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EVALUATING AMINOGLYCOSIDE RESISTANCE IN *E. COLI* ISOLATES FROM PNEUMONIA: A DUAL PHENOTYPIC AND GENOTYPIC APPROACH

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Abstract

Introduction: *Escherichia coli is a gram-negative facultative anaerobe bacteria of the family Enterobacteriaceae and causes a plethora of infections, both community and hospital-acquired. This is a Gram-negative bacillus, which is a facultatively anaerobic, organism with fermentative ability and is known for its pathogenicity. Main Antibiotic resistance mechanisms include; decreased expression and function of porins, modification of antibiotic targets, as well as enzymatic degradation of antibiotics.*

Objective: *The primary purpose of the study was to observe E. coli profiles of aminoglycoside-resistant E. coli isolated from clinical samples based on genotypic and phenotypic analysis.*

Methodology: *Isolation of bacterial pathogens was done as per CLSI guidelines as well as conventional microbiological techniques were used. The bacterial pathogens from the respiratory specimens were identified through culture and confirmed by PCR, after which DNA was extracted and the DNA bands were stained with the aid of gel electrophoresis. Primers for aminoglycoside resistance genes in Escherichia coli were obtained by PCR with the aid of specific primers of aminoglycoside resistance genes in Escherichia coli. The descriptive statistical analysis was therefore done using SPSS to produce a frequency distribution graph for patients of different ages. The study also presented percentage-based gender distribution, means and standard deviations and used bar charts to display the data.*

Results: *There were 42 males and 38 females among the 80 isolates obtained from patients. On microscopy, it was confirmed to be a Gram-negative, rod-shaped Bacillus. The organism showed clear biochemical differentiation through biochemical tests. About 85 % of the samples were resistant to aminoglycosides, carbapenems, cephalosporins and fluoroquinolones. Moreover, 80% of the bacterial isolate was resistant to colistin bacterial antibiotic. In samples of aminoglycoside-resistant Escherichia coli strains, the armA gene was identified in about 86%. The rest of the 14% (positive strains) were sensitive to aminoglycoside showing the absence of any bands in the electrophoresis test.*

Conclusion: *The results of the study confirm a highly studied bacterium, Escherichia coli, as a multidrug-resistant bacterium, especially against aminoglycosides and also reemphasize the importance of further research and practice of proper use of antibiotics. The need to design therapeutic approaches which would effectively address the increasing phenomenon of MDR in E. coli is therefore highlighted in the study as an essential step towards enhancing the nature and quality of treatment.*

Keywords: *Aminoglycoside resistance, DNA extraction, Escherichia coli, PCR*

INTRODUCTION

Escherichia coli (*E. coli*) is an extremely diverse organism of Enterobacteriaceae family which is not only a part of health beneficial gut microbiota but also an important pathogenic organism. It is associated with various types of infections such as urinary tract infections, blood stream infections and pneumonia especially in such patients as the elderly, immuno-compromised patients and patients with comorbid conditions (1, 2). Over the years, multidrug-resistant (MDR) *E. coli* strains have emerged creating a public

health concern as treatment regimens become more challenging and morbidity and mortality increases (3). In pneumonia cases aminoglycosides are preferred since they are effective in combating gram negative bacteria. However, the increasing trend of resistance of *E. coli* to aminoglycosides makes treatment challenging, leading to inferior clinical prognosis and costs of managing respiratory infections (4).

However, there are multiple approaches that lead to decrease in efficiency of aminoglycosides in *E. coli*; Among these it is important to mention enzymes that belong to the group of acetyltransferases, phosphotransferases and nucleotidyltransferases which modify the aminoglycoside molecules to pass a state when there is no possibility for their binding to the ribosomal RNA and, thus, the process of protein synthesis is interrupted (5). Further, single nucleotide changes in the 16S rRNA gene affect the structure of binding domain of a bacterial ribosome thus decreasing binding affinity of aminoglycoside (6). Alterations in the outer membrane of bacteria, which is regarded in most cases to be associated with the down-regulation of porin proteins, prevent the entry of aminoglycoside into the bacterial cell (7). In regards to pneumonia, this resistance is a problem as aminoglycosides are sometimes used for treating severe respiratory infections. As the resistance in *E. coli* increases as observed above not only limits the course of treatment but also leads to increased mortality, admission days and healthcare costs presenting a clinical problem.

