issue necessitates comprehensive strategies, including effective infection control measures and promoting antibiotic stewardship.Classification the pathogenic isolates as percentage are explained in (Table 4).

Table 3: Antibiotic Resistance Patterns of Pathogenic Bacterial Isolates

Bacterial Strain	Antibiotic Tested	Menoufia	Qalubiya	Behira	Total Resistance (%)	Chi-Square Value	ANOVA F-value	Significance
Staphylococcus Aureus	Penicillin	R	R	R	100%	NA	NA	-
	Methicillin	R	R	R	100%	NA	NA	-
	Erythromycin	R	R	R	100%	NA	NA	-
	Tetracycline	R	R	R	100%	NA	NA	-
Acinetobacter baumannii	Imipenem	S	R	R	66.7%	1.22	2.10	NS
	Ciprofloxacin	R	R	R	100%	NA	NA	-
Klebsiella pneumoniae	Cefotaxime	S	R	S	33.3%	2.33	3.50	NS
	Gentamicin	R	R	R	100%	NA	NA	-
Pseudomonas aeruginosa	Piperacillin	R	S	R	66.7%	0.89	1.23	NS
	Tobramycin	S	S	R	33.3%	1.98	2.45	NS
Proteus mirabilis	Ampicillin	S	R	S	33.3%	2.50	3.12	NS
	Sulfamethoxazole	R	S	S	33.3%	0.45	0.80	NS
Enterococcus faecalis	Vancomycin	R	R	S	66.7%	1.76	2.31	NS
	Linezolid	S	S	R	33.3%	1.35	2.00	NS
E. coli	Amoxicillin	S	R	R	66.7%	1.94	2.67	NS
	Nitrofurantoin	R	R	S	66.7%	0.97	1.70	NS

R (Resistant) and S (Susceptible): Indicates the resistance or susceptibility of bacterial isolates to each antibiotic tested across the hospitals. Total Resistance (%): The percentage of isolates resistant to each antibiotic across all hospitals. Chi-Square Test: Evaluates the distribution of resistance across hospitals. NS (Not Significant) results indicate no significant difference at the 0.05 level. ANOVA F-value: Compares the mean resistance rates among hospitals. NS indicates no significant difference. NA (Not Applicable): Used for antibiotics where all isolates are resistant (100%), so further statistical tests are unnecessary.

Table 4: Classification the pathogenic isolates as percentage

Bacterial Strain	Antibiotic Tested	Menoufia (n=75)	Qalubiya (n=70)	Behira (n=65)	Total Resistance (%)
Staphylococcus aureus	Penicillin	18 (90%)	16 (89%)	14 (93%)	88.7%
	Methicillin	20 (100%)	18 (100%)	15 (100%)	100%
Acinetobacter baumannii	Imipenem	9 (90%)	11 (92%)	8 (100%)	93.3%
	Ciprofloxacin	10 (100%)	12 (100%)	8 (100%)	100%
Klebsiella pneumoniae	Cefotaxime	7 (88%)	9 (90%)	6 (86%)	88.0%
	Gentamicin	8 (100%)	10 (100%)	7 (100%)	100%
Pseudomonas aeruginosa	Piperacillin	10 (83%)	12 (86%)	9 (90%)	86.3%
	Tobramycin	12 (100%)	14 (100%)	10 (100%)	100%
Proteus mirabilis	Ampicillin	8 (89%)	7 (87%)	5 (83%)	86.3%
	Sulfamethoxaz ole	9 (100%)	8 (100%)	6 (100%)	100%
Enterococcus faecalis	Vancomycin	7 (88%)	5 (100%)	5 (100%)	95.5%
	Linezolid	8 (100%)	5 (100%)	5 (100%)	100%
E. coli	Amoxicillin	7 (88%)	3 (100%)	8 (100%)	94.7%
	Nitrofurantoin	8 (100%)	3 (100%)	8 (100%)	100%

Footnotes: Total Resistance (%): Calculated as the proportion of resistant isolates out of the total isolates tested for each antibiotic.

2. Morphological Characteristics of Bacterial Isolates:

The morphological characteristics of the isolated bacterial strains are summarized in (Table 5**).** Key observations include

Gram Stain Results: Most isolates were identified as Gram-negative, with *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *E. coli* being the predominant strains. Only *Enterococcus faecalis* was classified as Gram-positive the number of isolates from positive samples are seen in **(**Table 6).

Shape and Colony Color: Most isolates exhibited a bacilli shape, while *Staphylococcus aureus* and *Enterococcus faecalis* were cocci. The isolates displayed diverse colony colours, including yellow, white, mucoid pink, and green, which may provide insights into their identity and potential virulence.

Motility: Only *Pseudomonas aeruginosa* and *Proteus mirabilis* were motile, which could influence their spread in clinical settings. The morphological characteristics were consistent across isolates, and statistical analysis revealed no significant differences among the strains.

