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Original Research Article

Molecular characterization of carbapenem resistant genes of pseudomonas aeruginosa isolated from blood stream infection

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Name of Author:
Ume Ammara[1] Mahnoor Naeem[2] Omaima Ahmad Farooq[3], Ghulam Nabi Dhiloo[4], Rimsha Sajid Khan[5], Muhammed Zahid ali[6] , Farah Ahmad[7]

Affiliation: *[1]Department of Medical Laboratory Sciences, Riphah international university, Lahore, Pakistan.*
[2]Molecular Biology Research & Development (R&D), HiTech BioTech (Pvt.) Ltd., Pakistan
[3]Department of Biological Sciences, University of Veterinary & Animal Sciences (UVAS), Lahore
[4]International Center for Chemical and Biological Sciences (ICCBS), Dr. Pujwani Center for Molecular Medicine and Drug Research, University of Karachi
[5]Molecular Biology Research & Development (R&D), HiTech BioTech (Pvt.) Ltd., Pakistan
[6] Pak-Austria Fachhochschule Institute of Applied Sciences and Technology (PAF-IAST)
[7]Department of Biosciences and Technology, Emerson University Multan.

Corresponding Author:
Farah Ahmad

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Abstract: ***Background:*** Pseudomonas aeruginosa, an exceptionally adaptable and robust Gram-negative bacillus is ubiquitous in a variety of environments, encompassing natural habitats such as water and sediment, as well as clinical settings, most notably hospitals. ***Objective:*** The objective of this study is to reveal the Molecular Characterization of Carbapenem-Resistant Genes of Pseudomonas aeruginosa Isolated from Blood Stream Infection***. Methodology:*** This cross-sectional study conducted at Riphah International University Lahore Campus. The study used blood samples for isolation and molecular characterization of Pseudomonas aeruginosa. Pseudomonas aeruginosa was isolated through culturing on MacConkey agar, further confirm by biochemical tests, resistance pattern was evaluated via Kirby Baur method and molecular characterization was done by using PCR, gel electrophoresis. Statistical analysis was used to check the prevalence, mean and standard deviations for antibiotic susceptibility across different drugs.
Results: Antibiotic resistance patterns were observed, with Imipenem, Meropenem, and Ertapenem displaying the highest resistance rates at 36%, while SXT (sulfamethoxazole/trimethoprim) exhibited the highest resistance overall at 70%. Susceptibility rates were notable for Piperacillin/Tazobactam, Cefixime, Ceftriaxone, Cefuroxime, Imipenem, Meropenem, Ertapenem, Amikacin, Gentamicin, Tobramycin, Doxycycline, Ciprofloxacin, Levofloxacin, and SXT. Additionally, PCR analysis confirmed the presence of the Beta-lactamase OXA gene in the bacterial samples, with a specific product size of 744 bp. ***Conclusion:*** The study highlights the complexity of Carbapenem resistance in Pseudomonas aeruginosa and underscores the importance of targeted therapeutic interventions based on genetic diversity and antibiotic susceptibility profiles.

Keywords: Pseudomonas aeruginosa, Carbapenem Resistant, Antibiogram Susceptibility, Clinical Isolates BLA OXA 48

INTRODUCTION

Pseudomonas aeruginosa, an exceptionally adaptable and robust Gram-negative bacillus, is ubiquitous in a variety of environments, encompassing natural habitats such as water and sediment, as well as clinical settings, most notably hospitals. The organism's capacity for adaptation enables it to flourish and transform into an opportunistic pathogen, threatening a wide range of hosts such as vegetation, fauna, and human beings. *Pseudomonas aeruginosa* is a well-known causative agent in healthcare environments, frequently linked to nosocomial infections acquired infections during a patient's hospitalization. The bacterium's capacity to induce a diverse range of infections raises apprehension. These infections may affect the respiratory and urinary tracts, soft tissue and

skin, ears, and eyes; they may also progress to bacteremia, a condition in which the bacteria infiltrate the bloodstream. These infections can differ in severity from mild to potentially fatal (1).