Thus, the combined use of phenotypic and genotypic evaluation of resistant *E. coli* isolates is valuable in studying the mechanisms of aminoglycoside resistance. Disk diffusion techniques, and broth micro dilution, enable the determination of antibiotic effectiveness as well as the ability to distinguish between resistant strains visually based on their growth (8). Other biochemical tests such as urease, indole production and citrate utilization tests may provide clear and detailed recognition of such isolates and help in their classification (9).

On the molecular level PCR sequencing and molecular typing techniques can be used to identify individual resistance genes and characterize genetic factors associated with the spread of resistance factors such as plasmids and transposons (10). By using these methodologies, the researchers are able to have a good view of the genetic mechanisms that underlie resistance to IGF inhibition and the molecular targets that can be exploited for treatment.

Several researches have pointed out the fact that HGT plays significant role in the transfer of aminoglycoside resistance genes among *E. coli.* Plasmids and integrative conjugative elements (ICEs) i.e. mobile genetic elements enable passage of resistance factors between the bacteria where resistance is quickly disseminated in clinical settings (11). A new resistant clone can appear in a short time, especially in settings where antimicrobial agents are heavily employed, exemplified by recurrent outbreaks of infections with antimicrobial-resistant pathogens (12). Predicting the mechanisms and the factors that control the selection of the resistant strains are important for implementing proper infection control methods and antibiotic protocol.

Considering the rising interest in aminoglycoside resistance, this research seeks to establish the phenotypic and genotypic profile of aminoglycoside resistant *E. coli,* sampled from pneumonic patients. Our long-term goal is to provide clinicians with insights into all aspects of resistance and with the precise types of genes that are carried by these isolates in order to facilitate the design of improved therapeutic approaches.

MATERIALS AND METHODS

This study is a cross-sectional observational research design which used survey data about a population at a certain point in the time. Data collection was done at the Life Science Laboratories of the inter-collaborative institutions under the IRB protocol. Nasal and throat secretions were obtained by swabbing from patients in selected clinical facilities in Lahore with cooperation from the microbiology and biotechnology departments of the many institutions.

INCLUSION AND EXCLUSION CRITERIA

Only *Escherichia coli* isolates from respiratory patients were used in this research. The specimens which were not collected from respiratory patients were not considered for this study. Distilled water was

used for preparation of all media and reagents used in this work. Equipment including glassware was thoroughly washed and then autoclaved with water at 121 °C for 15 minutes under 15 psi pressures before it was used.

ISOLATION OF BACTERIA

E. coli samples were isolated from the respiratory specimen of the patients. These samples were grown on normal microbiological media plates. For inoculation sheep blood agar and MacConkey agar were used. The culture plates were also incubated at a temperature of 37°C and the colonies on these plates were observed after the samples had been left for 24 hours. Colony morphology was assessed and subsequent bacterial colonies were streaked on the relevant enrichment nutrient agar plates to accomplish bacterial isolation. After 24 hours of incubation, colonies were purified; growth was noted and then reported for record. Based on their morphology, size, color, margins, surface texture, elevation, and overall colonial morphology, preliminary identification of the different potential pathogens was made. To study colony characteristics, cultures were left to incubate for not less than 18-24 hours. The variable nature of these characteristics was useful in diagnosing and categorizing the infections. By using light microscope, gram staining was performed and biochemical tests for the characterization of isolated bacteria like catalase, oxidase, citrate utilization, motility, urease, triple sugar iron (TSI) agar and antimicrobial susceptibility testing (AST) were also performed.

DNA EXTRACTION

Genomic DNA was extracted using the GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific, #1554M) from tissues following the mentioned procedures. PCR started with denaturation step at 94°C for 4 minutes followed by 35 cycles at 45seconds for denaturation at the same temperature. An annealing step was performed for 40 seconds at a temperature that is most suitable for the chosen primers. An extension phase was done at 72°C for 50 sec followed by a final extension at 72°C of five minutes. Gel Electrophoresis: The PCR was performed using 2% agarose gel and was run for an hour at 70V with Tris-Edta Buffer. Agarose gel was prepared to contain ethidium bromide with a concentration of 0.5 micrograms per ml. PCR products were visualized using ultraviolet light and their sizes estimated based on a 1 Kb DNA molecular weight marker. The PCR products that were selected underwent genome sequencing so as to get a comprehensive result of the genetic makeup.

