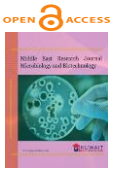

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Investigation of Class 1 Integrons (intl1) and Insertion Sequences Common Region (ISCR1) Genes Cassette in Multidrug Resistance Bacteria

Shadman Tariq Sadiq[1*]
[1]Department of Biology, College of Science, Tikrit University, Iraq

Abstract: The phenomenon of multidrug resistance (MDR) in bacteria represents a significant global health challenge, particularly in hospital environments where resistance genes proliferate through mechanisms such as class 1 integrons and ISCR1 elements. This study investigates the impact of these genetic elements in facilitating antibiotic resistance genes (ARG) dissemination among non-pathogens and key pathogens, specifically Pseudomonas aeruginosa and Staphylococcus aureus, which are notorious for causing nosocomial infections. Through isolation and identification of bacterial strains, antibiotic susceptibility testing, and genomic analysis, we demonstrate a concerning prevalence of resistance to critical antibiotics, particularly β-lactams, aminoglycosides, and fluoroquinolones. Our findings reveal that both Int1 and ISCR1 contribute to the genetic mobility of resistance traits, complicating treatment protocols and elevating healthcare costs due to ineffective therapies. The study emphasizes the urgent need for continued research into the mechanisms of antibiotic resistance dissemination and the development of novel antimicrobial strategies targeting these genetic elements. Furthermore, our results highlight the importance of understanding the historical context of antibiotic resistance gene mobilization as a means to inform healthcare practices and mitigate the rising threat of MDR bacteria on a global scale.

Keywords: Integrons, antibiotics mobile genes, ISCR1, antimicrobial resistance.

Research Paper

***Corresponding Author:**
Shadman Tariq Sadiq
Department of Biology, College of Science, Tikrit University, Iraq

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INTRODUCTION

The multi-drug resistance (MDR) phenomenon has become global problem for all environments that colonized with harmful bacteria [1]. In the past the resistance was limited to one or 2 drugs and by continuing, the problem was magnified due to mobiling and transforming resistance gene between different bacterial species in the same spot especially in hospital environment leading to forming a set of resistance gen hub loaded in one package called antibiotics gene cassette, the commone types of this resistance hub are int1 and iscr1 that responsible to many antibiotics resistance dissemination between human and environments [2-4].

The investigation of this kind of gene cassettes help scientist to assess the degree of bacterial harm resistance genes dissemination for any bacteria isolated from specific environment for example hospital, also gives an evidence about the history of antibiotics resistance that emerged in specific environment that lead to form this kind of resistance magnification threaten public health which lead to limiting therapeutic options and Complicate disease treatment by spreading antibiotic resistance [5]. The synergistic between both int1 (Integrase gene) and ISCR1 genetic elements interplay critical roles to facilitate the dissemination a set of antibiotic resistance genes especial genes that encode resįstance to β-lactam among bacterial populations, rendering conventional treatments ineffective as well as increase costs and elevate mortality rates [6-8].

This article delves into the functions, implications, and recent research surrounding Class 1 integrons and IS elements in bacteria isolated from hospitals as an examples of the most antibiotics resistance gen donation spot in the world finally elucidating integrin class 1-ISCR1 interactions to understand resistance dissemination and developing new and novel antimicrobials treatments that targets both gene cassette.

MATERIAL AND METHODS

Bacteria isolation and identification

Two important and common bacteria types, Pseudomonas aeruginosa and staphylococcus aureus

isolated and identified the most bacteria species that attracts attention for antibiotics resistance problem in the environment and medical fields, both them are responsible for nosocomial infection and antibiotics resistance gene emerging.

Isolates cultured on different selective and differential media to identified by biochemical tests and confirmed by commercial methods like VITEK-2, other routine practices was followed according to our previous works [9-12].

Antibiotics susceptibility

To insure the phenotypic resistance, antibiotics susceptibility via Kirby-Bauer protocol, 1966 [13] was performed using standard antibiotics disk diffusion expecting the availability of resistance genes located in *Int1 and ISCR1*.

