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CHARACATERIZATION OF AMINOGLYCOSIDE RESISTENT PSEUDOMONAS AERUGINOSA IN RESPIRATORY SPECIMENS: PHENOTYPIC AND MOLECULAR INSIGHTS

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Abstract

Introduction: *Pseudomonas aeruginosa, a bacterial pathogen belonging to the Pseudomonadaceae family, causes various infections, including nosocomial infections. It is a Gram-negative bacillus that is aerobic, non-fermentative, and noted for its virulence. Three primary mechanisms can confer antibiotic resistance in this bacterium: reduced permeability of porins, alteration of antibiotic targets, and enzymatic inactivation of antibiotics.*

Objective: *The main objective was to evaluate the phenotypic and genotypic characteristics of aminoglycoside-resistant Pseudomonas aeruginosa isolates from respiratory specimens.*

Methodology: *Bacterial pathogens were identified by following the CLSI guidelines and traditional microbiological procedures. After identification and confirmation of bacterial pathogens from respiratory specimens, DNA was extracted using the Miniprep bacterial DNA extraction kit, and the DNA bands were visualized by gel electrophoresis. Genes responsible for aminoglycoside resistance in Pseudomonas aeruginosa were amplified with specific primers using Polymerase Chain Reaction (PCR). Additionally, the descriptive statistical analysis was carried out using SPSS for generation of frequency distribution curve for the patients of different age groups. The study used gender wise percentages, means, standard deviations, and bar chart for the isolated data.*

Results: *Among 80 isolates from patients 42 are males and 38 are females. Microscopic examination revealed a Gram-negative, rod-shaped bacillus and motile. The organism demonstrated distinct biochemical characteristics through a battery of assays, including tests for catalase, oxidase, citrate, urease, SIM, and TSI. It tested positive for catalase, oxidase, and citrate utilization but negative for motility, urease, TSI, and indole production. Strains exhibiting resistance to aminoglycosides, carbapenems, cephalosporins, and fluoroquinolones accounted for approximately 85% of the samples. Additionally, about 80% of the bacteria were resistant to colistin. Pseudomonas aeruginosa strains resistant to aminoglycosides were found to carry the armA gene in approximately 86% of cases. The remaining 14% of the strains, which were sensitive to aminoglycosides, did not display bands in electrophoresis analyses.*

Conclusion: *The repercussions authenticate the pathogen's renowned multidrug resistance and highlight the need for more study and efficient antimicrobial superintendence. The results of this study highlight the significance to develop therapeutic options to resolve this multi-drug resistance in bacteria.*

Keywords: *Aminoglycoside, Medical microbiology, PCR, Pseudomonas aeruginosa*

INTRODUCTION

Gram-negative bacteria (GNB) have complex outer layers that contain lipopolysaccharides (LPS), lipoproteins, and phospholipids. These components, in combination with the cell wall, gram negative structure retain a layer known as an envelope. This envelope contains endotoxins and lipopolysaccharides.

Different types of porin proteins are found at the external membrane of bacterium that facilitates the passage of small water loving molecules into the cell (1). Medically important gram-negative coccobacillary bacteria, including *Hemophilus influenzae*, *Bordetella pertussis*, *Legionella pneumophila*, and *Pseudomonas aeruginosa*, are associated with respiratory tract infections (2). *Pseudomonas Aeruginosa,* a bacterial pathogen appurtenance to the *Moraxellaceae* class, is known to cause the variety of nosocomial infections. *Pseudomonas aeruginosa* is a consequential gram-negative, small coccobacillus with a pleomorphic and aerobic nature. It cannot ferment glucose, lactose, or sucrose. It has the potential to be a potent disease-causing agent. *P. aeruginosa* can easily stick to surfaces as it has peapods and pili (3, 4).