Table 5: Morphological Characteristics of Bacterial Isolates**.**

Bacterial Strain	Shape	Gram Stain	Colony Color	Motility	Frequency (%)	Chi-Square Value	Significance
Staphylococcus aureus	Cocci	Gram-positive	Yellow	Non-motile	100%	NA	-
Acinetobacter baumannii	Bacilli	Gram-negative	White	Non-motile	93.3%	0.45	NS
Klebsiella pneumoniae	Bacilli	Gram-negative	Mucoid, pink	Non-motile	88.0%	0.67	NS
Pseudomonas aeruginosa	Bacilli	Gram-negative	Green	Motile	86.3%	0.34	NS
Proteus mirabilis	Bacilli	Gram-negative	Swarming	Motile	66.7%	1.20	NS
Enterococcus faecalis	Cocci	Gram-positive	White	Non-motile	95.5%	0.89	NS
E. coli	Bacilli	Gram-negative	White	Motile	94.7%	0.78	NS

Footnotes: Frequency (%): Represents the percentage of isolates with the characteristic across the total samples. Chi-Square Test: Evaluates the distribution of morphological features across the isolates. NS (Not Significant) indicates no significant differences.

3. Physiological Characteristics of Bacterial Isolates:

Table 6, presents the physiological characteristics of the bacterial isolates. Key findings include:

Optimal Growth Temperature: Most isolates thrived at an optimal temperature of 37°C, except for *Acinetobacter baumannii*, which preferred 30°C.

pH Preferences: Most isolates exhibited optimal growth at a neutral pH of 7.0, while Proteus mirabilis preferred a slightly acidic pH of 6.8.

Oxygen Requirement: Most isolates were facultative anaerobes, allowing them to grow in oxygen-rich and oxygen-poor environments. In contrast, *Acinetobacter baumannii* required oxygen for growth. Statistical analysis indicated no significant differences in physiological characteristics among the bacterial strains.

Table 6: Physiological Characteristics of Bacterial Isolates

Bacterial Strain	Optimal Temperature (°C)	Optimal pH	Oxygen Requirement	Frequency (%)	ANOVA F-value	Significance
Staphylococcus aureus	37	7.0	Facultative anaerobe	100%	NA	-
Acinetobacter baumannii	30	7.5	Aerobe	93.3%	1.02	NS
Klebsiella pneumoniae	37	7.0	Facultative anaerobe	88.0%	0.89	NS
Pseudomonas aeruginosa	37	7.0	Aerobe	86.3%	1.15	NS
Proteus mirabilis	37	6.8	Facultative anaerobe	66.7%	1.67	NS
Enterococcus faecalis	35	7.5	Facultative anaerobe	95.5%	0.78	NS
E. coli	37	7.0	Facultative anaerobe	94.7%	0.90	NS

Footnotes: ANOVA F-value: Used to compare mean physiological characteristics (temperature, pH, oxygen requirement) among the strains. NS indicates no significant differences.

4. Biochemical Characteristics of Bacterial Isolates:

The biochemical characteristics of the isolated strains are summarized in (Table 7). Key results include:

Catalase and Oxidase Tests: Most isolates tested positive for catalase, indicating the presence of the enzyme that breaks down hydrogen peroxide. Notably, *Pseudomonas aeruginosa* was the only strain to test positive for oxidase.

Indole Production: *Proteus mirabilis* and *E. coli* were indole-positive, suggesting the ability to produce indole from tryptophan.

Citrate and Urease Utilization: *Acinetobacter baumannii* and *Klebsiella pneumoniae* demonstrated citrate-positive results, while *Klebsiella pneumoniae* and *Proteus mirabilis* were urease-positive, indicating their potential for survival in various environments. The biochemical characteristics were uniformly distributed among the isolates, with no significant differences identified through statistical testing.

Table 7: Biochemical Characteristics of Bacterial Isolates.

Bacterial Strain	Catalase	Oxidase	Indole	Citrate Utilization	Urease	Frequency (%)	Chi-Square Value	Significance
Staphylococcus aureus	Positive	Negative	Negative	Negative	Negative	100%	NA	-
Acinetobacter baumannii	Positive	Negative	Negative	Positive	Negative	93.3%	0.45	NS
Klebsiella pneumoniae	Positive	Negative	Negative	Positive	Positive	88.0%	0.67	NS
Pseudomonas aeruginosa	Positive	Positive	Negative	Negative	Negative	86.3%	0.34	NS
Proteus mirabilis	Positive	Negative	Positive	Negative	Positive	66.7%	1.20	NS
Enterococcus faecalis	Positive	Negative	Negative	Negative	Negative	95.5%	0.89	NS
E. coli	Positive	Negative	Positive	Negative	Negative	94.7%	0.78	NS

Footnotes: Frequency (%): Represents the percentage of isolates with the characteristic across the total samples. Chi-Square Test: Evaluates the distribution of biochemical characteristics among the isolates. NS (Not Significant) indicates no significant differences.

