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Molecular Characterization and Genetic Basis of Aminoglycosides Resistance in *Escherichia Coli* Isolates from Clinical Specimens of Islamabad Residents

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Abstract

Escherichia coli (E. coli) is a ubiquitous microorganism and a resident bacterium in the intestines of animals and humans. They are related to various intestinal and parenteral diseases. Due to the widespread and inappropriate use of antibiotics, *E. coli* is gaining resistance at the genetic level through horizontal gene transfer mechanisms. This study aims to decipher the antibiotic resistance pattern of clinical isolates of *Escherichia coli* with reference to aminoglycosides. Culturing of clinical isolates of E. coli on MacConkey and blood agar (n = 200). These isolates were identified by microscopy and biochemical examination, and confirmed at the genus level by API®20E kit. For gene confirmation, the *E. coli* housekeeping gene uidA was amplified by PCR. The antibacterial spectrum of the characteristic isolate was decrypted by Kirby-Bauer assay. Finally, PCR was used to screen aminoglycoside resistance determinants. According to our results, the distributions of *E. coli* samples were 70, 50, 10 and 70 in urine, pus, swab and stool respectively. Males (37.5%) in this study were found less infected with *E. coli* than females (62.5%). According to antibiotic susceptibility testing, the isolates are resistant to amikacin, ceftriaxone, cefotaxime, Ceftazidime, ciprofloxacin, Gentamicin, Moxifloxacin, Ampicillin, Cefepime, tetracycline, and Ceftaroline. 80% of the strains are resistant to Gentamicin, and 75% are resistant to amikacin. However, all isolates were sensitive to meropenem, imipenem and tigecycline. Our isolates contain 11.5% rmtB, 7% aadA1 and 8.5% aphA6 aminoglycoside resistance gene. In summary, our study describes the *E. coli* infection trends and resistance patterns in Islamabad, Pakistan. Further research is needed to screen other aminoglycoside resistance genes in our local environment.

Keywords: Isolates; Aminoglycosides resistance; Antibiotic; Housekeeping gene

Introduction

Theodor Escherichia (Bacteriologist) found bacteria in the stool of patient with diarrhea and named the strain of bacterium as coli communis (1). *Escherichia coli are* Gram- negative bacteria, known for their high growth rate. It is a member of the *Enterobacteriaceae family* and belongs to class *Gammaproteobacteria* (2). The complete genomic sequence of *E. coli* was first published in 1997. Its genome size is 4,639,221 base pair with 4288 protein-coding genes. Genome contains 80 genes encoding ABC transporters.

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The classification of E. *coli was* initially completed by serologic identification of O and H antigens. There are seven pathological types of enteric *Escherichia coli and* three types of extra-intestinal *Escherichia coli* (3). Most E. *coli strains are* not pathogenic and have a symbiotic relationship with their host by preventing the colonization of infective bacteria in the intestine (4).
Resistance to antibiotic is one the major issues. The resistance is due to the intensity of antibiotics used, the ecological and geographical characteristics of the prevailing microorganisms. Obviously, the chemicals that have been used are very effective against this microorganism, but now a variety of drugs resistant microorganism has been developed. Resistance is not limited to bacteria, but also in fungi and other parasites. Resistance to antibiotics mainly develops in hospitals. For example, in 1930, streptococcus pyogenes in the military hospitals developed resistance to sulfonamide drugs.
During 1950s and 1960s resistance to multiple drugs first developed in *E. coli, salmonella* and *Shigella*. The trend changed the 1970s when *Hemophilus influenza* becomes resistant to tetracycline. Similarly, *Neisseria gonorrhea* causes infection in the genitourinary tract and develops resistance to Ampicillin. The reason for this microbial resistance is due to the massive use of antibiotics, especially in developing countries. After the reappearance of tuberculosis in 1980s the disease is usually multidrug resistant and favored by HIV (5).
Amino glycosides are composed of a 2-deoxystreptamine ring and are connected to two or more sugars through glycosidic bonds. There are three main types of antibiotics resistance in bacteria, inherent, adaptive and acquired. The inherent reason is that genes exist in one generation and transferred to the next generation. Inherent chromosomal genes exist in the host chromosomes, such as AmpC and β-lactamase in G-vie bacteria. Acquired resistance is mainly due to the mutation or gene transfer of the target genes. The genes are transferred via transposons, bacteriophages and other mobile genes. The process of acquired change is through transduction, conjugation, and transformation (5). Antibiotic resistance is one the main issues in Pakistan. Therefore this study was conducted to determine the molecular characterization and genetic basis of aminoglycosides resistance in *Escherichia coli* isolates from Clinical specimens of Islamabad residents