The targeted antibiotics were Penicillin (P 10µg), Azithromycin (AZM 15µg), naldixic acid(NA 30µg), Chloramphenicol (C 10µg), Vancomiçin.(VA 30.µg),Ciprofloxacin (Cip 5µg), Trimethoprime (TMP 10 µg), Òxacillin (OX 5.µg), Nitrufurantoin (F.100)µg, Sulfamethoxazole plus Trimethoprim25 µg (SXT), 30 µg of Methicillin (MET), & Ceftazidime (CAZ).

DNA extraction and electrophoresis

Briefly, the bacteria organisms were cultured aseptically into 5 ml of sterilized and quality controlled broth and incubated for 18_24 hrs. at 37°C. Microbial cells graphically centrifuge-harvested at 8000-10,000 r.p.m for 5 min. The extraction was performed by using Gram positive DNA extraction kit and Gram negative DNA extraction kit followed by purification that quantified by Nano drop spectro, after polymerase chain reaction (PCR) running, the whole genomic (PCR Products) was confirmed phenotypically by electrophoresis run on 1% agarose gel (Figure 1), reference genomic control was E.coli genomic pre-extracted , while A blank control (distilled water).The project workflow is diagramed in the (Figure 2).

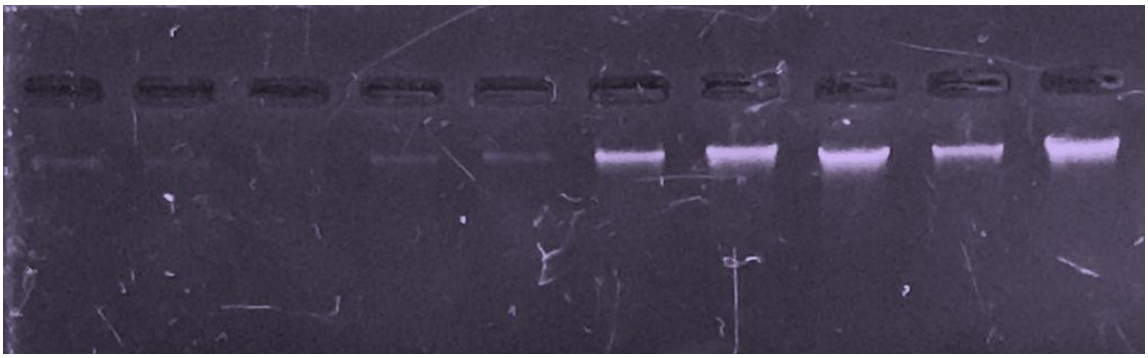

Figure 1: PCR product after extraction procedure

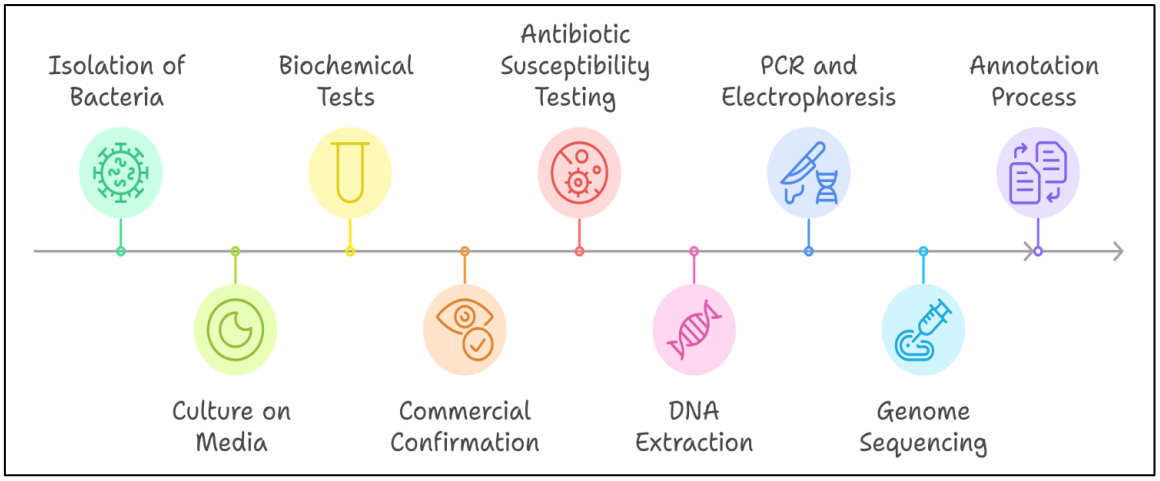

Figure 2: Schematic diagram of project workflow (current study)

Genomic identification

According to [3, 14-16]. Whole-genomic DNA of Int1 & ISCR1 was sequenced and the most platform used for this is by USA Pacific PacBio-Biosciences R.S II sequencing platform at Seoul, Republic of Korea Macrogen incorporation and annotated by Prokka vic bioinformatics.com, DNAStar (www.dnastar.com/) The most comprehensive sequence analysis tools on the market and the NCBI-PGAP server. The main incorporated gen loci in the int1 and diagrimized in (Figure 3) while ISCR1 cassette are diagrimized in (Figure 4).

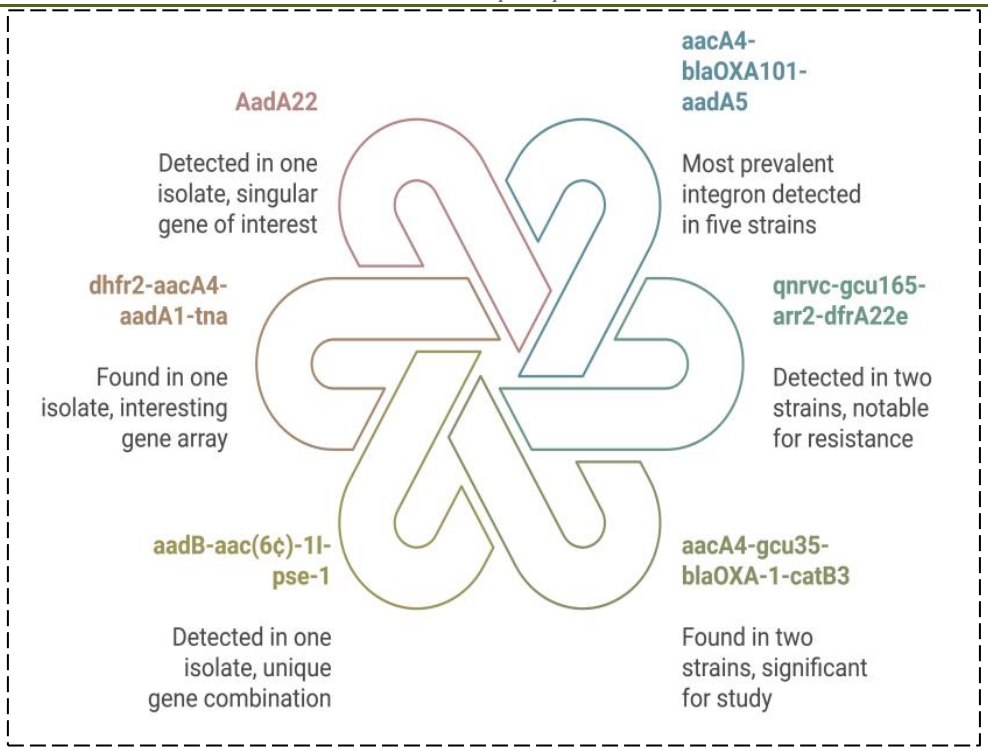

Figure 3: Schematic map of Int1 gen cassette (current study)