DISCUSSION

Our study revealed that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were the most commonly isolated bacterial strains from nosocomial infections across all three governorates. This is consistent with prior research highlighting these organisms as major contributors to hospital-acquired infections worldwide (Aman S., and Mittal D. 2022).

For instance, similar studies from Egypt and other countries have reported the dominance of nosocomial infections, particularly wound infections. , especially Methicillin-Resistant (MRSA), has been a persistent issue in healthcare settings due to its high transmission rate and resistance to first-line antibiotics. The widespread occurrence of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is also not surprising, as these bacteria are notorious for causing opportunistic infections in immunocompromised patients and are often associated with hospital environments such as intensive care units (ICUs) (Di Domenico EG.*et al.,* 2022).

Moreover, the predominance of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in our study aligns with research indicating that these bacteria have a remarkable ability to survive in hospital environments due to their resistance to desiccation and disinfectants, thereby facilitating their spread. Previous studies have shown that *Acinetobacter baumannii* species can form biofilms on medical devices, which protects them from the host immune response and antibiotic treatment. Similarly, *Pseudomonas aeruginosa* is well-known for its ability to form biofilms and exhibit quorum sensing, a cell-to-cell communication mechanism that regulates gene expression related to virulence factors and antibiotic resistance (Al-Dahmoshi H, *et al.,* 2020).

Our study demonstrated high levels of antibiotic resistance across all isolated strains, with *staphylococcus aureus* exhibiting 100% resistance to methicillin, indicating the prevalence of MRSA in the studied regions. This finding echoes several previous studies that have reported MRSA as a major nosocomial pathogen with resistance rates exceeding 90% in various countries, particularly in the Middle East and North Africa (MENA) region. The mechanism behind MRSA resistance is primarily due to acquiring the *mecA* gene, which encodes a penicillin-binding protein (PBP2a) that has a low affinity for beta-lactam antibiotics. This allows MRSA to survive with antibiotics like methicillin and other beta-lactams (Ekambaram SP, *et al.,* 2016).

In addition to MRSA, our study's 100% resistance of *Acinetobacter baumannii* to ciprofloxacin and Klebsiella pneumoniae to cefotaxime is alarming. Studies have attributed the high resistance rates in these pathogens to the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases, enzymes that can hydrolyze a wide range of antibiotics, including cephalosporins and carbapenems. *Acinetobacter baumannii* spp. Often harbour resistance genes such as *blaOXA*, *blaNDM*, and *blaVIM*, which confer resistance to carbapenems, one of the last-resort antibiotics used to treat infections caused by MDR bacteria. Furthermore, the resistance of *Klebsiella pneumoniae* to cefotaxime is likely due to the presence of ESBLs, which are commonly found in this pathogen and are capable of degrading third-generation cephalosporins (Bradford PA, *et al.,* 1997).

In our study, *Pseudomonas aeruginosa* exhibited high resistance to piperacillin and tobramycin, consistent with previous studies highlighting the adaptive mechanisms of this pathogen. *Pseudomonas aeruginosa* has a variety of resistance mechanisms, including the

overexpression of efflux pumps (e.g., MexAB-OprM) that actively expel antibiotics from the bacterial cell and modify antibiotic targets through mutations. Moreover, the presence of resistance genes such as *blaVEB* and *blaIMP* in *Pseudomonas aeruginosa* can contribute to resistance against beta-lactams and aminoglycosides (Shaaban MT, *et al.*, 2011; Kanthakumar A, and Jayavarthinni M. 2022).

The resistance of *Enterococcus faecalis* to vancomycin (VRE) observed in this study aligns with the global trend of increasing VRE infections in hospitals. VRE strains possess the *vanA* and *vanB* gene clusters, which alter the target site of vancomycin, thereby reducing its binding affinity and rendering the antibiotic ineffective. This resistance mechanism poses a significant clinical challenge, as vancomycin is often used as a last-line treatment for infections caused by gram-positive cocci, such as *Enterococcus faecalis* (Lee K, Jang S-J, Lee HJ, Ryoo N, Kim M, Hong SG) 2004;19(1):8–14, (Miller NJ, and Rice-Evans CA. 1997).

The consistent biochemical profiles of these bacterial strains support their use in diagnostic microbiology. The indole and urease tests are particularly valuable for distinguishing between species in clinical settings. Although no significant differences were found in the biochemical characteristics ($p > 0.05$), the cumulative data provides a comprehensive understanding of their metabolic capacities, aiding in their identification and the formulation of targeted treatments (Altheide ST. 2020).

Conclusion

Based on the findings of this study, it is evident that nosocomial infections in the studied Egyptian governorates are primarily caused by a few dominant bacterial strains, namely *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. These pathogens exhibit high levels of antibiotic resistance, particularly to commonly used antibiotics, posing a significant threat to public health.

Declarations:

Ethical Approval: Not applicable.

Conflicts of Interest: The author declares no conflicts of interest.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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