Pseudomonas aeruginosa possesses potent invasive virulence components such as lipopolysaccharide, phospholipases, and exterior layer protein A (OmpA), which binds to host epithelium and powerhouse of cells (mitochondria) once it binds, and promotes a powerhouse of cell (mitochondrial) malfunction and distension. This was demonstrated by proclamation to cytochrome c oxidase, a haemoprotein that causes apoptosome development. All of these events contribute to apoptosis. Pathogens are the uppermost polypeptide that is most prevalent. OmpA is also important for complement resistance and biofilm formation. Biofilm production is an essential pathogenic mechanism and a distinguishing feature of *P. aeruginosa* (5). A bio-film is a considerable amount of exterior micro-bacterial cells enclosed in an extracorporeal polymeric substance matrix. *P. aeruginosa* has a faster rate of biofilm development than another category. Several genetic codes influence the production of *P. aeruginosa* bio-films, and the organism has a valuable link within biofilm constitution and "antimicrobacterial resistance" (6, 7).

The extrusion of a destructive substrate from cytoplasmic cells into the neighboring environment is facilitated by efflux pumps, which are shipping proteins. These proteins are also present in bacteria and may be gram-positive or gram-negative bacteria. Acinetobacter also do virulence through efflux pump (8). Antimicrobacterial resistance has been pragmatic in the mainstream of germs, and then it is especially hazardous in hos acquired contaminations caused by drug- resilient ESKAPE pathogens. Groups of antimicrobial drugs used against pathogens include β-lactams, aminoglycosides, cephlosporins, quinolones, tetracyclines, and penicillin (9).

Aminoglycosides are wide-spectrum drugs accustomed cure illnesses provoked by many bacterial pathogens. Aminoglycosides are composed of an amino alcohol ring and amino sugar fragments connected by a ligand-sugar bonding. These decisively charged amino functional groups can interrelate with anionically particles, specifically the non-coding RNA of the bacteriological 30S and 50S subunits of ribosomal protein, inhibit porin polypeptide combinations, and result in cell termination (10). *P. aeruginosa* is remarkably antibiotic-resistant, and some isolates are resistant to all known antibiotics, which is why it is a multidrug-resistant organism (MDR). The more impermeable outside membrane of the organism and its exposome to a vast pool of resistance genome may both contribute to its capacity for broad antibiotic resistance in Acinetobacter. Phase-dependent antibiotics that can be eradicated are called β-lactams. These antibiotics bind penicillin-binding proteins that inhibit peptide cross-linkage, that sort of interrupt bacterial cell coat biosynthesis (11).

Carbapenems are the sole leading momentous group of β-lactam antibiotics. Carbapenem resistant is disreputable due to its capacity to be tolerated in health care settings, elude host immunity, develop new pathways for antibiotic resistance, and dodge therapeutic responses. In P. *aeruginosi*, carbapenems are typically resilient (12). Recently, colistin-resistant strains of *P. aeruginosa* have been identified. Lipopolysaccharide (LPS) structural modifications and the existence of plasmaforming-carrying genes (mcr-1, mcr-2, mcr-3, and mcr-4.3) cause this resistance. The *Pseudomonas Aeruginosa* pathogen is highlighted by the CDC and WHO (13).

This study aims to appraise the phenotypic and genotypic detection of aminoglycoside-resistance *Pseudomonas Aeruginosa* isolates from respiratory specimens.

MATERIALS AND METHODS

This cross- sectional study belongs to the type observational study that analyzed data from population, or a representative subject, at a specific period of time. The survey was settled in the Life

Sciences Laboratories at Inter-collaborative Institutions after the approval of IRB. Respiratory Samples were collected from the designated clinics settings in Lahore and analyzed at microbiology and Biotechnology departments inter-collaboratively at different Institutes. Analytical tests Quality Control were assessed via Agha Khan laboratories. Samples included respiratory cases including naso-pharynx (NP) and guedel pattern airway swabs, nasal mid-turbinate (NMT) swabs, tracheal and lower respiratory tract washing, bronchial brushing specimens, and phlegm. In this study, we included only *Pseudomonas Aeruginosa* isolates from respiratory patients, whereas, the specimens other than respiratory patient samples were excluded. In the manufacture of all media and reagents, distilled water was used. Before usage, glassware was professionally cleaned, artificially heat-dried, and fumigate. The manufacturing reagents and glassware were sterilized for 15 minutes at 121°C and 15psi of pressure. Isolation of bacterial isolates: The strains of bacterium were obtained with respiratory specimens. The whole cases were treated on traditional microbiological culture plates. For inoculation, sheep blood culture medium, and MacConkey agar were utilized. On 37°C, culture plates were placed for incubation. Colonies were inspected after 24 hours of incubation, colony features were evaluated, and isolated bacterial colony was refined on corresponding nutrient plates. Purification of separated colonies was done using fastidious mediums. After one-day, segregated colonies showed pure growth. The characteristics of the colony were reported and compared for the last report. These segregated micro biomes were applied to additional research. On culture media, bacteria's culture features and colony shape were observed. Based on-these culture characteristics, pathogens were primarily identified and described.

