

A Preliminary Study on Antibiotic Resistance and the Detection of Co-Resistance Genes to Aminoglycosides and Fluoroquinolones in Gram-Negative Bacterial Isolates

Lubna Abd Muttalib Alshalah¹, Ghadah Ali Al-Oudah^{2,*} , Noor Salman³ , Zainab Adil Ghani Chabuck⁴, Amin Adeeb⁵

¹Environmental research and study center, University of Babylon, Hillah 51001, Babylon, Iraq.

²Clinical pharmacology, Pharmacy College, Al-Mustaqbal University, Hillah 51001, Babylon, Iraq.

³Department of Biology, Faculty of Science, Babylon University Babylon, Hillah 51001, Babylon, Iraq.

⁴Department of Medical Microbiology, College of Medicine/ University of Babylon, Hillah 51001, Babylon, Iraq.

⁵Medical University of Wrocław, Faculty of Medicine and Dentistry, Piasts of Silesia, Wrocław, Poland.

Corresponding Author Email:

ghada.ali@uomus.edu.iq

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ABSTRACT

Antibiotic resistance poses a major challenge among pathogenic bacteria, particularly Gram-negative types. Early detection of this resistance is crucial to prevent its horizontal transfer between different bacterial species. This preliminary study aimed to establish the antibiogram and detect specific genes responsible for co-resistance to aminoglycosides and fluoroquinolones commonly found in Gram-negative bacteria. The study evaluated four different clinical species of Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes*.

The susceptibility of these isolates to antibiotics spanning seven antimicrobial categories was determined by measuring inhibition zone diameters using the disk diffusion method. Additionally, the resistance genes *parC* and *ant(2'')-Ia* were detected using conventional polymerase chain reaction (PCR). The preliminary findings revealed high resistance percentages across all isolates, ranging from 50% to 100% against most tested antibiotics. The Multiple Antibiotic Resistance (MAR) index exceeded 0.2 for all isolates, ranging between 0.64 and 0.82, though not reaching 1.0.

Furthermore, all isolates were positive for both aminoglycoside and fluoroquinolone resistance genes, highlighting that *ant(2'')-Ia* is primarily responsible for aminoglycoside resistance in these isolates. This preliminary study underscores that the MAR index serves as a reliable indicator for the spread of antimicrobial resistance. The molecular identification of resistance genes remains a crucial hallmark for recognizing resistant isolates and implementing effective control measures.

Keywords: Preliminary study, Antibiotic resistance, Gram-negative bacteria, Aminoglycoside resistance, Fluoroquinolone resistance.

1. Introduction

Aminoglycosides antibiotics are either natural or semisynthetic compounds that derived from actinomycetes; prevalently found, vigorously active against wide spectrum of bacteria (G- and G+) [1]. Moreover, these antibiotics are powerful, broad-spectrum via prevent synthesis some molecule like protein. A first member of this group to be presented for routine clinical usage in humans. Additional members of aminoglycosides were applied over the consecutive years including neomycin, kanamycin, gentamicin, netilmicin, tobramycin, and amikacin.

Enterobacteriaceae, like, *E. coli*, *K. Pneumoniae* and *K. oxytoca*, *Enterobacter cloacae* and other species inhibited by some antibiotic like aminoglycosides are mostly effective against species of Enterobacteriaceae [2,3]. The mechanism of Aminoglycosides includes binding to specific site (A-site) found on the 16S ribosomal RNA of the 30S ribosome, and prevent translation (protein synthesis) [4].

Resistance against aminoglycosides appears in many different mechanisms include:

- (1) modifying enzymes,like Aminoglycoside Phosphoryltransferase (APH), Aminoglycoside Acetyltransferase (AAC), and Aminoglycoside Nucleotidyltransferase (ANT) .
- (2) Methylation enzyme by a family of ribosomal methyltransferase enzymes.
- (3) Mutation subunit.
- (4) Efflux pumps[5,6].

By active release of antibiotic from bacterial cell and [5] alteration the bacterial cell membrane permeability and prevention or reduction passage of some compounds such as antibiotics (aminoglycosides) [7,8].

There was an effective and detective mechanism that has been used to detect resistant bacterial sources called multiple antibiotic resistance (MAR) index. MAR index can be defined as calculate the ratio of number of resistant antibiotics by microorganisms to the total number of antibiotics which exposed in this experiment. When MAR index values more than 0.2 indicate high threat source of resistant where antibiotics are often used [9,10].The aim of this research was to detect profile of antibiotic susceptibility for some Gram negative isolates and determine some aminoglycoside antibiotic resistance genes.