STATISTICAL ANALYSIS

To analyze quantitative data descriptive statistical analysis was run on SPSS, to obtain frequency distribution curves of variety of age groups. The study provided the gender-wise pre and post-test percentage, mean, standard deviations and the bar charts for the isolated data as well. To reduce sample bias, subjects were drawn across the age spectrum, and specific clinical environments were outlined. Population size with respect to gender is displayed in graph to show the gender wise proportion of antibiotic resistance.

Despite the relatively small sample size, these results give the most up-to-date information on aminoglycoside resistance in *Escherichia coli*. In general, the results of the study presented in Table I in the gender-wise percentages, means, standard deviations, and bar charts result in clear analysis of the formulated hypotheses and point out trends and patterns. With the findings of this preliminary research, there is potential for future investigations of crucial domains of antibiotic resistance as indicated in this study.

RESULTS

Comparing the sex distribution in the selected households, where the study members can be represented by fifty persons, the researchers observed the approximate equality with the male sex dominating weakly, 54%, compared to female, 46%. Furthermore, analysis by age groups I got a varied picture with the mainstream of old aged adults falling in the category that formed 36% of the whole sample.

Children and young adults accounted for 22% respectively, while 20% was from the middle aged adult population. Prominent microscope features were identified in the bacterium which induced focused study with biochemical diagnostics testing for *E. coli* identification can be seen in Table I.

Table I. Biochemical diagnostic testing for *Escherichia coli* identification

Test	Expected Result for *E. coli*	Purpose of the Test
Gram Staining	Gram-negative (Pink Rods)	Differentiates between species
Oxidase Test	Negative	Determines the presence of cytochrome c oxidase enzyme
Catalase Test	Positive	breaks down hydrogen peroxide
Indole Production	Positive	Confirms the ability to convert tryptophan into indole
Methyl Red Test	Positive	Indicates glucose fermentation with stable acid production
Voges-Proskauer	Negative	Tests for acetoin production from glucose fermentation
Citrate Utilization	Negative	Tests the ability to use citrate as the sole carbon source
Urease Test	Negative	Detects urease enzyme, which hydrolyzes urea to ammonia
Triple Sugar Iron (TSI)	Acid/Acid	Differentiates bacteria based on glucose, lactose/sucrose fermentation, gas production, and H2S production
Motility Test	Positive	Confirms the motility of the organism (presence of flagella)
Lactose Fermentation	Positive	Confirms lactose fermentation
Nitrate Reduction Test	Positive	Nitrate reduction
ONPG Test	Positive	Confirms the presence of β-galactosidase for lactose fermentation

In terms of age, the research respondents formed an array of ages. Since it was apparent that our results were broken down into clear age brackets, we grouped them into four age groups. Extended age group classifications are young adults, middle-aged population, the elderly, and children. Fig. 1 shows the histogram of population of each age group. Cumulative, 50 participants are in four groups by ages: 11 children, 11 young adults, 10 middle aged adults, and 18 old aged adults. Overall 50 samples from the individuals with gender frequency of 54% (n=27) male and 46% (n=23) female were recorded in bar chart as shown in Fig. 1.