For colonies characteristics observe, minimum 18hr to 24hr timeworn/incubated culture were examined. The color, size, margins, surface, texture, elevation, and overall appearance were grossly examined by naked eye. These variable characteristics assisted in identifying and categorizing the infections. Slides were gram stained and for biochemical characterization of isolated bacteria Catalase, Oxidase, Citrate utilization, Motility, Urease, TSI motility and AST were performed. Gene Jet Genomic DNA Purification Kit (Thermo Fisher Scientific#1554M) was used to implement DNA extraction as per manufacturer protocols. The PCR procedure began with a rigorous 4-minute first denaturation phase at a scorching 94 °C. After that, there were 35 cycles of denaturation at the identical heating frequency for a brief 45 seconds, which was repetitive in an appropriate way. Afterwards, during the annealing phase, the molecules discovered their ideal match and warmed up for a comfortable 40 seconds. For a quick 50 seconds, the extension phase felt like a sprint at 72 °C. All of it culminated in a magnificent crescendo that included an incredible final extension step, performed for five glorious minutes at 72°C.

GEL ELECTROPHORESIS

The final results were determined by chromatography on a 2% agarose gel performed at 70V for one hour. Within an environment of Tris-EDTA buffer, 0.5µg/ml of the compound ethidium bromide was injected into the aforementioned gel. The diameters of the PCR amplicons were then visible on the gel when it was placed under a UV light. Based on the migration pattern of a reference 1k-bp DNA ladder, these sizes were calculated. Certain PCR results were sent for genome sequencing in order to increase precision even more, guaranteeing a thorough comprehension of the genetic makeup.

STATISTICAL ANALYSIS

The descriptive statistical analysis was carried out using SPSS for generation of frequency distribution curve for the patients of different age groups. The study used gender wise percentages, means, standard deviations, and bar chart for the isolated data. In order to get minimized biasedness, this sample size contains a diversity of age groups and specified clinical settings places.

Although the sample size is small, it provides preliminary data on aminoglycoside resistance in *Pseudomonas aeruginosa*. Gender-wise percentages, means, standard deviations, and bar charts offer a clear, analysis of the data, enabling us to identify significant trends and patterns. This foundational research sets the stage for larger, more comprehensive studies by highlighting critical areas for further investigation.

RESULTS

In the demographic sorting of the study members, accounting fifty persons, a near- even split between genders was perceived, with males accounting for 54% and females 46%. Moreover delineation by age groups revealed a diverse illustration, with the mainstream falling into the set of old-age adults, constituting 36% of the total sample. Young adults and children each encompassed 22% of the participants, while middle-aged adults established the remaining 20%.The investigation into bacterium demonstrating pink-colored, short, wide, gram-negative coccobacillus morphology exposed notable microscopic characteristics. Through numerous tests, including catalase, oxidase, citrate, urease, SIM, and TSI tests, the organism displayed distinct metabolic traits. Conspicuously, it showed positive reactions for catalase and citrate utilization, while testing negative for oxidase, urease, indole production, and motility. Additionally, TSI testing indicated alkaline slants and butts with non-fermentative properties. Further examination using VITEK MS recognized specific microbial gears, corroborating the outcomes from biochemical tests. Antibiotic sensitivity testing unveiled a regarding pattern of resistance, particularly against aminoglycosides as per tobramycin, gentamicin, and amikacin. Gel electrophoresis confirmed the presence of the ArmA gene in the preponderance of specimens, signifying a genetic basis for the pragmatic resistance. This inclusive inspection underscores the significance of indulgent both the phenotypic and genotypic physiognomies of microbial isolates for effective treatment strategies.

