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E. coli's escape route: AcrAB –TolC efflux-associated aminoglycoside resistance in clinical UTI isolates

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Aminoglycosides remain important therapeutic options, but rising resistance threatens their efficacy. Among urinary tract infections (UTIs) were the most common bacterial infections, and *Escherichia coli* is their leading reason. The AcrAB-TolC efflux pump overexpression was recognized as a multidrug resistance mechanism in the gram-negative bacteria (G-ve). To conclude the profile of resistance of urinary *E. coli* samples to aminoglycosides with to investigate the presence of the genes *acrA* and *acrB* of the efflux pump in isolates resistant to all tested aminoglycosides. Twenty samples from clinical UTI patients were isolates at medical city hospital in Baghdad from July 2025 to December 2025. *E. coli* was identified by cultured on Eosin methylene blue (EMB) with MacConkey agar then confirm test via biochemical test (catalase & oxidase). Susceptibility to amikacin (AK), gentamicin (GN) and tobramycin (TOB) were tested via disk diffusion method. Isolates resistant to all three antibiotics were screened for *acrA* and *acrB* via PCR technique. Of twenty samples, six isolates were selected to PCR test according to their resistance to all three antibiotics were found that two isolates harboring genes separately and only one isolate harboring both resistance genes, when the isolates gave resistance rate 70% (14/20) for amikacin and 80% (16/20) percentage rate for the two antibiotics (GN & TOB) separately. The presence of *acrA* and *acrB* in five isolates from six isolates screened to triple aminoglycoside- resistant isolates suggests that AcrAB efflux may contribute to resistance in urinary *E. coli*. Expression studies or efflux inhibitor assays are needed to confirm a causal role.

Keywords

Escherichia coli,
Urinary Tract Infection,
Aminoglycosides,
Efflux Pump,
Acrab-Tolc

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Introduction:

Aminoglycoside group (AG)consider as antibiotic branch based on chemical structure which exhibit a several characteristics whereas make a useful for antibacterial therapies (1). Bacteria uptake AG via triple stages energy independent and two energy dependent phases, usually these antibiotics binds to proteins in specific region where they interfering with the formation of initial complex of peptide chain or inducing mRNA misreading and may be caused the dissociation of polysomes into nonfunctional monosomes, the overall effects are irreversible and lethal (bactericidal) for the bacterial cells (2).

Genetic change in microbes could be reduced or eliminate the effectiveness of AG but with long period of exposure to them led to generate a new mechanism of resistance (developed or acquired) under selective pressure (3). Whereas different AG resistance patterns include modification enzymes, low outer membrane permeability, target modification (16s rRNA) and active efflux pump (EP). Ejection antibiotic via EP were mostly chromosomal genes code and belong to the five classes superfamily, which recognized as tripartite structures in the gram-negative bacteria and the most common types coded in *E. coli* as AcrAB-TolC system, which consists of three proteins AcrB, AcrA and TolC located in inner membrane, periplasmic membrane and inner membrane respectively (4). Different substrate was moved from the cell to outside via two binding proteins of AcrB due to conformational changes subsequently in a mechanism with functional rotating to finishes required interaction with AcrA and TolC to march out the substrate (5).

Material and methods

Twenty *E. coli* isolates from medical city in Baghdad hospital from July 2025 to December 2025, from midstream urine of patients suffering from UTI. The primary diagnosis test based on phenotypic character of colony shape, colony texture, color and edges on EMB and MacConkey agar then transported to microscopic slide with gram stain to detect shape and arrangement of the cell finally confirm with standard biochemical tests (catalase and oxidase tests).

Antimicrobial susceptibility test

All isolates were cultured on Mueller Hinton agar for disk diffusion methods for classified isolated according to CLSI (2022) guideline their resistance to AG for thee antibiotic Amikacin (AK/ 30mg), Gentamicin (GN/ 30mg) tobramycin (TOB/ 10mg) with used stander strain ATCC35218 from Central Public Health Laboratory, Baghdad.