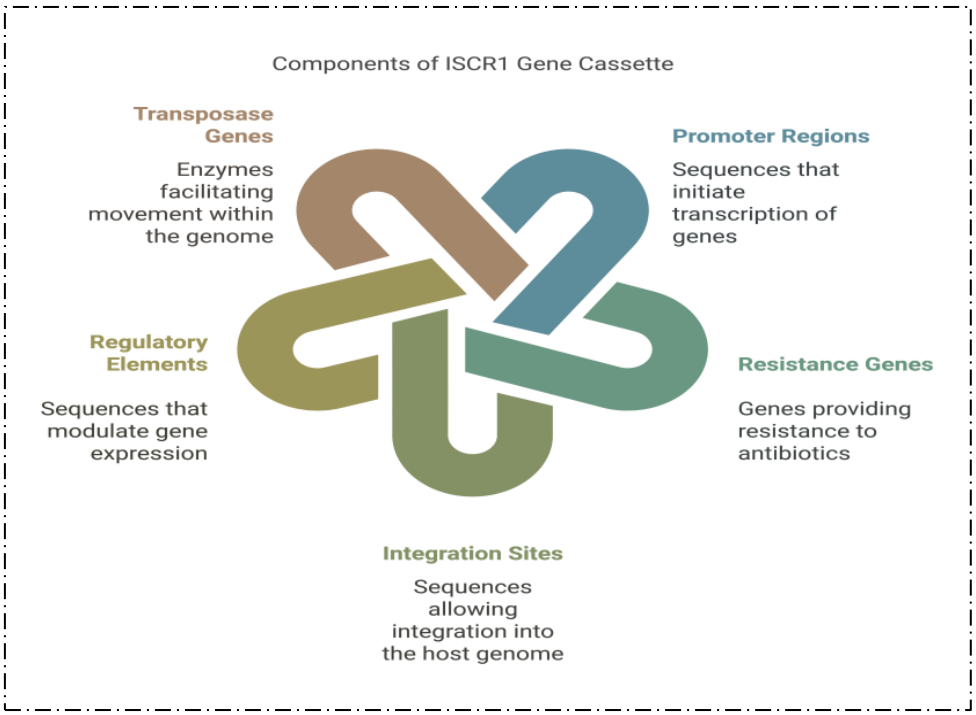

Figure 4: Schematic map of ISCR1 gen cassette (current study)

Primers and PCR condition

To determine where *P.aeruginosa* and *S.aureus* carry Int1 and ISCR1 genes a set of primers used pre designed and provided by promega (USA) Table 1.

PCR running for 30 cycle; Initial denaturation 94°C for 3 min, denaturation at 94°C for 30s, annealing at 55°C for ISCR and 60°C for Int1 , extension at 72°C for 45 s, final extension at 72°C for 10 min. The final volume of PCR mix was 20 µL composed of (master mix 10 µL, F primer 0.5 µL, R primer 0.5 µL, nuclease-free DW 6 µL and DNA template was 3 µL).

Table 1: Primers of IntI1 and ISCR1

Antıbiotic name	Gene primer name	Primer sequence	Amplicon size	Anealing C
Class 1 ıntegron	Int1	5'- GTTCGGTCAAGGTTCTG 3' 5'- GCCAACTTTCAGCACATG 3'	923	60
insertion sequence common region	ISCR1	:5- CGC CCACTCAAACAAACG-3 :5-GAG GCTTTG GTGTAACCG-3	180	55

RESULTS AND DISCUSSION

As a revolutionary gene-capturing technology, *ISCR1* and *Int1* elements can mobilize any adjacent DNA sequence as ISCR1 on of the most importance part of Int1. The mobilization mechanism of these gene.cassette provides flexibility to transfer horizontally in-between different SPP. of bacterial genus. The researchers discovered about 19.members of this platform family most of them in Gram-negative pathogens. The majority of these genes strongly associated with Antibiotics resistance-AR and responsible for the rapid translocation of bacterial multi-drug resistance. Regarding to IS*CR1, this* elements also associated with AR genes as part of complex class 1 integrons-int1 [17-20].