2. Materials and Methods:

Samples: The study evaluated four different clinical species of Gram-negative bacteria: 24 isolates (6 for each species: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes*) were obtained from the College of Science for Women / University of Babylon, Iraq. Definitive identification and diagnosis depend on phenotypic and biochemical characteristics [11].

2.1. Antibiotic susceptibility Test

The disc diffusion technique was conducted to detect the antibiotic susceptibility pattern of isolates against eleven different antibiotics that belong to seven antimicrobial categories (Table 1). Inhibition zones were read post-incubation (24 h, 37), recorded, and compared to CLSI [12].

Table 1. Antibiotic types with their groups and concentrations that used in this study

Group	Sub-group	Symbol	Conc.
Fluoroquinolones	Ciprofloxacin	CIP	5
Fluoroquinolones	Levofloxacin	LEVO	10

Fluoroquinolones	Norfloxacin	NOR	30
Phenicol	Chloramphenicol	C	30
Aminoglycoside	Amikacin	AK	10
Aminoglycoside	Tobramycin	TM	10
B-lactam combination	Amoxicillin–clavulanate	AUG	30/10
B-lactam combination	Imipenem–Relebactam	IMR	10
Penicillin	Piperacillin	PRL	100
Quinolone	Nalidixic acid	NA	25
Carbapenem	Meropenem	MEM	10

2.2. Pattern of Multiple Antibiotic Resistance Indices (MAR) indices:

- The (MARI) was intended according to the Krumperman formula [9]:

$$\text{MARI} = a/b$$

where a =total number of resistant antibiotics by microorganisms

b = total number of antibiotics which exposed.

2.3. DNA Extraction and Resistance Genes Amplification by PCR:

The whole genomic bacterial DNA and detection of aminoglycoside resistance genes were extracted depended on the manufacturer instruction (IntronBio/Korea) . PCR procedure was performed to amplify genes as mentioned in Table 2.

Table 2. Primer Pair Sequences and Amplicon Size and PCR condition of each Primer

Primer name	Sequences	Amplicon size (BP)	PCR condition	References
parC-F	CACTACCGGTTGGTTGCTCT	811	Step 1: 95°C, 2min Step 2: 95°C, 30 sec Step 3: 59°C, 30 sec Step 4: 72°C, 90 sec Step 5: 72°C, 5 min Step 6: 4°C	Al-Zuhairy, et al., 2020
parC-R	CGGTGGTTATCAGCGCATTG			
ant(2'')-la-F	TCCAGAACCTTGACCGAAC	700	Step 1: 95°C, 2min Step 2: 95°C, 1 min Step 3: 62°C, 1 min Step 4: 72°C, 1 min Step 5: 72°C, 10 min Step 6: 4°C	Soleimani, et al., 2014
ant(2'')-la-R	GCAAGACCTCAACCTTTTCC			

3. Results and Discussion:

Antimicrobial resistance is a natural occurrence in microorganisms, occurring naturally over time, usually through several means like genetic changes, drug efflux, down regulation of receptors, excretion of inactivating enzymes; mismanagement and over-use of different antimicrobials' types, as these drugs are frequently taken without advice or counsel of a professional, or it may be applied as growth promoters for animal feeds [15].

The selected four Gram-negative bacterial isolates showed an antibiotics' profiles as in table 3, that showing high resistance percentages against most of used antimicrobial types with percentages ranging

from 50 to 100%; with 100% resistance against Ciprofloxacin and Norfloxacin, Amikacin and Tobramycin, Amoxicillin-Clavulanate and Nalidix Acid. While regarding susceptibility to Imipenem-Relebactam and Meropenem that showed 100% sensitivity for both types.