GRAM STAINING

Pseudomonas aeruginosa are gram negative, facultatively anaerobic, non-fermentative, motile and long, slender blighted rods that possess an absolute requirement of oxygen for growth. Identification is normally tended by the fact that it forms round colonies which fluoresce greenish in color. Mucoid strains are especially common in patients suffering from cystic fibrosis.

BIOCHEMICAL TESTING

Table I represents different biochemical tests performed to confirm the bacterial species.

Table I. Biochemical Tests

Sr. No	Tests		Results	Interpretation
1	Catalase Test		Positive	Bubbling
2	Oxidase Test		Positive	Not have the cytochrome c that oxidase test reagent
3	Citrate Test		Positive	Utilize citrate as a carbon source
4	Urease Test		Negative	No ammonia production
5	SIM TEST	Sulphur	Negative	-
		Indole	Negative	No indole production
		Motility	Negative	Non-motile
6	TSI TEST	Slant	Alkaline	Non-fermentation
	(Triple Sugar Iron)	Butt	Alkaline	Non-fermentation
7	Gas production		Negative	-
8	H_2S production		Negative	-

GENDER FREQUENCY DISTRIBUTION

Fifty individuals in all, with 54.0% (N = 27) of them being male and 46.0% (N = 23) being female, were enrolled in the study as shown in Fig. 1(a).

FREQUENCY OF AGE GROUP

An array of age groups comprised the research's respondents. We divided our findings into four distinct categories according to age. Age groupings include young adults, middle-aged people, elderly individuals, and children. Each age group's population is represented by the histogram in Fig. 1 (b). In all, 50 participants are divided into 4 age groups, 11 are children, 11 are young adults, and 10 are middle-age adults and 18 old- age adults.

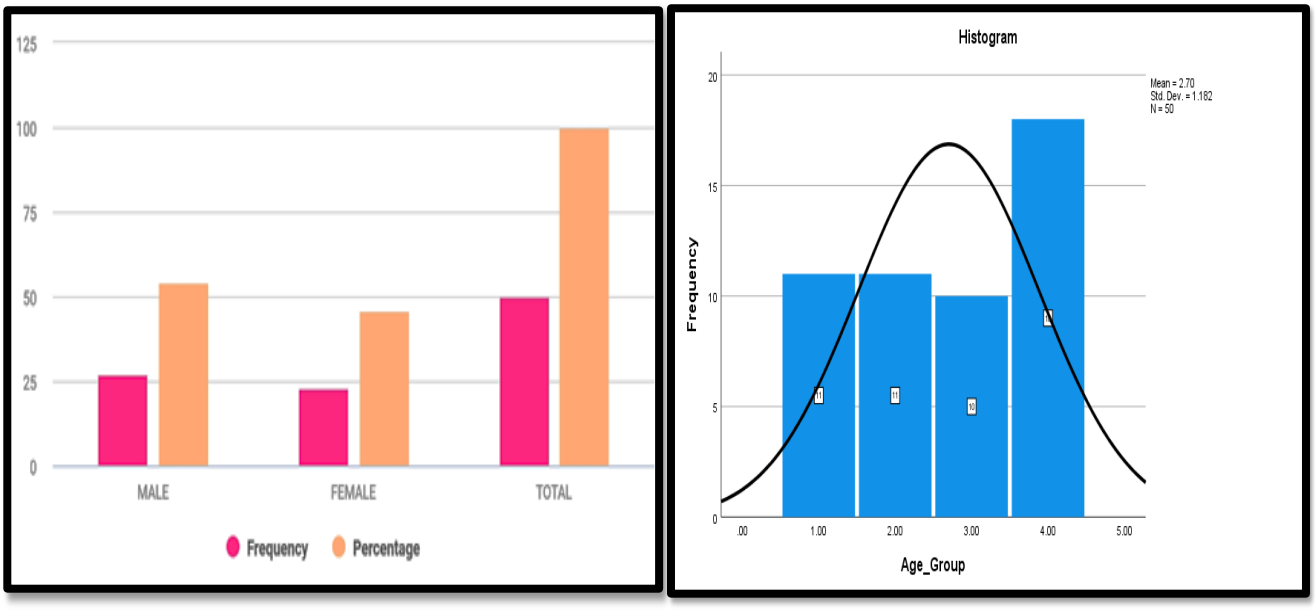

Fig. 1 (a). Bar chart showing gender frequency distribution **(b).** Histogram showing frequency of age group using SPSS