Material and methods

Samples

Our investigation included a total number of 200 isolates of *E. coli*, collected at National Institute of Health Islamabad from August 2020 to January 2021.

Isolation and identification

Specimens including, urine, pus and stool samples were used for inoculation on the MacConkey agar, blood agar followed by incubation at 37C° overnight. Positive growth of samples was identified by the standard laboratory methods using gram staining technique for morphological characteristics and biochemical indicative tests including catalase, indole, oxidase, methyl red, Vogues Proskauer and analytical profile test (6).

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Antimicrobial susceptibility

To check the susceptibility of the isolates, 12 antibiotics were used according to the standard method of disc diffusion which was recommended by Clinical and Laboratory standards institute.

Molecular characterization and detection of E.coli

Extraction of DNA of *E.coli*

Heating method was used for extraction of DNA. Firstly, Eppendorf tube was filled with 200ul distilled water and put single colony of *E. coli*. This work was done in bio-safety cabinet and Eppendorf tube vortexes for 30seconds. Furthermore, the tube was punctured and kept in hot water (96C°) for 10mins and frozen at 4C° for 5mint, followed by centrifugation at 12500rpm for 5 minute. The 150ml supernatant was collected in separate tube. The extracted DNA was used for PCR.

Molecular characterization and detection of *E.coli* for identification of its virulent strains

Molecular characterization of the convalescence *E. coli* were carried out by conventional PCR by using specific primer Amino glycoside O-nucleotidyltransferase Ant3Ia (*aad*A1), Amino glycoside O-phosphotransferase Aph3VIa (*aph*A6) gene and 16S rRNA (guanine (1405)-N (7)) -methyltransferase (*arm*B) were detected.

Results

In this study a total of 200 isolates of *E. coli* were included. According to our results, the distributions of *E. coli* samples were 70, 50, 10 and 70 in urine, pus, swab and stool respectively. Males (37.5%) in this study were found less infected with *E. coli* than females (62.5%). According to antibiotic susceptibility testing, the isolates are resistant to amikacin, ceftriaxone, cefotaxime, Ceftazidime, ciprofloxacin, Gentamicin, Moxifloxacin, Ampicillin, Cefepime, tetracycline, and Ceftaroline. 80% of the strains are resistant to Gentamicin, and 75% are resistant to amikacin. However, all isolates were sensitive to meropenem, imipenem and tigecycline. Our isolates contain 11.5% rmtB, 7% aadA1 and 8.5% aphA6 aminoglycoside resistance gene.