Table 3. Antibiotic Susceptibility pattern of the study isolated against eleven antibiotic types by Disk-Diffusion Test

Antibiotic types	E. coli	Pseudomonas	Enterobacter	Klebsiella	Resistance Percentage %
Ciprofloxacin	R	R	R	R	100
Levofloxacin	S	S	R	R	50
Norfloxacin	R	R	R	R	100
Chloramphenicol	R	R	S	R	75
Amikacin	R	R	R	R	100
Tobramycin	R	R	R	R	100
Amoxicillin–Clavulanate	R	I	R	I	0
Imipenem–Relebactam	S	S	S	S	0
Piperacillin	S	I	S	S	50
Nalidixic Acid	R	R	R	R	100
Meropenem	S	S	S	S	0

High percentages of multidrug resistance distribution faces countries a serious challenge in safeguarding their populations' health and specifies a thoughtful requirement for wide-based, native anti-microorganisms resistance observation and organization of actual involvements to control multiple resistance in such pathogens. MAR in microbes is almost usually related by the occurrence of external factor like extra- chromosome of DNA (plasmids) which usually have resistance genes (one or more), it can encode as pecific trait (antibiotic resistance) in bacteria [16].

Results of table-3 were utilized to calculate (MAR) indexes of study isolates to the used antibiotics according to MARI-Krumperman equation; as the obtained results summarized at table (4) which showed that, all the four study isolates (100%) had an index more than 0.2 to be from a high risk sources; while none had an index of 1.0, being resistant to all antibiotics it was tested against. The progression of resistant strains led to a weakening of the antibiotics used in the treatment of bacterial infection. [17].

MAR indexing method have some characteristics like high cost, fast and easy to perform. Simple and does not need particular application and extraordinary costly tools especially when compared to other methods such as genotypic characterization. Knowledge of these points such as the antibiotic intake and multidrug antibiotic resistances which are especially important in nosocomial infections is essential to developing and reviewing effective containment programs and audit of such programs, in addition to all that, MAR index is significant in analyzing health risk, as well as to check the extent of antibiotic resistance. The benefit of MAR index analysis to distinguish isolates from diverse sources by means of antibiotics that are usually used in treatment of infectious cases [10,18].

All isolates from this research showed a MAR index greater than 0.2 indicating that their source commonly used antibiotics, or the organism's previous exposure to antimicrobial agents. In other words, isolates are have high resist to antibiotic may be from inpatients and outpatients or even from community acquired pathogens. In developing countries the hazard of antimicrobial resistance is mainly dangerous because of high burden of infectious disease in addition to concomitant high rate of poverty and delay of

constrains the access to newer, more effective antimicrobial agents [19].

A certain study was recorded to be multidrug resistant respectively,, that showed about 33%, 42%, 60% of clinical isolates of *Klebsiella* spp, *Escherichia* spp, and *Pseudomonas* spp and addition 62% of *Klebsiella* isolates were ESBL producers [20]. Moreover, lots of other studies revealed high ratio of resistance against different antimicrobial agents as [21-24].

Table 4. Multiple Antibiotic Resistance Indices of Isolated to Select Antibiotics

Bacterial spp.	Multiple antibiotic resistance (MAR) index
<i>E. coli</i>	0.64
<i>Pseudomonas</i>	0.73
<i>Enterobacter</i> spp.	0.64
<i>Klebsiella</i>	0.82

Additionally, regarding detection of Aminoglycosides resistance genes; results presented that wholly isolates that were already resistant to aminoglycosides group members (Tobramycin and Amikacin) also were positive for resistance genes and gave the amplicon products of both these genes as in figure 1 and 2.

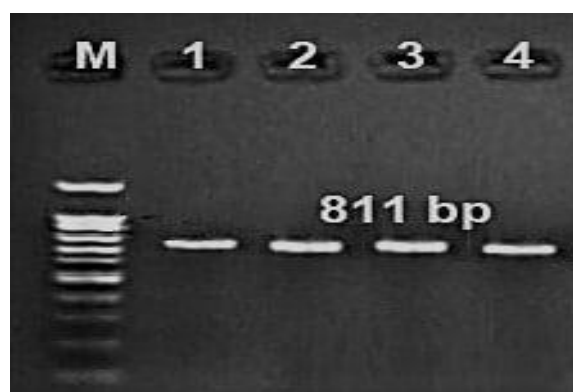


Figure1. Agarose Gel Electrophoresis of *parC* gene amplicon after traditional PCR amplification in the four clinical bacterial isolates. Lane M=100 bp DNA Ladder (molecular marker), lanes 1-4 refer to the bacterial isolates' number.



Figure2. Agarose Gel Electrophoresis of *ant(2'')-Ia* gene amplicon after traditional PCR amplification in the four clinical bacterial isolates. Lane M=100 bp DNA Ladder (molecular marker), lanes 1-4 refer to the bacterial isolates' number.