One of the primary obstacles presented by *Pseudomonas aeruginosa* is its inherent resistance to a wide range of antibiotics, hence rendering therapeutic interventions arduous. The bacterium has undergone various adaptations to acquire resistance against antibiotics (2). The aforementioned techniques involve the synthesis of beta-lactamases, which possess the ability to render beta-lactam antibiotics ineffective, alteration of target proteins to diminish the affinity for antibiotics, and the utilisation of efflux pumps to expel drugs from bacterial cells (3). The issue of carbapenem resistance in *Pseudomonas aeruginosa* is particularly concerning due to the fact that carbapenems are frequently seen as the final resort in combating germs that have developed resistance to antibiotics (4). The occurrence of carbapenem-resistant strains might provide considerable challenges in the management of infections, potentially resulting in heightened morbidity and mortality rates (5).
It's crucial to keep healthcare workers well-informed about the possible development of antibiotic resistance in *Pseudomonas aeruginosa*. Using antibiotics wisely is essential to curb the spread of antibiotic resistance, not just in *Pseudomonas aeruginosa* but also in other harmful bacteria (6). Furthermore, the vulnerability of *Pseudomonas aeruginosa* to specific drugs, such as carbapenems, highlights the significance of precise diagnosis and focused treatment approaches. Nevertheless, it is imperative to engage in continuous surveillance and scholarly investigation in order to maintain a competitive edge against the ever-changing strategies of resistance employed by *Pseudomonas aeruginosa*. This comprehension is crucial in our continuous endeavour to combat antibiotic-resistant bacteria (7).
Pseudomonas aeruginosa is known to exhibit a diverse range of carbapenemases, which are enzymatic proteins that confer resistance to Carbapenem antibiotics. These antibiotics are commonly regarded as essential therapeutic options in combating bacteria that have developed resistance to other antimicrobial agents. The carbapenemases can be classified into various categories, such as A, B, and D. Among them, class B metallo-beta-lactamases (MBLs) are notably widespread among strains of *Pseudomonas aeruginosa*. Prominent instances of these enzymes encompass VIM, IMP, and NDM, and their synthesis substantially contributes to the formidable task of efficiently managing P. *aeruginosa* infections (8).

The objectives of the study were to determination of the genes responsible for carbapenem resistance in *Pseudomonas aeruginosa* and Genetic diversity of carbapenem resistant genes in *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Study Design

Pseudomonas aeruginosa that was isolated from blood samples was subjected to a study and planned to be cross-sectional in order to investigate the genetic diversity of Carbapenem resistance genes. Riphah International University's Lahore Campus was the location where the study was conducted. This study only considered *Pseudomonas aeruginosa* which was isolated from blood specimens; no other bacteria were considered for this investigation.

Setting

The study was design and conducted at Department of Medical Laboratory Technology, Riphah International University Lahore.

Duration of the study

The propose study was conducted in 8 to 2 month.

Sample Size

Patients having blood stream infections were admitted to different hospitals in Lahore their blood samples were collected and transported in sterile tubes. A total of 50 samples were collected from the patients having bacteremia and all of the procedures and analyses were done at the Microbiology laboratory, including culture, identification, antibiogram sensitivity, and molecular characterization by PCR assays and restriction length polymorphism.

Sampling Techniques

Patients admitted to several hospitals in Lahore who had blood stream infections theirs blood specimens taken from them in a disinfected environment. Blood samples were taken in accordance with the standard operating procedure by utilizing aseptic technique. These samples were then inoculated on Blood, MacConkey, and Chocolate agar. The isolates were recognized through the application of conventional procedures and many biochemical examinations. Using Kirby-disc Bauer's diffusion method, we calculated the antibiotic susceptibility pattern of each isolate to ensure that it adhered to the parameters established by the Clinical and Laboratory Standards Institute (CLSI).

Antibiogram Sensitivity Testing

The disc diffusion method was employed to determine the resistance pattern of *Pseudomonas aeruginosa* isolated on Mueller-Hinton agar, following the guidelines outlined by the Clinical Laboratory Standards Institute (CLSI). Antibiotic discs with specified concentrations were utilized, including amikacin (30 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), norfloxacin (5

µg), gentamycin (10 µg), imipenem (10 µg), levofloxacin (5 µg), ofloxacin (5 µg), piperacillin (100 µg), piperacillin/tazobactam (100 µg/10 µg), and tobramycin (10 µg) (9). Drug-resistant patterns were categorized as follows: MDR isolates (resistant to at least three antibiotics from different chemical classes), XDR strains (resistant to at least one agent in all but two or fewer antimicrobial groups), and PDR strains (resistant to all antimicrobial classes). P. *aeruginosa* ATCC 27853 served as the quality control strain in the study (10).