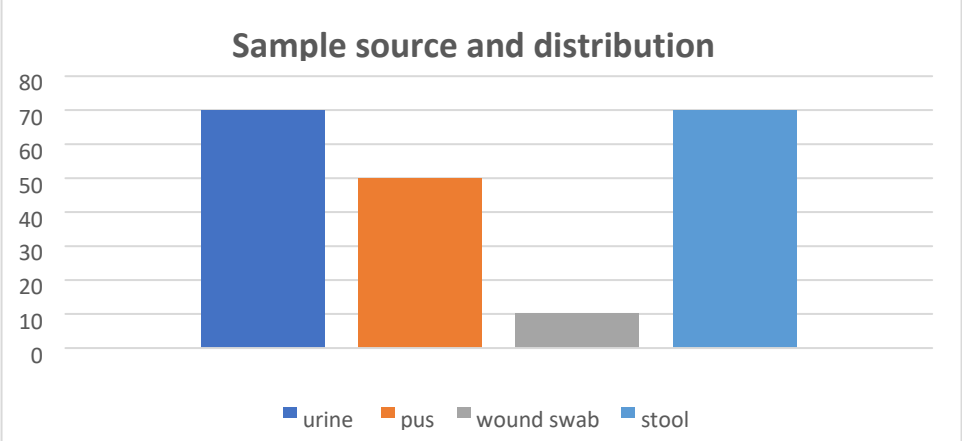

Figure 1: Distribution of isolates on the basis of sample source

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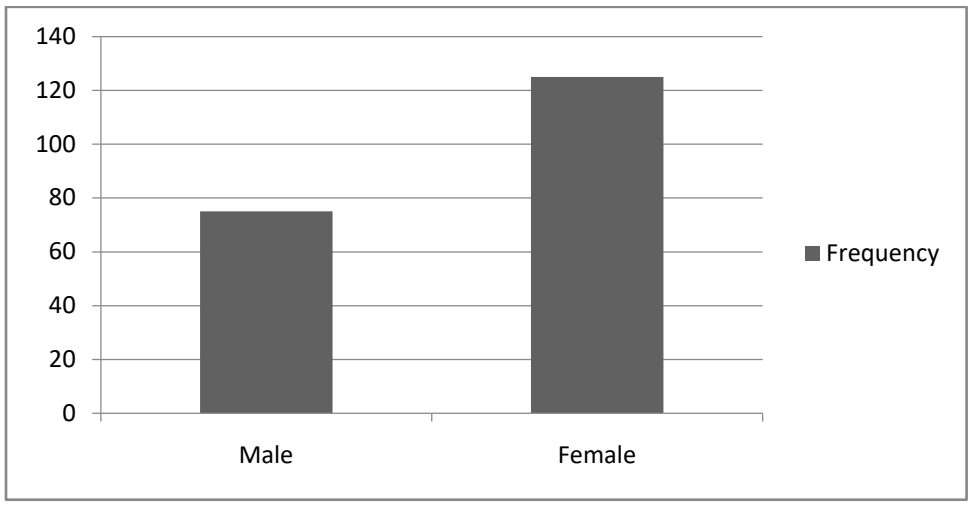

Figure 2: Gender wise distribution of samples

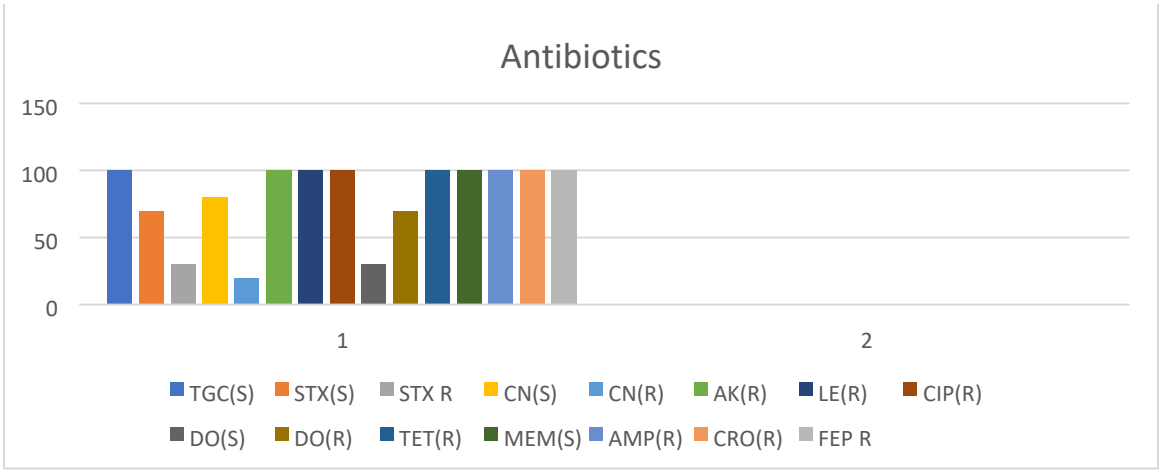

Figure 3: Antimicrobial susceptibility test of recovered *E.coli* by disc diffusion method