Phenotypic resistance consider a first step to explore these kinds of platform, although some resistance genes are latent but in case *int1* and *iscr1* the phenotypic highly reflexed genotypic due to massive genes loci associated to cassette. The result of antibiotics resistance by Kirby_Bauer method revealed that both S.aureus and P.aeruginosa isolates were resistant to many antibiotics including Penicillin (P 10µg), Azithromycin (AZM 15µg), Naldixic acid (NA 30µg), Chloramphenicol (C 10µg), Ciprofloxacin (Cip 5µg), Trimethoprime (TMP 10 µg), Vancomycin (VA 30 µg), Oxacillin (OX 5 µg), Nitrufurantoin (F 100 µg), Sulfamethoxazole_Trimethoprim (SXT 25 µg), Methicillin (MET 30 µg), Ceftazidime (CAZ 30 µg) at once Figure (5); this can classified as an extensively drug-resistant comparing to other organisms out of hospital environment we see different result , the organisms from hospital more virulent and resist to many antibiotics due to continuous interaction with multi drugs persisted in the hospital environment leading to difficulty in remediation by classic processes.

Many studies revealed the same results, Liu *et al*., attained that 113 isolates expressed phenotypic resistance, The highest prevalence of resistance isolates was to betal_lactam antibiotic with percentage varied from (93.33%) for imipenim and (86.67%) for meropenem, as well as Flouriqinolon, for example levofloxacin reached about (56.67%), and ciprofloxacin (53.33%) [18]. General about -84.2%- of tested strains were classified as M.D.R organisms. Rezae reported that all isolates that reached 99.3% were resistant to amoxicillin [15] . Ong'era *et al*., 2023 expressed that the beta_lactam highest resistance was 78.7% for _amoxicillin, followed by 76% for ceftazidime and 49.3% for oxacillin. These results can be evaluate the potential association of various factors: antibiotic usage, gender, hospital visits, and the occurrence of M.D.R bacteria in nares, for example our finding showed high resistance to Betalactam group due to randomly intakes of antibiotics drugs [21].

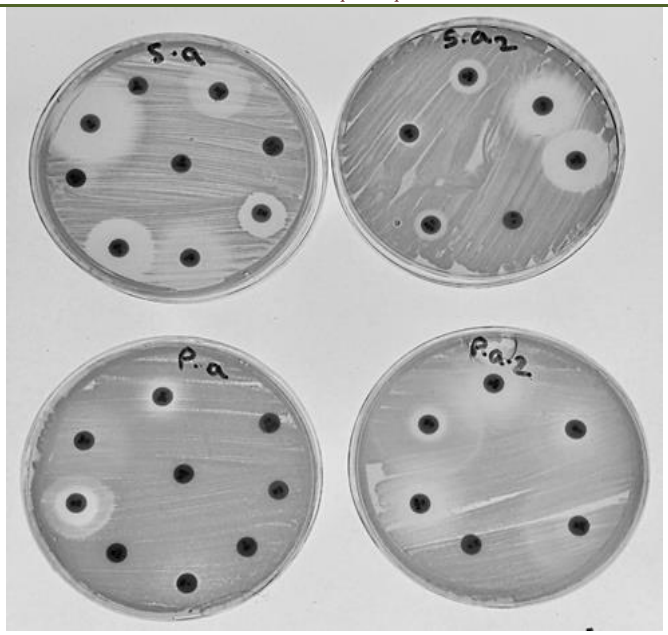

Figure 5: Phenotypic resistance of P.aeruginosa (p.a) and S.aureus (s.a) to selected antibiotics revealing multidrug resistance

According to previous genomic findings, the phenotypic results revealed that 100 % of the isolates contained betalactamase producing genes involving bla_{CTX-M}, bla_{TEM}, bla_{SHV} and *bla* OXA-21, and about 85 % of the isolates contained amenoglycosides acetyltransferase genes. The percentages was near due to many genes producing β-lactamases in *int1* are also express resistance toward different classes of allied antibiotics belong to other classification like aminoglycosịdes and fluoroquịnolones, similar finding concluded by [22].