Genotypic detection

DNA extracted from all twenty isolates according to commercial DNA extraction kit (Promega, USA) then measured by Nano-drop 2000 (Thermos, Fisher Scientific, USA) as a PCR template for amplifications to detect harboring resistance EP genes (*acrA* and *acrB*) with using of gene specific primers as *acrA* F. CTCTCAGGCAGCTTAGCCCTAA, R. TGCAGAGGTTCAGTTTTGACTGTT with 107bp from Alpha DNA co. (Canada) and *acrB* F. AGCCCGCATGGATTTGA, R. GGCATACGGGAAGAAGT with 107bp from Oligo Data South Africa. Analyzing of gel electrophoresis with ethidium bromide as stain to interpretation the PCR results. PCR conditions were optimized via multiple repeats to annealing temperatures and number of cycles from 43 to 62 and 30 to 35 respectively according to primers with final volume of PCR mixture 25 µl as in table 1 and 2.

Table 1: PCR mixture used for amplification different genes in the current study final volume 25 µl.

Free nuclease water	Master mix (1x)	F+R primers volume (µl) (from 10pmol)	Final concentration of primer	template DNA	Target gene
4.5 µl	12.5 µl	1.5	0.6pmol	5µl	*acrA*
2.5 µl	12.5 µl	2	0.8pmol	6 µl	*acrB*

Table 2: reaction condition of PCR.

Final extension	elongation	Annealing	Denaturation	No. of cycles	Initial denaturation	Genes
10 min / 72°C	72°C / 1 min	43°C/30 sec	95°C / 1 min	33	94°C / 5 min	*AcrA*
4 min / 72 °C	72°C /30 sec	56°C / 1 min	95°C / 15 sec	30	95°C / 5 min	*AcrB*

Results

Phenotypic detection

All isolates were cultured on selective and differential media as in EMB and MacConkey agar whereas look like small, dry, no mucous colony with black to greenish metallic sheen on EMB agar and lactose ferment pink colony on MacConkey agar. For biochemical test gave negative react to oxidase and positive result in catalase test.

Antimicrobial susceptibility test

Tested was based on disk diffusion methods for three AG of antibiotic when appeared that resistance rate for AK 70% (14/ 20) whereas gave 80% (16/20) resistance for two others antibiotics (GN and TOB) separately as in figure 1.