GEL ELECTROPHORESIS

Three aminoglycosides were examined in the study's specimens: gentamicin, tobramycin, and amikacin. In 86% of the cases under investigation, the antibiotic- resistant gene ArmA was found. It's intriguing to note that all samples carrying the armA gene showed notable rebellion against the aminoglycosides that were being studied (gentamicin, amikacin, and the antibiotic tobramycin). This implies that among the bacteria isolates that possess the ArmA gene for these antibacterial medications, resistance is highly prevalent. In Fig. 2 bands of armA gene are shown on gel electrophoresis against different band sizes.

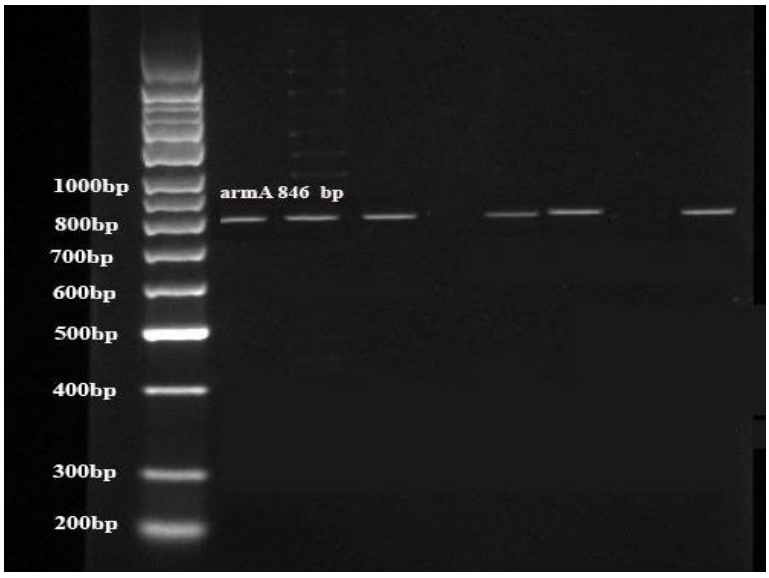

Fig. 2. Bands of armA gene on gel electrophoresis

DISCUSSION

This study reported the allelotypic and molecular portrayal of aminoglycoside resistance *Pseudomonas Aeruginosa* isolate from respiratory specimens at Chughtai Institute of Pathology, Lahore. Antibiotic resistance is exceedingly common internationally, which is consistent with today's developments and spotlights the pivotal need for innovative antimicrobial tactics and efficient aliment control methods. A worrying degree of resistance to several antibiotics, including TZP, FEP, CAZ, IPM, MEM, AK, CN, TOB, CIP, LEV, and DO, is revealed by the use of antibiotic responsiveness testing (16). This is consistent with

Pseudomonas Aeruginosa's well-known multidrug resistance, which has been observed worldwide and poses a serious problem in health care settings (15). Identifying the molecular foundation of resistance through genomic investigations is crucial, as demonstrated by the nature of resistance seen in aminoglycosides, which is likely due to the contribution of the armA gene. In *Pseudomonas Aeruginosa*, similar results have been documented in the literature, linking the armA gene to aminoglycoside resistance (17, 18).