Finding by [3] showed that Int1 harboring about many *β*-lactamase gene, about 21 AMR- genes including *aph.(3')-IIb,, strB, aac.(6')-Ib5, , dfr.A1, strA, aad.B as well as* many particular antimicrobial resistance gene copies including 4 copies of *sul1gen*, 3.copies of *bla.*IMP-1 and 2.copies of *aac(6')-Ib5*. Regarding to previous study which indicated the domination of imipenem and metallo-s-lactamase gene to be identified in *P. aeruginosa* ST-964, the majority of this project explained in conclusions part.

CONCLUSIONS

Class 1 integrons and ISCR1 elements play critical roles in the evolution and dissemination of antibiotic resistance. Understanding these genetic elements is crucial for addressing the growing threat of multidrug-resistant bacteria.

Continued research and innovative strategies are essential for combating this pressing public health concern.

One of the most importance point of this investigations is discovering the history of AMR gen mobilization, in other word consider biological archives in health institute or any environment that countered antibiotics.

The assessment of Int1 and ISCR1 gene cassette can help in risk management for health issues in medical field hence the presence of IS*CR1* and class 1 integrons represent the availability many resistance genes as a result indicate the size of antibiotics resistance problem and by the way can lowering the cost of treatment, minimizing routine investigations and preventing future crises finaly development of appropriate AB resistant solution strategies.

The development of antibiotic resistance genes can open the road to develop cross resistance of other associated microorganisms, for example development resistance to other material like heavy metals leading to difficulties in remediation of medical waste bacteria containing.

Our findings build the basis to integrate allied studies to solve antimicrobial resistance for any targeted community to understand the socio-economic factors influencing the dissemination of antibiotịcs resịstance in specific community to building up on efforts to mitigate resistance crisis at both of national and global scales, finally we recommend more investigation about gene cassettes in different health institutes environment to record the majority of antibiotics resistance genes disseminations.

Conflict of Interest: No conflict of interests.

Ethical Approval: Not required.