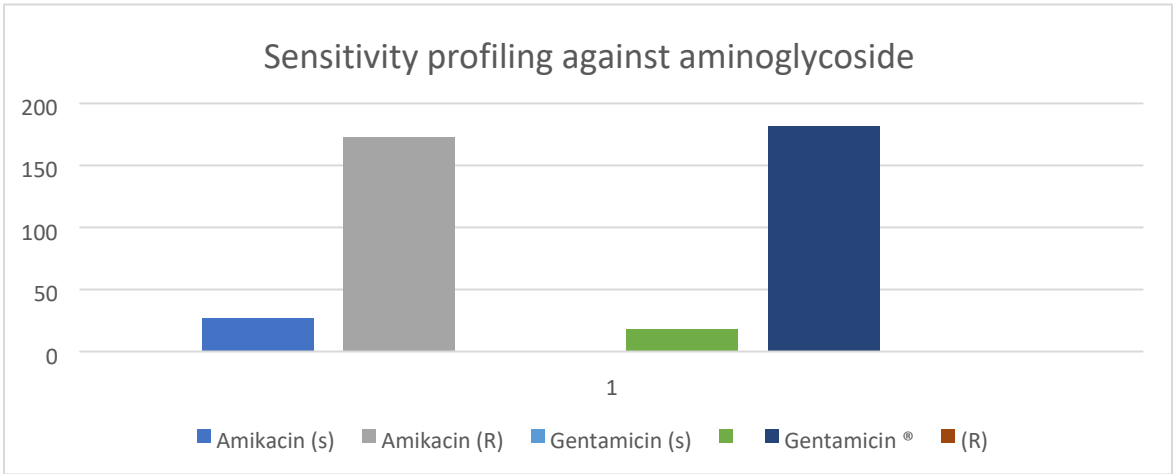

Figure 4: Molecular detection of oligo enzyme nucleotide transferase gene (aada1), phosphotransferase gene (apha6), and methyl transferase gene (rmtb) resistance against aminoglycoside by using conventional pcr

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All the resistant isolates were tested by PCR using specific primers for genes aadA1, aphA6 and rmtB. Meanwhile entire strains were observed and amplification of 792bp, 797bp and 769bp specific fragments for each gene was detected. 14 ,18,23 isolates were positive out of 200, and remaining strains were negative visualized on ethidium bromide-stained agarose gel of 1.5 % concentration as displayed in figures A,B,C respectively.