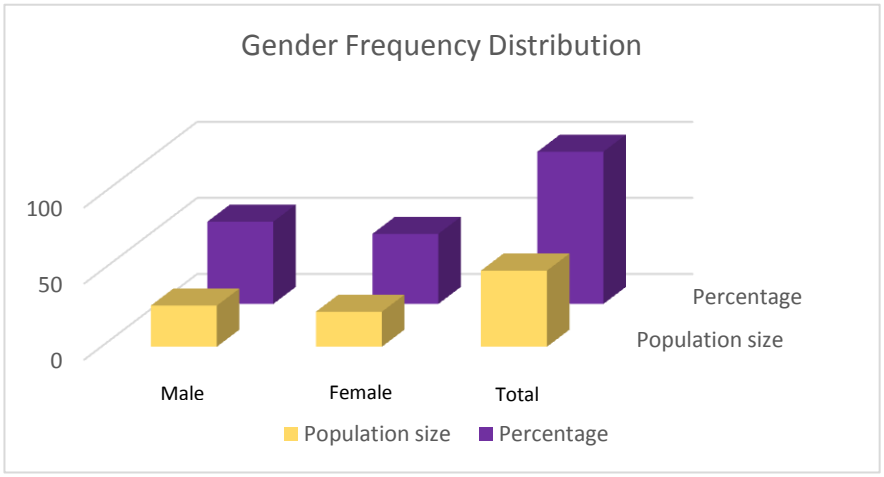

Fig. 1. Age groups showing histogram made by SPSS

DETECTION OF ArmA GENE BANDS VIA GEL ELECTROPHORESIS

Three aminoglycosides were examined in the study's specimens: of which are gentamicin, tobramycin and amikacin. In the present study analysis of antibiotic resistant gene ArmA was done in 86

percent of the total cases under consideration. What also can be found quite antsy is that all samples with the presence of the ArmA gene had a highly raised level of resistance to the aminoglycosides that were under work which include gentamicin, amikacin and tobramycin antibiotic. This means, that of the bacterial isolates with the ArmA gene for these antibacterial medications, resistance for the above is highly present. In Fig. 2 ArmA gene bands are represented against various standardized bp bands (Graded Controls) of gel electrophoresis.

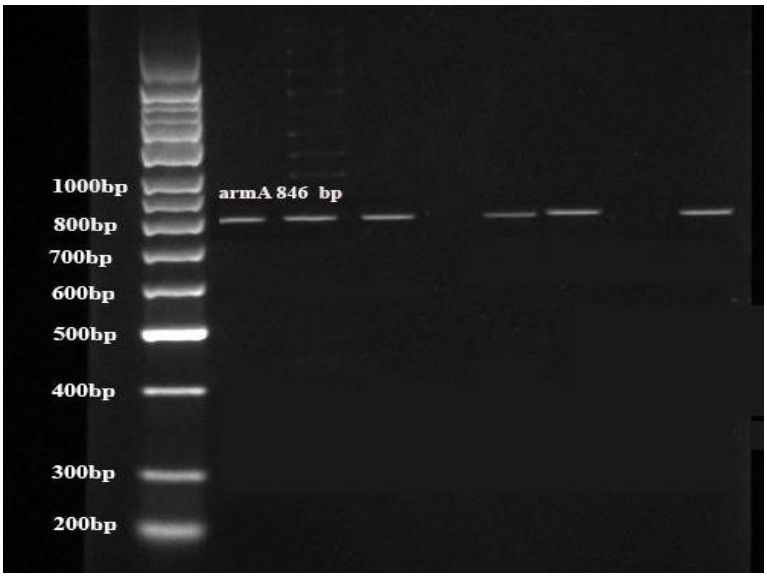

Fig. 2. Bands (846 bp) of armA gene on gel electrophoresis

Clearly, the phenotypic resistance patterns identified were fairly consistent with the genotypic aminoglycoside resistance markers. Of 50 *E. coli* isolates, 85% showed phenotypic resistance to aminoglycosides which include gentamycin, tobramycin, and amikacin. Consequently, molecular probing showed that 86% of these phenotypic MLSs contained the armA gene, encoding for a methyltransferase associated with high-level aminoglycoside resistance. Furthermore, both, aac(3)-IIa and aph(3')-VIa gene carries were associated phenotypical resistance patterns to aminoglycoside which strengthens the evidence of the relation between the presence of these genes and aminoglycoside resistance. Of the remaining 14 % isolates, PCR test did not show the presence of any detectable resistance genes; these isolates were sensitive to aminoglycoside further illustrating the link between the genes and resistance in the bacteria.

DISCUSSION

This work reveals a detailed analysis of factors associated with aminoglycoside resistance in *Escherichia coli* isolates from pneumonia patients and reports the phenotypic and genotypic resistance profile. One of the current issues that are threatening lives globally is antibiotic resistance, especially in *Escherichia coli* and thus there is a need to advance different aspects of AMR and put in place better standard control measures. Cross-resistance in terms of gentamicin, tobramycin, amikacin, and others indicates the need for surveillance and the development of better therapeutic strategies (13, 14).