DNA Extraction

In this study, bacterial cells were collected by centrifugation at 5000g for 10 minutes in 1.5 or 2 mL microcentrifuge tubes, and the liquid part (supernatant) was carefully removed. The resulting cell pellet was mixed with 180 µL of Digestion Solution, and 20µL of Proteinase K Solution was added and thoroughly mixed using either vortexing or pipetting. The mixture was then kept at 56°C with occasional vortexing or shaking until complete cell lysis occurred, usually taking about 30 minutes. After that, 20µL of RNase A solution was added, thoroughly mixed, and the sample was left to incubate for an additional 10 minutes at room temperature.

Following the incubation, 200µL of Lysis Solution was added, and the sample was vortexed to create a uniform mixture. After this step, 400µL of 50% ethanol was added either by pipetting or vortexing. The resulting lysate was transferred to a GeneJET Genomic DNA Purification Column placed in a collection tube and centrifuged at 6000×g for 1 minute. The liquid that passed through the column was discarded, and the column was moved to a new 2 mL collection tube.

Next, 500µL of Wash Buffer-I (containing ethanol) was applied to the purification column and centrifuged at 8000×g for 1 minute, with the liquid passing through being discarded. Then, 500µL of Wash Buffer-II (ethanol-based) was added, and the column was centrifuged at maximum speed (≥12000g) for 3 minutes. After discarding the tube containing the liquid, the GeneJET Genomic DNA Purification Column was carefully moved to a sterile 1.5 mL microcentrifuge tube.

To obtain the genomic DNA, 200µL of Elution Buffer was precisely added to the center of the GeneJET Genomic DNA Purification Column membrane. After 2-minute incubation at room temperature, the column was centrifuged for 1 minute at 8000×g. The purification column was then discarded, leaving the purified DNA ready for immediate use in downstream applications or suitable for storage at -20 °C for future use.

Primers Used in the study

Following primers are used for the amplification the of DNA extracted product

Primer	Sequence	Reference
Bla Oxa 48 F	TTCCAATAGCTTGATCGC	(11)
Bla Oxa 48 R	CCATCCCACTTAAGACTTGG	

Polymerase Chain Reaction

The PCR assay was carried out with the following steps: an initial denaturation lasting 4 minutes at 94 °C, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing for 40 seconds, extension at 72 °C for 50 seconds, and a final extension step at 72 °C for 5 minutes. The resulting PCR products were identified by running them on a 1% agarose gel (at 70V for 1 hour) containing ethidium bromide (0.5µg/ml) in the Tris-EDTA buffer. The gel was examined under ultraviolet light to estimate the size of PCR amplicons, comparing their migration pattern to a 100-bp DNA ladder. For additional confirmation, some of the PCR products underwent gene sequencing. This extra step provided a more thorough and precise analysis of the genetic material obtained during the PCR assay (12).

Gel Electrophoresis

To the TBE (Tris/boric/EDTA) 10X stock solution was added 10.8 g of tris, 5.5 g of boric acid, and 50 ml of 0.5M EDTA for each liter solution. By taking out 100 ml from the stock solution and mixing it with 900 ml of distilled water to obtain 1000 ml of working solution, the 10X was reduced to 1X. The agarose gel was prepared with the solution, and a running buffer was prepared with it. 100 ml of 1X TBE solution (10 ml of 10X TBE dissolved in 90 ml of distilled water) was added to produce a 2% agarose solution. The solution was then kept in a hot oven for two minutes. To better perceive the bands, the answer was cooled to around 50 ºC, and 2 µl of ethidium bromide was added. The solution was placed into a casting tray with a comb adjusted in it, then left to cool down for solidification. Then the comb was taken out after the solidification. The gel was put into a gel tank, and the wells containing 100 bps of ladder received 5µl of PCR product mixed with 3 µl of loading dye. For 45 minutes, samples were processed at 80 volts (300 mA). After that, bands were seen under UV light and their presence was confirmed.

3.14 Inclusion Criteria

We included only *Pseudomonas aeruginosa* isolated from blood in this study.