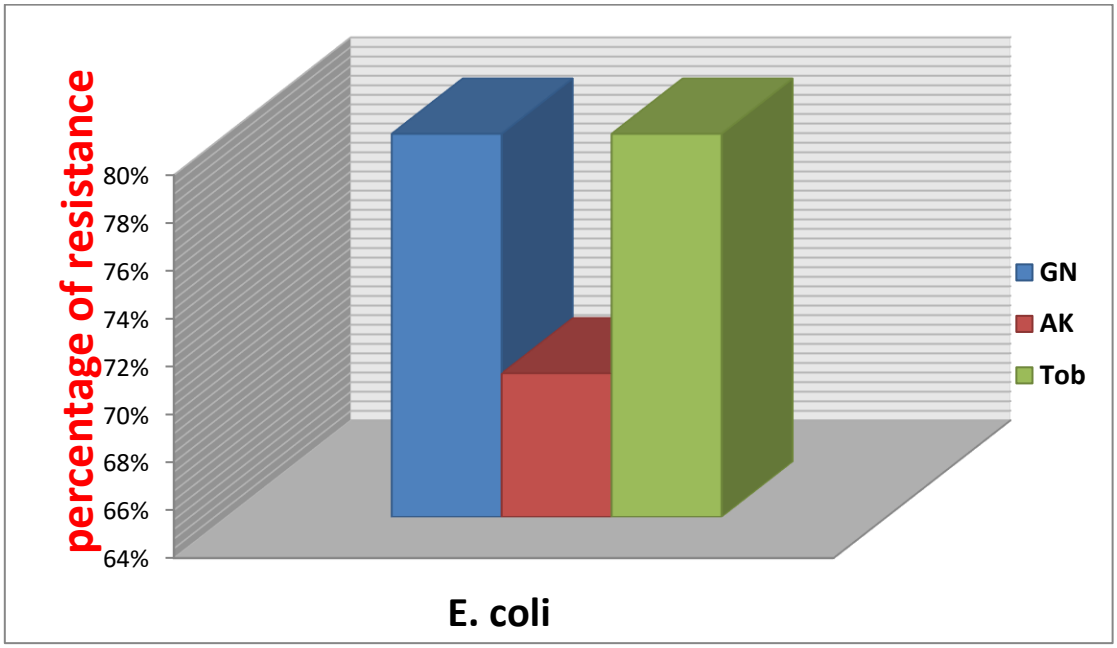


Figure 1: the percentage of *E. coli* resistance to AG

Genotypic detection for AG resistances of EP genes

Six isolates of *E. coli* were selected depending on their resistance to three AG antibiotic (AK, GN and TOB) in combined, among these six isolates analysis two genes (*acrA and acrB* with size 107 bp to each one) were detected in isolates to realize the molecular basis of resistance experiential study's isolates as in figure (2) and figure (3) which shows the PCR product for two genes.

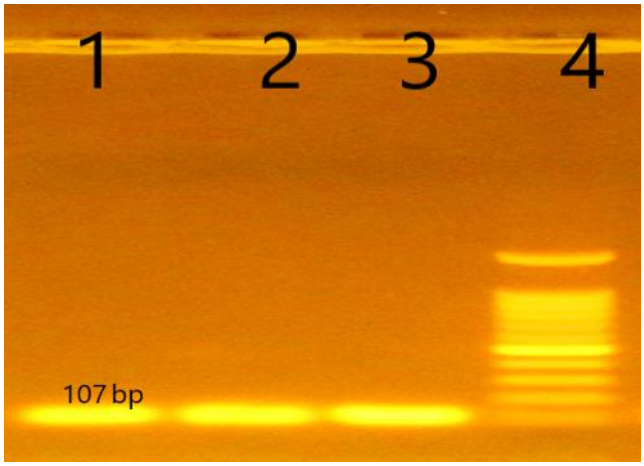


Figure 2: agarose gel electrophoresis for *acrA* PCR products (107 bp). Line 4 as DNA ladder (1000 bp), three line 1, 2 and 3 as a positive result.

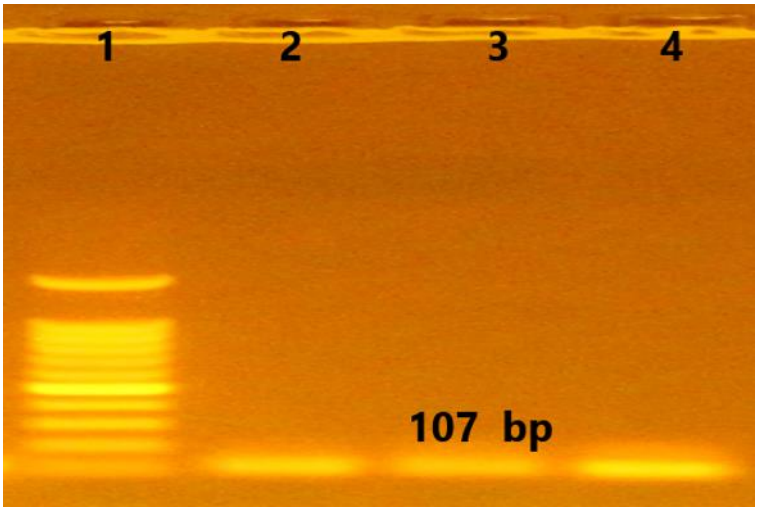


Figure 3: gel electrophoresis for *acrB* PCR products (107 bp) as shown that line 1 refer to DNA ladder and three other line (2, 3 and 4) as positive results.

Additionally, the antimicrobial susceptibility test showed that 6 out 20 studies of *E. coli* isolates were resistance to three AG antibiotic (E^4, E^5, E^6, E^{14}, E^{19} and E^{20}) and 5 out of 20 isolates resistance to two AG antibiotic (E^1, E^2, E^{10}, E^{15} and E^{18}) when others have resistance to only one AG antibiotic, the harboring resistance genes found only in E^4, E^5 and E^{19} for *acrA*, whereas *acrB* gene found in E^6, E^{14} and E^{19} as in figure (4).