Altogether, 50 respiratory samples were collected in our study, 27 participants were male, and 23 participants were female. This is going with agreement with other studies which also reveal similar demographic characteristics of patients who develop pneumonia due to *E. coli* (15). The species identification and its characterization involved colony characteristics on different media, biochemical patterns, and antibiotics sensitivity profiles. The high resistance rates observed in our study highlight the importance of investigating antimicrobial resistance at both the phenotypic and genotypic levels to better understand its practical implications and develop effective strategies to combat it (16).

Ensuring the maximal species-level identification and antibiotic susceptibility testing added further credibility to the results obtained with the help of methods including VITEK MS and VITEK 2 compact. The conventional techniques used such as colony morphology and gram staining supported the results obtained

through molecular techniques thus proving that combining the different approaches can enhance the overall characterization of bacteria (17).

A significant outcome of the present investigation was the identification of presence of more than one gene related to aminoglycoside resistance among the study isolates. These included aac(3)-IIa, aph(3′)-VIa, and armA genes, which were noted among the *E. coli* isolates showcasing a genetic profile for resistance (18). Other studies have also associated the existence of these resistance determinants to the high level of aminoglycoside resistance in *E. coli* strains in different clinical settings (19, 20). For example, the armA gene identified by our study has been said to be associated with high-level resistance to aminoglycosides revealing a concerning future for treatment of pneumonia due to *E. coli.*

When comparing with other studies, the identified resistance rates differ. For example, earlier studies revealed that netilmicin and gentamicin had high resistance profiles, while their susceptibilities varied depending on the region and clinical traditions (21, 22). According to this study, gentamycin displayed highest percentage of resistance of 94% thus having the highest incidence of resistance to tobramycin and amikacin. This raised a concern on the need to enhance the strength of antibiotic stewardship in health facilities to avoid emergence of these strains.

Thus, the results of the present study extend the current research on aminoglycoside resistance in *E. coli* and the necessity to expand continued research and monitoring for acquiring the updated data for treatment protocols. The phenotypic and genotypic analysis used in this work may provide useful information for understanding the resistance mechanisms that are crucial for setting up the effective therapeutic interventions and improving the overall wellbeing of the patients.

CONCLUSION

This study offers valuable insights into the phenotypic and genetic characteristics of aminoglycoside resistance in *E. coli* isolated from pneumonia cases. The findings reinforce the multidrug-resistant nature of this microorganism and underscore the need for ongoing research into the growing issue of antimicrobial resistance. The work also highlights significant correlations between aminoglycoside antibiotics and bacterial resistance mechanisms, emphasizing the necessity of tracking resistance trends over time.

Given the rising incidence of aminoglycoside-resistant *E. coli* in pneumonia patients, it is essential for healthcare providers, clinicians, and policymakers to intensify efforts in strengthening antibiotic stewardship programs. Such measures can help reduce the unnecessary use of antibiotics that contribute to the development of resistance. Moreover, the development of advanced diagnostic tools to accurately identify intrinsic resistance genes for aminoglycosides would greatly enhance the timely and precise application of interventions. Improving infection control practices and advancing targeted antimicrobial stewardship will be crucial in addressing this critical challenge in microbial infections.

Limitations:

This research highlights the need for a revised approach to addressing aminoglycoside resistance, particularly in *E. coli* isolates from pneumonia cases. However, the study has certain limitations that must be considered: the small sample size and regional focus restrict the generalizability of the findings. Additionally, the diagnostic techniques employed may have overlooked other relevant forms of resistance, which are crucial in understanding the broader scope of aminoglycoside resistance. The subsequent studies should enroll a large sample size to obtain the data that is more generalized and collect the isolates from different parts of the world to check the geographical differences as well. Currently there is a great need to come up with new aminoglycoside derivatives as well as enhance efforts of controlling infections leading to resistance. Efforts to control the growth of antibiotic-resistant *E. coli* and other multi drug resistant organisms in healthcare facilities require international cooperation.

Conflict of Interest:

Authors have no conflict of interest.

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