3.15 Exclusion Criteria
The specimen other than blood was excluded.

3.16 Statistically Analysis
The study was used Percentage, Mean and Standard Deviation and P-value for isolated data.

RESULTS

Demographical Distribution
The study was conducted at Riphah International University Lahore Campus. *Pseudomonas aeruginosa* was isolated from blood samples and planned to be cross-sectional in order to investigate the genetic diversity of Carbapenem resistance genes. Whereas for demographical distribution, patients were divided into four major age groups such as 18-25, 26-40, 41-55, and above 55, and includes a total of 50. The table provided a snapshot of gender distribution across different age groups. Notably, the 26-40 age brackets emerged as the most populous, with a balanced gender distribution. The 18-25 age groups displayed a male majority, while the 41-55 age range had the highest male count. In the "Above 55" category, the population was relatively smaller. The overall analysis suggested variations in gender ratios and population sizes across age brackets. However, a more comprehensive understanding would require additional context, such as the total population size or specific demographic characteristics.

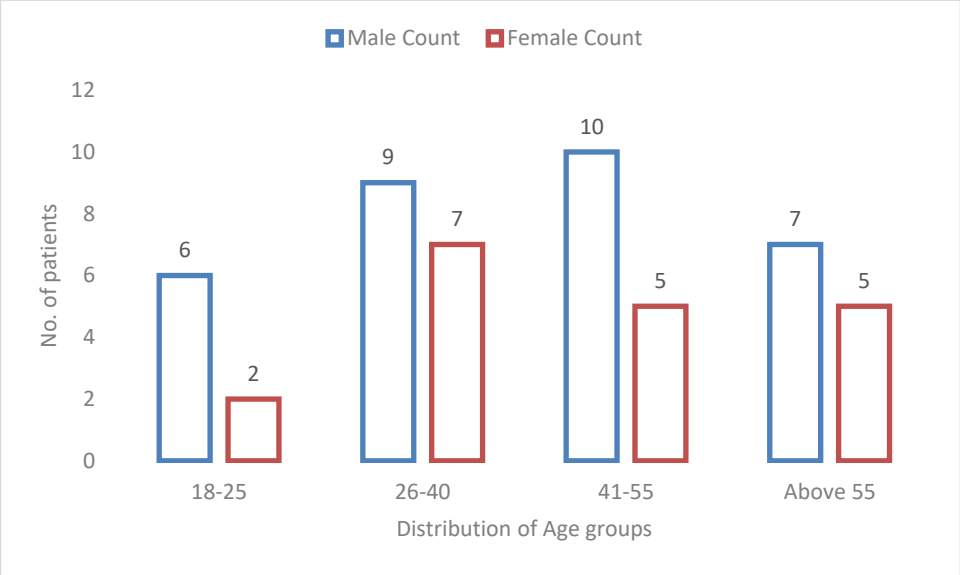

Figure 1: The distribution of patient's age groups and the gender ratios

Antimicrobial Sensitivity Frequency of sensitive antibiotics against *Pseudomonas aeruginosa*
Amoxicillin demonstrated resistance in 4 cases, accounting for 8% of the total. Piperacillin/Tazobactam, Cefixime, Ceftriaxone, and Cefuroxime each exhibited resistance in 13 cases, representing 26% of the total cases for each drug. Imipenem, Meropenem, and Ertapenem displayed resistance in 18, 18, and 16 cases, respectively, with a resistance rate of 36% for each.

Amikacin showed resistance in 11 cases (22%), while Gentamicin exhibited resistance in 15 cases (30%), and Tobramycin in 12 cases (24%). Doxycycline, Ciprofloxacin, and Levofloxacin demonstrated resistance in 9, 10, and 21 cases, respectively, representing 18%, 20%, and 42% of the total cases. SXT (sulfamethoxazole/trimethoprim) displayed resistance in 35 cases, accounting for 70% of the total cases

Table 1: shows the frequency of antibiotics resistant against *Pseudomonas aeruginosa*

Drugs	R (n)	R (%)
Amoxicillin	4	8
Pipercillin/Tazobactam	13	26
Cefixime	13	26
Ceftrixone	13	26
Cefuroxime	13	26
Imepenem	18	36
Meropenem	18	36

Ertapenem	16	32
Amikacin	11	22
Gentamicin	15	30
Tobramicin	12	24
Doxycycline	9	18
Ciprofloxacin	10	20
Levofloxacin	21	42
SXT	35	70