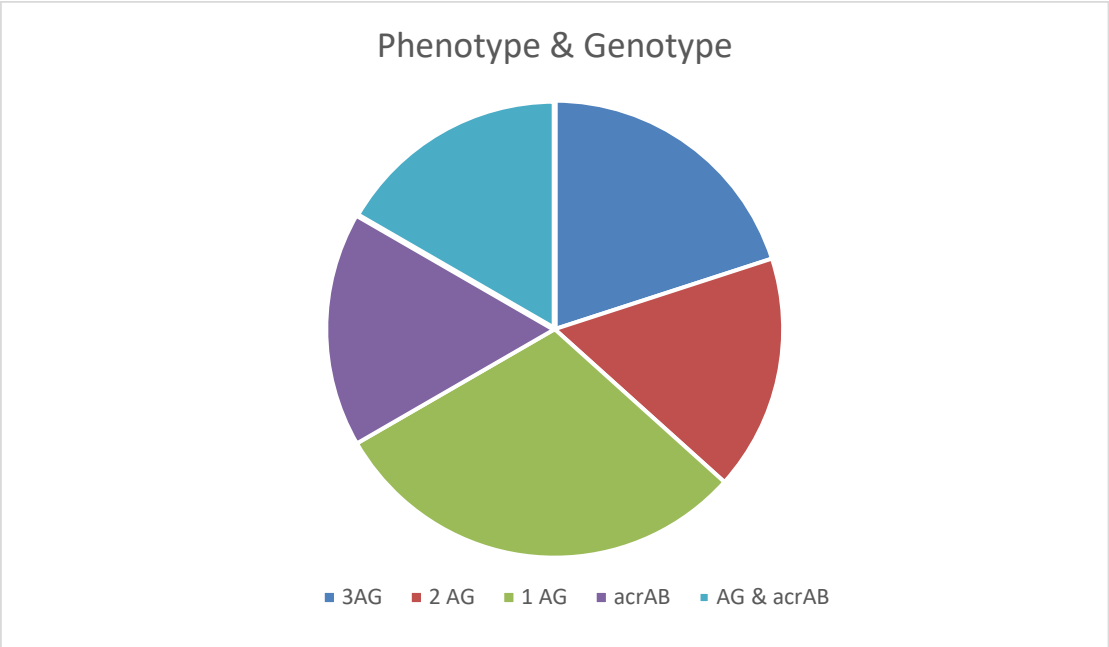


Figure 4: phenotype and genotype detections

Discussion

The Infectious Disease Society of America has recognized that UTIs as common disease in hospitals with different genders and age of patients, that threat the health caused via consequently complications by linked to diabetes mellitus which enhance the increase of acute infections in kidney (cystitis, pyelonephritis and absecss), approximately 80-90% of total UTIs were recorded as *E. coli* from all infections (6). A total *E. coli* strains were isolated from hospitals during the study period represented a high resistance rates to AG antibiotics and more frequently isolates in UTI. The data illustrated by El Aila and El Aish, (7) where the most distribution of *E. coli* in UTIs, many local and international studies were consistent with our study which presented cause the UTI was *E. coli*. Our analyzed that phenotypic characters and genotypic resistances patterns of *E. coli* isolates according to antimicrobial susceptibility test for all isolates via disk diffusion test when found that among of isolates have resistance 70% (14/ 20) for AK and 80% (16/20) as a higher percentage rate (GN and TOB), so the AK was remaining as the most effective one against *E. coli* from this collection as in figure (1). Notably that research done by Fadhil, *et. al.*, (8) which detected very lower of GN resistance rate as 7% and these result reverse to our study. also for AK found that reached to 10.6% and 9.58% with 27.2% and 18.56% for GN in the researches done by El Aila and El Aish, (7) and Al-Arosi. *et. al.*, (9) respectively. The reason may be long illness length, high cost of healthcare and superior morbidity and mortality, presented a severe challenge in setting of hospital and community (10). In addition to being have abilities to colonize on the urinary tract which facilities by expression of surface adhesion proteins particularly fimbriae with P-fimbriae as associated in pathogenesis of uropathogenic *E. coli* (UPEC) (11).