Aminoglycosides and Fluoroquinolones are useful in the management of bacterial infections. Wide use of antibiotics assisted the development of resistance, as well as other issues such as how to regulate the hygiene and organization of the hospital, and the subsequent extent of bacterial strains or determinants of resistance. Controlling antibiotic consumption is an important component of combating multidrug resistance and documenting the importance of infection control programmes. In the end, there must be cooperation between local and international policies by relying on the rules governing antibiotic policies and international or national recommendations and standards.

Several studies were concerning with the detection of different aminoglycosides and Fluoroquinolones resistance genes and getting various high percentages of these genes [25-28]. Moreover, a great frequency distribution of AMEs was recorded previously in Gram-negative bacteria such as *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* [29].

4. Conclusion

A high prevalence of genes conferring resistance to aminoglycosides was observed; specifically, genes such as "parC" and "ant(2")" were identified in 100% of the tested isolates, with Multiple Antibiotic Resistance (MAR) indices. Although these indicators reflect local resistance patterns, a major limitation of this study is the small sample size ($n=24$). There is an urgent need for comprehensive future studies involving larger bacterial cohorts from multiple medical centers (multicenter studies) to provide clearer insights for infection control efforts and antimicrobial policies.

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References

1. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: an overview. Cold Spring Harb Perspect Med. 2016;6(6):a027029. <https://doi.org/10.1101/cshperspect.a027029>
2. Aggen JB, Armstrong ES, Goldblum AA, Dozzo P, Linsell MS, Gliedt MJ, et al. Synthesis and spectrum of the neoglycoside ACHN-490. Antimicrob Agents Chemother. 2010;54(11):4636-42. <https://doi.org/10.1128/AAC.00572-10>
3. Landman D, Babu E, Shah N, Kelly P, Bäcker M, Bratu S, et al. Activity of a novel aminoglycoside, ACHN-490, against clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from New York City. J Antimicrob Chemother. 2010;65(10):2123-7. <https://doi.org/10.1093/jac/dkq278>

4. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob Agents Chemother*. 2000;44(12):3249-56. <https://doi.org/10.1128/AAC.44.12.3249-3256.2000>
5. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev*. 1993;57(1):138-63. <https://doi.org/10.1128/mr.57.1.138-163.1993>
6. Yamane K, Wachino J, Doi Y, Kurokawa H, Arakawa Y. Global spread of multiple aminoglycoside resistance genes. *Emerg Infect Dis*. 2005;11(6):951-3. <https://doi.org/10.3201/eid1106.040924>
7. Niu H, Yu H, Hu T, Tian G, Zhang L, Guo X, et al. The prevalence of aminoglycoside-modifying enzyme and virulence genes among enterococci with high-level aminoglycoside resistance in Inner Mongolia, China. *Braz J Microbiol*. 2016;47(3):691-6. <https://doi.org/10.1016/j.bjm.2016.04.003>
8. Bodendoerfer E, Marchesi M, Imkamp F, Courvalin P, Böttger EC, Mancini S. Co-occurrence of aminoglycoside and β -lactam resistance mechanisms in aminoglycoside-non-susceptible *Escherichia coli* isolated in the Zurich area, Switzerland. *Int J Antimicrob Agents*. 2020;56(1):106019. <https://doi.org/10.1016/j.ijantimicag.2020.106019>
9. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl Environ Microbiol*. 1983;46(1):165-70. <https://doi.org/10.1128/aem.46.1.165-170.1983>
10. Mthembu MS. The usefulness of multiple antibiotic resistance (MAR) indexing technique in differentiating faecal coliform bacteria from different sources [MSc thesis]. KwaDlangezwa: University of Zululand; 2008.
11. Forbes BA, Sahm DF, Weissfeld AS. *Bailey & Scott's diagnostic microbiology*. 12th ed. St. Louis (MO): Mosby Elsevier; 2007.
12. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*. 28th ed. CLSI supplement M100. Wayne (PA): CLSI; 2018.
13. Al-Zuhairy A, Al-Dahmoshi H. Aminoglycosides and fluoroquinolones resistance genes among uropathogenic *E. coli* in Hilla City, Iraq. *Drug Invention Today*. 2022;13(2).
14. Soleimani N, Aganj M, Ali L, Shokoohizadeh L, Sakinç T. Frequency distribution of genes encoding aminoglycoside modifying enzymes in uropathogenic *E. coli* isolated from Iranian hospital. *BMC Res Notes*. 2014;7:842. <https://doi.org/10.1186/1756-0500-7-842>
15. Ghadiri H, Vaez H, Khosravi S, Soleymani E. The antibiotic resistance profiles of bacterial strains isolated from patients with hospital-acquired bloodstream and urinary tract infections. *Crit Care Res Pract*. 2012;2012:890797. <https://doi.org/10.1155/2012/890797>
16. Daini OA, Ogbolu OD, Ogunledun A. Quinolones resistance and R-plasmids of some gram negative enteric bacilli. *Afr J Clin Exp Microbiol*. 2005;6(1):14-20.
17. Yah SC, Yusuf EO, Eghafona NO. Pattern of antibiotic usage by adult populations in the city of Benin, Nigeria. *Sci Res Essays*. 2008;3(3):81-5.
18. Guan S, Xu R, Chen S, Odumeru J, Gyles C. Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. *Appl Environ Microbiol*. 2002;68(6):2690-8. <https://doi.org/10.1128/AEM.68.6.2690-2698.2002>
19. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect Dis*. 2005;5(8):481-93. [https://doi.org/10.1016/S1473-3099\(05\)70189-4](https://doi.org/10.1016/S1473-3099(05)70189-4)