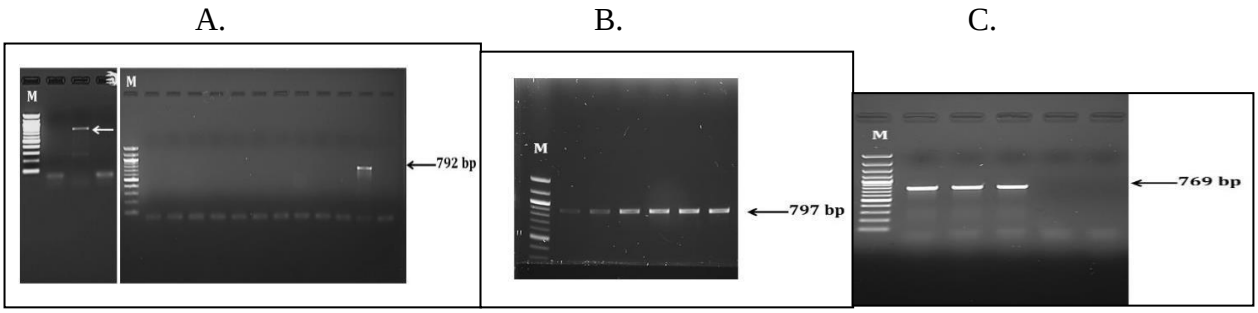

Figure 5: Bands obtained after gel electrophoresis

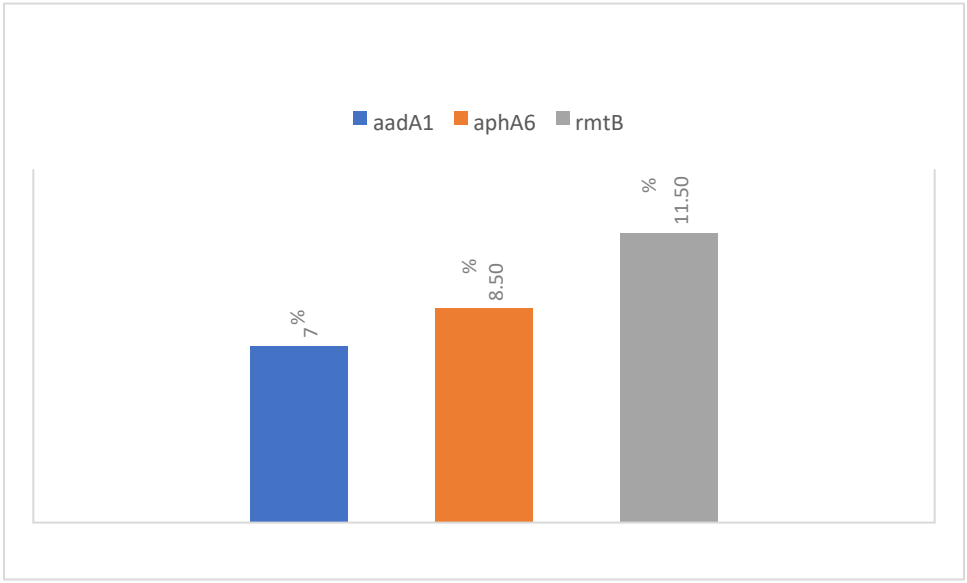

Figure 6: Pattern of gene resistance

Discussion

In humans, *E. coli* develop various diseases such as blood stream infections, food poisoning, otitis media, purulent wounds and other complex diseases (7). *Escherichia coli* is ubiquitous organism and are related to different infections of humans and animals. It contains various proteins related to virulence, including host surface binding protein, hemolysin, fimbrin, and fibrin, which play an important role in pathogenicity (8). In this study, isolates were resistant to amikacin, ceftriaxone, cefotaxime, Ceftazidime, ciprofloxacin, Gentamicin, Moxifloxacin, Ampicillin, Cefepime, tetracycline, and Ceftaroline. 80% of the strains are resistant to Gentamicin, and 75% are resistant to amikacin. However, all isolates were sensitive to meropenem, imipenem and tigecycline. A study was conducted in India to investigate the

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antibiotic resistance of *E. coli*, and the results showed that 63% of isolates were resistant to Amikacin (9). In Canada, Diarra et al. conducted a quality level screening of poultry and reported that, like our study, 25% of the isolates are resistant to Gentamicin (10). Unlike our research, Saenz et al. reported that *Escherichia coli* of human and veterinary origin are highly resistant to Gentamicin (11).

Bacteria are rapidly developing a variety of mutations that enable them to withstand the toxicity of aminoglycoside. Members of the Enterobacteriaceae have evolved different mechanisms to control the toxicity of aminoglycoside. These include drug efflux pumps (12), changes in membrane permeability (13), mutations in ribosomal target sites (14), and modification of ribosomal target sites by enzymes (15).

Our isolates contain 11.5% rmtB, 7% aadA1 and 8.5% aphA6 aminoglycoside resistance genes. Contrary to our study, a previous study done in India reported that rrs, aacC1, aphA3, aacC and aphD are the frequent aminoglycoside resistance genes (16). A previous study done by Soleimani et al in 2017 in Iran reported that only aac3-IIa and ant2-Ia are responsible for aminoglycoside resistance (17). Another study done by Shin et al., in South Korea reported that aminoglycoside resistance is caused by aph3-Ia, aph3-Ib, aac3-Iva and ant2-Ia (18). These studies show that the determinants of aminoglycoside resistance vary from region to region. The major limitation of our study is small sample size. A study having large sample size should be conducted for better results. Further research is needed to screen other aminoglycoside resistance genes in our local environment.

Conclusion

In conclusion, our study represented that *E. coli* mediated infections are increasing in the populated city of Pakistan. The bacteria are developing resistance against multiple antibiotics. Moreover, the aminoglycoside resistance in our region is different from our neighboring countries on a genetic basis. Further work is necessary to delineate other resistance mechanisms in our settings.

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