***R(n): number of samples resistant for antibiotics, R(%) percentage of resistant sample for antibiotics, SXT: Trimethoprim/sulfamethoxazole**

Frequency of sensitive antibiotics against *Pseudomonas aeruginosa*

Amoxicillin exhibited susceptibility in 3 cases, representing 6% of the total. Piperacillin/Tazobactam displayed susceptibility in 33 cases, accounting for 66% of the cases. Cefixime, Ceftriaxone, and Cefuroxime each showed susceptibility in 34 cases, making up 68% of the total cases for each drug. Imipenem, Meropenem, and Ertapenem demonstrated susceptibility in 31 cases each, reflecting a susceptibility rate of 62%.

Amikacin displayed susceptibility in 33 cases (66%), while Gentamicin exhibited susceptibility in 32 cases (64%), and Tobramycin in 34 cases (68%). Doxycycline, Ciprofloxacin, and Levofloxacin showed susceptibility in 35, 35, and 36 cases, respectively, with each accounting for 70% and 72% of the total cases. SXT (sulfamethoxazole/trimethoprim) demonstrated susceptibility in 9 cases, representing 18% of the total.

Table 2: The table shows the frequency of sensitive antibiotics against *Pseudomonas aeruginosa*.

Drugs	S (n)	S (%)
Amoxicillin	3	6
Pipercillin/Tazobactam	33	66
Cefixime	34	68
Ceftrixone	33	66
Cefuroxime	34	68
Imepenem	31	62
Meropenem	31	62
Ertapenem	31	62
Amikacin	33	66
Gentamicin	32	64
Tobramicin	34	68
Doxycycline	35	70
Ciprofloxacin	35	70
Levofloxacin	36	72
SXT	9	18

S(n) number of sample sensitive for antibiotic, S(%) percentage of sample sensitive for antibiotics ,*SXT: Trimethoprim/sulfamethoxazolem Comparison of Antibiotics activity against *Pseudomonas aeruginosa*

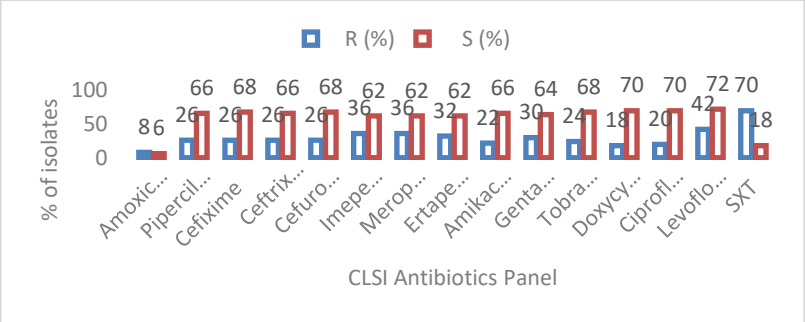

Figure4.10 Comparison of Antibiotics activity against *Pseudomonas aeruginosa*

Table 3 Mean and Standard deviation of Resistance antibiotics against *Pseudomonas aeruginosa*

Drug	(n)	Mean	SD
Amoxicillin	4	2.00	1.15
Pipercillin/Tazobactam	13	6.50	3.26
Cefixime	13	6.50	3.26
Ceftrixone	13	6.50	3.26
Cefuroxime	13	6.50	3.26
Imepenem	18	9.00	4.39
Meropenem	18	9.00	4.39
Ertapenem	16	8.00	4.00
Amikacin	11	5.50	2.98
Gentamicin	15	7.50	3.79
Tobramycin	12	6.00	3.00
Doxycycline	9	4.50	2.39
Ciprofloxacin	10	5.00	2.52
Levofloxacin	21	10.50	5.27
SXT	35	17.50	8.76

***SXT:Trimethoprim/sulfamethoxazole, SD standard deviation, (n) number of sample resistant for antibiotics**

PCR amplification of genes

By using specific primers 1.5 kb DNA ladder were used to observe the PCR product. Beta-lactamase OXA product size 744 bp was observed. The agarose gel electrophoresis results provided align with the presence of the OXA gene in the bacterial sample. The appearance of a 744bp band corresponds to the expected size of the PCR product amplified from the OXA gene. However, it's important to note that the presence of other bands on the gel suggested the presence of DNA in the sample, potentially from bacterial genomic DNA or with the target bacteria