In our study, 50 respiratory specimens were included. 54.0% (27) were gents and 46.0% (23) were women. In 2021 a study was conducted in the city of Ismailia the north eastern city of Egypt which included the 52 subjects from ICU and it revealed the similar results as our research. In our research, the pathogen must be identified and characterized using the colony morphology on various agar media, biochemical test results, and antibiotic sensitivity patterns. The organism's resistance to several antibiotics is highlighted in the study, which is alarming considering the potential therapeutic ramifications. Operating VITEK MS for microbiological identification gives the research a more up-to-date and accurate perspective while the experiment comported by the colony morphology, unique biochemical reactions, and Gram staining have been employed to recognize colonies. Finally, tests for identifying species plus medications testing were accomplished by VITEK 2 Compact 5. Infections with Pseudomonas species are very serious neuropathology and CNS disorders seen with various clinicopathological studies diagnosed by gene expressions and neurotropic analysis (18, 20, 22).

We were interested in researching *P. aeruginosa* strains in this contrasting investigation carried out in tertiary care medical centers in Faisalabad and Lahore, Pakistan. Specifically, we focused on 148 strains in the comparative study and 50 strains in our study, which were obtained from an assortment of clinical tests, mainly respiratory fluid samples. The sequencing techniques used in the two investigations were different: our study used Gram staining, colony morphology, biochemical testing, VITEK MS, and gel electrophoresis for the armA gene, whereas the comparison study used morphological tests, API 20E, and multiplex PCR. Furthermore, by CLSI and FDA criteria, the comparison research used MIC assessments and disc diffusion assessment for vulnerability to antibiotics, whereas our investigation used a disc dispersion technique for assessing responsiveness and susceptibility disintegration. ArmA amplifying genes and species-level authentication were carried out (19).

Netilmicin was a particularly efficacious antibacterial in the initial research, with a 68% resistance rate, while ciprofloxacin had the highest resistance incidence at 100%. This was determined by an extensive evaluation of immunity to antibiotics. The results of the minimum inhibitory concentration (MIC) test showed that gentamicin, tobramycin, and streptomycin were significantly resistant, with 94% of the samples showing resistance. Netilmicin showed the highest susceptibility, at 20%. APH(3')- VIa (aphA6), ANT(2″)-Ia (aadB), ANT(3″)-Ia (aadA1), AAC(6')-Ib (aacA4), ArmA, and AAC(3)-IIa (aacC2) were among the resistance alleles to aminoglycosides that were also found in this research. Conversely, experiment examined the antibiotic resistance patterns and socioeconomic background of fifty *P. aeruginosa* strains that were isolated from respiratory samples. It was carried out at Agha khan Labs. The results revealed a significant incidence of antibiotic resistance, with notable incidences of resistance for AK, CIP, TZP, FEP, CAZ, IPM, and MEM. Remarkably, the ArmA gene's gel electrophoresis data showed a startling 86% presence, suggesting a possible link to resistance to aminoglycosides including gentamicin, tobramycin, and amikacin.

CONCLUSION

In a nutshell this work provides important new information about the characteristics, ascertainment, and patterns of antimicrobial resistance of *Pseudomonas Aeruginosa*. The repercussions authenticate the pathogen's renowned multidrug resistance and highlight the need for more study and efficient antimicrobial superintendence. The results highlight the detailed information of the interplay between the antibiotic's action on bacteria and bacterial resistance mechanism and the significance of ongoing monitoring for resistance to antibiotics, highlighting the necessity of focused conciliation and the creation of substitute therapeutic approaches to tackle the escalating problem of multidrug resistance in microbial infections.

LIMITATIONS AND FUTURE PROSPECTS:

This research still forces the idea of having new tactics for the management of antimicrobial resistance and resource to a global fight against antibiotics. Nevertheless, the study is a valuable source of knowledge that cannot be disregarded when striving to devise appropriate coping mechanisms for this urgent issue within the sphere of healthcare. This study has some drawbacks such as a restricted number of participants and the study conducted only in one region. The use of certain diagnostic techniques might also overlook other mechanisms of resistance. Therefore, future research studies should increase the sample size and consider having the increase the sample's representativeness. Also, the addition of whole-genome sequencing could offer a better understanding of the resistance underlying factors. Implementing new approaches to the creation of AMs and enhancing the infection-control practices are the urgent needs to account for the increasing rates of antibiotic resistance. Therefore, it is important to have global cooperation to counter this threat that is on the rise.

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