Sources of Funding: Not required

REFERENCES

1. Assefa, M. (2022). Multi-drug resistant gram-negative bacterial pneumonia: etiology, risk factors, and drug resistance patterns. *Pneumonia*, *14*.
2. Lorestani, R. C., Akya, A., Elahi, A., & Hamzavi, Y. (2018). Gene cassettes of class I integron-associated with antimicrobial resistance in isolates of *Citrobacter spp*. with multidrug resistance. *Iranian journal of microbiology*, *10*(1), 22–29.
3. Kumpangcum, S., Anek, P., Khamnoi, P., Prommeenate, P., & Phannachet, K. (2023). Complete genome sequence of Pseudomonas aeruginosa PA99 clinical isolate from Thailand carrying two novel class 1 integrons, In2083 and In2084. *Journal of Global Antimicrobial Resistance*, *33*, 97-100.
4. Dragon, D., Jansen, W., Dumont, H., Wiggers, L., Coupeau, D., Saulmont, M., ... & Daube, G. (2024). Conventionally Reared Wallon Meat Lambs Carry Transiently Multi-Drug-Resistant Escherichia coli with Reduced Sensitivity to Colistin Before Slaughter. *Animals*, *14*(20), 3038.
5. Othman, R. M., & Alaa Abdulkaliq, H. U. S. S. A. I. N. (2024). Integrons Detection in Multi-Drug Resistance Escherichia Coli Isolated from Clinical and Environment Sources in Basrah, Iraq. *Assiut Veterinary Medical Journal*, *70*(183), 90-104.
6. Mazel, D. (2006). Integrons: Agents of bacterial evolution. *Nature Reviews Microbiology*, *4*(8), 608-620.
7. Stokes H. W., & Gillings M. R. (2011). Gene flow, mobile genetic elements, and the recruitment of antibiotic resistance genes into Gram-negative bacteria. *FEMS Microbiology Reviews*, *35*(5), 790-805.
8. Partridge S. R., & Tsafnat G. (2011). Identifying antimicrobial resistance genes in bacterial genomes. *Journal of Antimicrobial Chemotherapy*, *66*(9), 2065-2074.
9. Mohammed, M. S., Sadiq, S. T., & Mohammed, D. S. (2024). Changes in Staphylococcus aureus Infection before and after the COVID-19. *Medical Journal of Babylon*, *21*(Suppl 2), S313-S314.
10. Sulaiman, S. Q., Sadiq, S. T., & Farhan, M. S. (2024). Biosynthesis of silver nanoparticles from Inocutis tamaricis and its antimicrobial and antibiofilm activity. *GSC Biological and Pharmaceutical Sciences*.
11. Mohammed, M. S., Sadiq, S. T., & Mohammed, D. S. (2023). Prevalence of Methicillin Resistance S.aureus (MRSA) and Vancomycin Resistance S.aureus (VRSA) among Iraqi Patients: a Comparative Study. *South Asian Research Journal of Applied Medical Sciences*.
12. Sadiq, S. T., & Yildirim, H. (2024). Antibacterial, Antifungal, Antioxidant Activities Assessment of Some Newly Registered Plant Extracts Collected from Izmir City. *Recent Advances in Biology and Medicine*.
13. Kirby, W. M., & Bauer, A. W. (1966). Antibiotic Susceptibility Testing by a Standardized Single Disc Method. The American. *J Clin Path*, 45, 493-496.
14. Rui, Y., Lu, W., Li, S., Cheng, C., Sun, J., & Yang, Q. (2018). Integrons and insertion sequence common region 1 (ISCR1) of carbapenem-non-susceptible Gram-negative bacilli in fecal specimens from 5000 patients in southern China. *International journal of antimicrobial agents*, *52(5)*, 571-576.
15. Rezaee, M. A., Sheikhalizadeh, V., & Hasani, A. (2011). Detection of integrons among multi-drug resistant (MDR) Escherichia coli strains isolated from clinical specimens in northern west of Iran. *Brazilian Journal of Microbiology*, *42*, 1308-1313.
16. Ahmadian, L., Haghshenas, M. R., Mirzaei, B., Norouzi Bazgir, Z., & Goli, H. R. (2020). Distribution and Molecular Characterization of Resistance Gene Cassettes Containing Class 1 Integrons in Multi-Drug Resistant (MDR) Clinical Isolates of *Pseudomonas aeruginosa*. *Infection and drug resistance*, *13*, 2773–2781.
17. Deng, Y., Zeng, Z., Liu, J., & Chen, Z. (2009). *Wei sheng wu xue bao = Acta microbiologica sinica*, *49*(8), 987–993.
18. Liu, M., Ma, J., Jia, W., & Li, W. (2020). Antimicrobial Resistance and Molecular Characterization of Gene Cassettes from Class 1 Integrons in Pseudomonas aeruginosa Strains. *Microbial Drug Resistance*, *26*, 670-676.
19. Li, W., Ma, J., Sun, X., Liu, M., & Wang, H. (2022). Antimicrobial Resistance and Molecular Characterization of Gene Cassettes from Class 1 Integrons in Escherichia coli Strains. *Microbial Drug Resistance*, *28*, 413-418.
20. Toleman, M. A., Bennett, P. M., & Walsh, T. R. (2006). ISCR elements: novel gene-capturing systems of the 21st century?. *Microbiology and molecular biology reviews: MMBR*, *70*(2), 296–316.
21. Ong'era, E., Kagira, J. M., Maina, N.W., Kiboi, D., Waititu, K. K., Michira, L., & Ngotho, M. (2023). Prevalence and Potential Risk Factors for the Acquisition of Antibiotic-Resistant Staphylococcus spp. Bacteria Among Pastoralist Farmers in Kajiado Central Subcounty, Kenya. *BioMed Research International*, *2023*.
22. Doi, Y., Wachino, J. I., & Arakawa, Y. (2016). Aminoglycoside resistance: the emergence of acquired 16S ribosomal RNA

methyltransferases. *Infectious Disease Clinics*, *30*(2), 523-537.