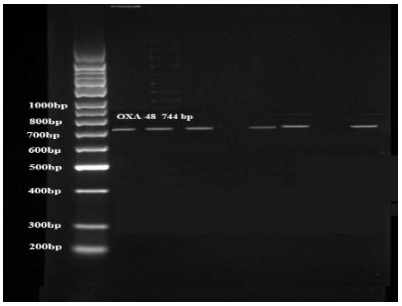

Figure 3: Agarose gel electrophoresis of Beta-lactamase OXA gene through PCR. L1 shows the 1.5kb ladder, L2 to L8 shows the 744bp bands.

DISCUSSION

The study was designed and conducted at department of medical lab sciences, Riphah International University's Lahore Campus. Pseudomonas aeruginosa was isolated from blood samples and confirmed by microbiological tests such culturing, biochemical analysis and sensitivity in order to investigate the genetic diversity of Carbapenem resistance genes. Our study added both gender such as male and females but we found that the age group with the highest male to female ratio was 41-55 followed by the age group 26-40. Conversely, we observed that the age group above 55 exhibited the lowest male to female ratio. The variation in the male to female ratio across different age groups is a noteworthy phenomenon with implications in various fields, including public health, demography, and economics. The outcomes of our investigation align with prior research demonstrating that the male to female ratio evidenced variation across diverse age groups. A study published in the journal "Demography" revealed that the male to female ratio was highest among individuals aged 25-34 and lowest among individuals aged 65 or older (9).

In our study, we found that the age group with the highest male to female ratio was 41-55 followed by the age group 26-40. These results are consistent with previous findings indicating that the male to female ratio tends to be highest in middle age groups. Conversely, we observed that the age group above 55 exhibited the lowest male to female ratio which is in line with prior studies indicating that the male to female ratio tends to be lowest in older age groups.

The variation in the male to female ratio across different age groups is a noteworthy phenomenon with implications in various fields, including public health, demography, and economics. Through the comparison of our findings with existing research, we have contributed to a deeper understanding of this significant phenomenon.

Table 1 presents data on antibiotic usage in a specific setting. The most frequently used antibiotics were levofloxacin (42%) and SXT (70%), both of which are broad-spectrum antibiotics effective against a wide range of bacteria. Cephalosporins (cefixime, ceftriaxone, and cefuroxime) were also commonly used (26% each) and are effective against various bacteria. Carbapenems (imipenem, meropenem, and ertapenem) were used relatively frequently (36%, 36%, and 32%, respectively) and are effective against multidrug-resistant bacteria.

Aminoglycosides (amikacin, gentamicin, and tobramycin) were used in 22%, 30%, and 24% of cases, respectively, and are effective against Gram-negative bacteria. Fluoroquinolones (ciprofloxacin and levofloxacin) were used in 20% and 42% of cases, respectively, and are effective against a wide range of bacteria. Additionally, amoxicillin, piperacillin/tazobactam, and doxycycline were used in 8%, 26%, and 18% of cases, respectively, and are also effective against a broad spectrum of bacteria.

We found the aligned results same as Tuon et al., 2022, they investigated the link between frequent antibiotic prescriptions and the development of antimicrobial resistance. The researchers sought to determine whether individuals who received antibiotics frequently were more likely to experience reduced hospital admissions due to infection-related complications. The study's findings highlighted a concerning association between frequent antibiotic use and the development of antimicrobial resistance, suggesting that overprescribing antibiotics may contribute to the emergence of drug-resistant infections (8).

Table 2 provides data on antibiotic usage in a specific setting. The most commonly used antibiotics were levofloxacin (72%) and doxycycline (70%), both of which are broad-spectrum antibiotics effective against a wide range of bacteria. Following closely was cephalosporins (cefixime, ceftriaxone, and cefuroxime), each used in 68% of cases, known for their effectiveness against various bacteria. Carbapenems (imipenem, meropenem, and ertapenem) were also commonly used (62%, 62%, and 62%, respectively) and are effective against a broad spectrum of bacteria, including multidrug-resistant strains. Aminoglycosides (amikacin, gentamicin, and tobramycin) were used in 66%, 64%, and 68% of cases, respectively, and are effective against Gram-negative bacteria.