The resistance genes profile of efflux pump in the isolates of this study strongly link with resistance to aminoglycoside antibiotics via multiplicity ways in E. coli isolates which harboring the AcrAB-TolC belong tofamily of RND, epitomizes the major efflux pump (8). PCR products via electrophoresis analysis for *acrA and acrB* genes in six isolates which selected according to AG resistance by disk diffusion methods as appeared these isolates harboring two genes except one isolate (E^{20}). Among these 6 isolates, *acrA* gene was detected in three isolates (E^{4}, E^{5} and E^{19}) (15%) as in figure (2) and *acrB* gene detected in three isolates also (E^{6}, E^{14} and E^{19}) (15%) as in figure (3). Our research was inconsistent with previous study done by Maleki. *et. al.*, (12) in Iran where 82.9% of isolates harboring *acrA* gene and Ali & Al-Dahmoshi. (13) which identified *acrA* and *acrB* genes in 50 (100%) and 43 (86%) isolates respectably, also in Basra city search done by Nasir *et al.*, (14) where the percentage of harboring

reached to 78% for each *acrA* and *acB* genes. Interestingly, our PCR products detected both *acrA* and *acrB* genes together in only one isolate (E^{19}) which refer that has a unique gene character and required more confirm and deeper to other genes detections, this result unclosed to other researcher (12, 13, 14) also with search done in Egypt via Gawadet *et. al.*, (15). In the other hand, there are four isolates (E^4, E^5, E^6 and E^{14}) harboring only one gene from efflux pump that may be reason to resistance phenotypically while five isolates (E^1, E^2, E^{10}, E^{15} and E^{18}) gave resistance to two antibiotics rather than three antibiotics but don't screen for resistance efflux pump genes as in figure (4).

Whilst, genes percentage of identified were lower than data in phenotypic resistance data, that recommended to harboring multi mechanisms of resistance to drugs which requires further studies. Phenotypically resistance in some isolates, but no AG genes were recognized additionally, the isolate was resistant to three AG antibiotic (E^{20}), while not detected any resistance genes. the discrepancy apparent of the isolates may be carry genes of resistance but unexpressed because distant from promoter or associated with a weak promoter in an integron or may be have mutation on their genetic sequences (16).

On the other hand, there are other factors of resistance as target modification, decrease outer membrane permeability and enzymatic inactivation, also required to screened for other possible resistance determinant as plasmid, transposons and integron (17), whereas bacteria acquired their new resistance through horizontal gene transfer mechanisms that permit faster adaptation to pressure of antimicrobials (18). So the influence of biofilm regulated significantly the AcrAB-TolC efflux pump system that the role of system to prevent accumulation of toxic substances for many exposure intracellular antibiotics (19). Scape bacteria inside the macrophages also contributed as a pathogenesis and consider as virulence factor combining with other factors correlated together to infected human and facilities to forming mucoid biofilm character to adhere on the urine catheter to cause UTI disease (20).

Conclusion

Our study focusses on the relatively high rate of recurrence of *E. coli* isolation in hospital of Iraq as well as the high popularity of resistance AG rates were increasing to commonly usage antibiotics and UTI were highpoints the growing challenge of antimicrobial resistance in clinical practice so must be limiting effective treatment options and greater in therapeutic failure. Furthermore, our study suggests the combination of resistance mechanism detection to obtain a clear genetic view to bacteria thus, achieving therapeutic results in recording time.

Ethical permission

A search is accompanied succeeding the receiving of contestant consent and ethical agreement from ethics committee of the environmental health sciences department, college of Energy and Environmental Sciences at the University of AL-Karkh of sciences.

Acknowledgment

My study completed in the environmental health sciences department, college of Energy and Environmental Sciences at the university of AL-Karkh of sciences.

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