20. Olayinka AT, Onile BA, Olayinka BO. Antibiotic susceptibility and plasmid pattern of *Pseudomonas aeruginosa* from the surgical unit of a university teaching hospital in north central Nigeria. *Int J Med Med Sci*. 2009;1(3):79-83.
21. Kim SH, Wei CI, Tzou YM, An H. Multidrug-resistant *Klebsiella pneumoniae* isolated from farm environments and retail products in Oklahoma. *J Food Prot*. 2005;68(10):2022-9. <https://doi.org/10.4315/0362-028X-68.10.2022>
22. Lee DS, Lee SJ, Choe HS. Community-acquired urinary tract infection by *Escherichia coli* in the era of antibiotic resistance. *Biomed Res Int*. 2018;2018:7656752. <https://doi.org/10.1155/2018/7656752>
23. Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyanova DR, Lubin SA, et al. Virulence factors and antibiotic resistance of *Klebsiella pneumoniae* strains isolated from neonates with sepsis. *Front Med (Lausanne)*. 2018;5:225. <https://doi.org/10.3389/fmed.2018.00225>
24. Eltai NO, Yassine HM, Al Thani AA, Abu Madi MA, Ismail A, Ibrahim E, et al. Prevalence of antibiotic resistant *Escherichia coli* isolates from fecal samples of food handlers in Qatar. *Antimicrob Resist Infect Control*. 2018;7:78. <https://doi.org/10.1186/s13756-018-0369-2>
25. Vanhoof R, Nyssen HJ, Van Bossuyt E, Hannecart-Pokorni E. Aminoglycoside resistance in Gram-negative blood isolates from various hospitals in Belgium and the Grand Duchy of Luxembourg. *J Antimicrob Chemother*. 1999;44(4):483-8. <https://doi.org/10.1093/jac/44.4.483>
26. Samadi N, Pakzad I, Monadi Sefidan A, Hosainzadegan H, Tanomand A. Study of aminoglycoside resistance genes in *Enterococcus* and *Salmonella* strains isolated from Ilam and Milad hospitals, Iran. *Jundishapur J Microbiol*. 2015;8(4):e18102. <https://doi.org/10.5812/jjm.18102>
27. Plattner M, Gysin M, Haldimann K, Becker K, Hobbie SN. Epidemiologic, phenotypic, and structural characterization of aminoglycoside-resistance gene *aac(3)-IV*. *Int J Mol Sci*. 2020;21(17):6133. <https://doi.org/10.3390/ijms21176133>
28. Kishk R, Soliman N, Nemr N, Eldesouki R, Mahrous N, Gobouri A, et al. Prevalence of aminoglycoside resistance and aminoglycoside modifying enzymes in *Acinetobacter baumannii* among intensive care unit patients, Ismailia, Egypt. *Infect Drug Resist*. 2021;14:143-50. <https://doi.org/10.2147/IDR.S290584>
29. Nasiri G, Peymani A, Farivar TN, Hosseini P. Molecular epidemiology of aminoglycoside resistance in clinical isolates of *Klebsiella pneumoniae* collected from Qazvin and Tehran provinces, Iran. *Infect Genet Evol*. 2018;64:219-24.

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