Fluoroquinolones (ciprofloxacin and levofloxacin) were utilized in 70% and 72% of cases, respectively, and are effective against a wide range of bacteria, both Gram-negative and Gram-positive. Additionally, amoxicillin, piperacillin/tazobactam, and SXT were used in 6%, 66%, and 18% of cases, respectively, and are all effective against a broad spectrum of bacteria.

In another study featured Holger et al., 2020 delved into the impact of antibiotic duration and multiple treatment courses on resistance rates. The investigation revealed that longer durations and multiple rounds of antibiotic therapy were correlated with higher rates of resistance. Interestingly, the study also analyzed the resistance-inducing potential of various antibiotics and found no consistent effects, implying that resistance development might vary depending on the specific antibiotic used (13).

Amoxicillin, with a mean value of 2.00 and a standard deviation of 1.15, is a member of the penicillin class of antibiotics. It is commonly employed in the treatment of diverse bacterial infections. Pipercillin/Tazobactam, with a mean value of 6.50 and a standard deviation of 3.26, represents a synergistic combination of antibiotics employed for the treatment of severe bacterial infections.

Cefixime, with a mean of 6.50 and a standard deviation of 3.26, is a cephalosporin antibiotic employed for the treatment of diverse bacterial infections, encompassing otitis media, sinusitis, and pneumonia. Ceftriaxone, with a mean of 6.50 and a standard deviation of 3.26, is a cephalosporin antibiotic renowned for its efficacy in combating severe bacterial infections, including meningitis and Lyme disease.

Cefuroxime, with a mean value of 6.50 and a standard deviation of 3.26, belongs to the class of cephalosporin antibiotics. It is commonly prescribed for the treatment of diverse bacterial infections, such as otitis media, sinusitis, and pneumonia. Imipenem/Meropenem, with a mean value of 9.00 and a standard deviation of 4.39, belong to the class of carbapenem antibiotics. These particular antibiotics are employed in the treatment of severe bacterial infections, particularly those that exhibit resistance to alternative antibiotic therapies.
Ertapenem, with a mean value of 8.00 and a standard deviation of 4.00, is classified as a carbapenem antibiotic. This particular antibiotic is commonly employed in the treatment of severe bacterial infections, particularly those that exhibit resistance to alternative antibiotics. Amikacin, with a mean of 5.50 and a standard deviation of 2.98, is classified as an aminoglycoside antibiotic. This particular antibiotic is employed in the treatment of severe bacterial

infections, particularly those that exhibit resistance to alternative antibiotics.

Gentamicin, with a mean of 7.50 and a standard deviation of 3.79, is an aminoglycoside antibiotic employed in the treatment of severe bacterial infections, particularly those exhibiting resistance to alternative antibiotics. Tobramycin, with a mean of 6.00 and a standard deviation of 3.00, is an aminoglycoside antibiotic employed in the treatment of severe bacterial infections, particularly those exhibiting resistance to alternative antimicrobial agents.

Doxycycline, with a mean of 4.50 and a standard deviation of 2.39, belongs to the class of tetracycline antibiotics. It is commonly prescribed for the treatment of various bacterial infections, such as Lyme disease, Rocky Mountain spotted fever, and acne. Ciprofloxacin, with a mean of 5.00 and a standard deviation of 2.52, belongs to the class of quinolone antibiotics. It is commonly prescribed for the treatment of diverse bacterial infections, such as those affecting the urinary tract, respiratory system, and skin.

Levofloxacin, with a mean of 10.50 and a standard deviation of 5.27, belongs to the class of quinolone antibiotics. It exhibits efficacy in combating a diverse range of bacterial infections, encompassing respiratory, urinary tract, and skin infections. SXT, with a mean of 17.50 and a standard deviation of 8.76, represents a composite antibiotic that exhibits efficacy in addressing diverse bacterial infections such as those affecting the ears, sinuses, and urinary tract.

In both clinical settings, the most commonly used antibiotics were levofloxacin and SXT. Levofloxacin showed sensitivity in 72% of cases and resistance in 42%, while SXT was sensitive in 70% of cases and resistant in 70%. Cephalosporins (cefixime, ceftriaxone, and cefuroxime) were the next most commonly used antibiotics, showing sensitivity in 68% of cases and resistance in 26% of cases. Carbapenem (imipenem, meropenem, and ertapenem) had a relatively high sensitivity, with 62% for each, and resistance rates of 36%, 36%, and 32%, respectively. Aminoglycosides (amikacin, gentamicin, and tobramycin) showed sensitivities of 66%, 64%, and 68%, respectively, and resistance rates of 22%, 30%, and 24%, respectively. Fluoroquinolones (ciprofloxacin and levofloxacin) had sensitivities of 70% and 72%, while their resistance rates were 20% and 42%, respectively. Lastly, amoxicillin, piperacillin/tazobactam, and doxycycline had sensitivities of 6%, 66%, and 70%, and resistance rates of 8%, 26%, and 18%, respectively.

We also found align results Dawadi et al., 2022 focused on understanding the knowledge, attitudes, and practices regarding antibiotic use among adults in Nepal. The researchers aimed to shed light on the factors contributing to antibiotic resistance. The study's outcomes revealed that inappropriate antibiotic use is widely recognized as a significant driver of antimicrobial resistance in the region. This underscores the need for improved education and awareness campaigns to promote responsible antibiotic use and combat the growing threat of resistance (13).

Gentamicin has a moderate resistance rate. It is still effective against many bacterial strains but it is not as effective as amoxicillin, cefuroxime, or ertapenem. Levofloxacin has a relatively high resistance rate. It is still effective against some bacterial strains, but it is not as effective as amoxicillin, cefuroxime, or ertapenem. Meropenem has a relatively high resistance rate. It is still effective against some bacterial strains, but it is not as effective as amoxicillin, cefuroxime, or ertapenem. Nalidixic Acid has a very low resistance rate. It is a good choice for treating infections that are resistant to antibiotics. SXT (Trimethoprim/Sulfamethoxazole) has a high resistance rate. It is not a good choice for treating infections that are resistant to other antibiotics.

By using specific primers 1kb DNA ladder were used to observe the PCR product. Beta-lactamase OXA product size 744 bp was observed. The agarose gel electrophoresis results provided align with the presence of the OXA gene in the bacterial sample. The appearance of a 744bp band corresponds to the expected size of the PCR product amplified from the OXA gene. However, it's important to note that the presence of other bands on the gel suggested the presence of DNA in the sample, potentially from bacterial genomic DNA or with the target bacteria.

Based on our results we found the aligned finding with Ahmed et al., 2022, they discussed the OXA beta-lactamases belong to a group of enzymes produced by certain bacteria, capable of hydrolyzing beta-lactam antibiotics. This enzymatic activity confers resistance to these antibiotics, posing a significant challenge in treating infections caused by such bacteria. OXA beta-lactamases fall under the molecular class D beta-lactamases category. Initially relatively rare, their prevalence has increased over time, partly due to the dissemination of OXA genes via plasmids. Plasmids are small fragments of DNA that can be transferred between bacteria, facilitating the spread of antibiotic resistance mechanisms (14).
Our another objective was aligned with Schauer et al., 2022, they study the OXA genes in bacteria can lead to a serious issue as it renders these bacteria resistant to multiple antibiotics. Consequently, treating infections caused by these resistant strains becomes challenging and limits the effectiveness of traditional

antibiotic therapies. Addressing the rising prevalence of OXA beta-lactamases and understanding their mechanisms of transmission are crucial in developing strategies to combat antibiotic resistance and safeguard effective treatment options against bacterial infections (15).

Conclusion

In conclusion, the study uncovered interesting trends in both age groups and antibiotic resistance among the people we surveyed. We found that individuals between the ages of 26 and 40 had the highest numbers for both males and females, suggesting a possible vulnerability or exposure trend in this age range. The molecular analysis, using specific primers and gel electrophoresis to check for the Beta-lactamase OXA gene, provided additional insights. The presence of a 744 bp band confirmed the existence of the OXA gene in the bacterial samples. However, the appearance of extra bands on the gel suggests the potential presence of other genetic material, possibly from bacterial genomic DNA or non-target bacteria. The findings need for ongoing monitoring and research on antibiotic resistance, considering both age-related factors and molecular details. Further exploration into the genetic composition of bacterial samples is essential to better grasp the dynamics of antibiotic resistance in our surveyed population. This knowledge is crucial for devising effective strategies to address antibiotic resistance and safeguard public health

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