INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 22, No. 6, p. 47-53, 2023

RESEARCH PAPER OPEN ACCESS

Antibiotic Resistance and Integron Prevalence among Multidrug-Resistant Bacterial in Bangladesh. Molecular Pathology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Arnaba Saha Chaity[1], Dipa Roy, Tamanna Nasrin, Ariful Haque*[1]

[1]Molecular Pathology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh, Chaity

[2]Molecular Pathology Laboratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

[3]Genetics and Molecular Biology Laboratory, Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh

[4]Molecular Pathology Laboratory Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Key words: Extended-spectrum β-lactamase, Integron integrase 1, Multidrug-resistant and β-lactam antibiotics

http://dx.doi.org/10.12692/ijb/22.6.47-53 Article published on June 05, 2023

Abstract

Antibiotic-resistant bacterial strains are widespread in hospitals and intensive care units. This poses a serious threat to human health as the effectiveness of many antibiotics has been diminished by the emergence of resistant strains. The overuse of β-lactam antibiotics has led to the rise of antibiotic-resistant bacteria, including Extended-Spectrum β-Lactamase (ESBL) producing strains. However, ESBL screening is not commonly performed in Bangladesh, despite the growing prevalence of antibiotic resistance. Multidrug-resistant strains, particularly those carrying the Integron integrase 1 gene is responsible for antibiotic resistance. Horizontal integron transfer is one of the key factors that can contribute to the emergence of multidrug-resistant (MDR) bacteria. In this study, antibiotic sensitivity tests were conducted using 25 antibiotics. It was found that *E. coli* and *Klebsiella* both showed resistance to Aztreonam, Ampicillin/Sulbactam, Amoxyclav, Cefepime, Cefepime/Tazobactam, Ampicillin and Cefotaxime antibiotics. Our findings suggest that integron is common among MDR isolates and that they can be used to identify MDR isolates. As a result of the possibility of a widespread outbreak of MDR isolates, molecular surveillance and integron sequencing in other parts of the country is advised. The purpose of this study is innovation to create new antibiotics and alternative treatments to address antibiotic-resistant bacteria.

* **Corresponding Author:** Ariful Haque ✉ haque@ru.ac.bd

Introduction

Antimicrobial resistance poses a growing threat to human health and medical treatments and rising multidrug-resistant nosocomial infections in hospitals. Prompt treatment is crucial for vulnerable individuals at risk of bacterial infections. Viral infections are more likely to be the source of localized redness, swelling, and discomfort (Murray *et al.*, 1998).

Antimicrobial resistance in bacteria is a complicated process involving a variety of different mechanisms. Susceptible bacteria can develop resistance through mutations or the transfer of resistance genes found on mobile DNA elements like integron (Barlow *et al.* 2004, Normak & Normak 2002). This, integron play a crucial role in the spread of antibiotic resistance, especially in Gram-negative bacteria by attaching to mobile genetic elements like transposons and conjugative plasmids, resistance integron transmit antibiotic resistance. (Mazel, 2006). Integron share antibiotic resistance gene cassettes predominantly through convergent evolution. Mobile integron has had a considerable impact on the spread of antibiotic resistance, particularly in Gram-negative bacteria (Davies *et al.*, 2010). From the pool of these environmental genetic elements that is available, Integron has amassed a wide variety of resistance genes. Furthermore, their prevalence has significantly increased, increasing the possibility of interactions with other DNA and the emergence of additional, more complex mobile elements that are resistant to different antibiotic classes, disinfectants, and heavy metals (Gillings & Stokes 2012).

Class 1 integron are major contributors to antibiotic resistance in Gram-negative bacteria like Klebsiella and E. coli. They evolve due to exposure to selection agents in human-populated areas and natural environments, acquiring advantageous genes. The presence of resistance gene cassettes within class 1 integron is linked to antibiotic resistance. Addressing this problem requires essential research and intervention to combat the spread of antibiotic-resistant microorganisms. Ongoing development and dissemination of antibiotic resistance in Gram-negative bacteria like *Klebsiella* and E. coli class 1 integron play a crucial role. Their evolution is fueled by persistent exposure to selection agents in both human-populated areas and natural environments, leading to the acquisition of beneficial genes. The presence of resistance gene cassettes within class 1 integron is closely associated with antibiotic resistance. To effectively combat antibiotic-resistant microorganisms, it is essential to prioritize continuous research and innovation. Class 1 integrons are commonly found in various Gram-negative bacteria such as Acinetobacter, Vibrio, Aeromonas, Proteus, Burkholderia, Alcaligenes, Campylobacter, Enterobacter, Citrobacter, Klebsiella, Mycobacterium, Pseudomonas, Serratia, Salmonella, Shigella, and Escherichia coli (Yu G, Li Y and Liu X. 2013). Several studies have investigated prevalence of integron in MDR *Escherichia coli* and *Klebsiella* isolates around the world. These studies have identified a significant link between the presence of integrons and antibiotic resistance.

The study of integron aims to effectively combat antibiotic resistance by developing precise drugs that can be tailored to individual patients at a low cost, resulting in faster recovery times. With the emergence of antibiotic-resistant bacteria posing a significant threat to public health, there is a growing global interest in exploring integron profiles for the design of novel drugs that can safeguard both human and economically valuable animal populations. By understanding the intricate mechanisms of integron, scientists are hopeful of discovering innovative drugs that can tackle antibiotic resistance, thereby protecting human health and ensuring the well-being of economically important animals. This renewed focus on integron holds the potential to revolutionize the field of medicine and pave the way for personalized and cost-effective antibiotic treatments.

Materials and methods

Isolation and culture of bacteria

25 pathogenic bacterial samples were collected from the laboratory of Rajshahi Medical College in Luria

Broth (LB) agar plate. The samples were collected using a sterile loop and kept in incubator overnight at 37°C. Next day colonies of all the bacteria were found and culture in Extended Spectrum Beta-Lactamase (ESBL) media for screening and kept it at 37°C for overnight. Of those samples which were culture in ESBL media only 15 samples were grown on those media. Blue and purple color colony-forming bacteria were identified.

Antibiotic sensitivity test

Finding the most effective antibiotic for a condition is made easier with the aid of an antibiotic susceptibility test. The disc diffusion test, also referred to as the Kirby-Bauer test for antibiotic susceptibility, is a well-liked and long-used technique.

The most popular agar to use in this test for antibiotic susceptibility is Mueller-Hinton agar. Sensitivity of antibiotics the isolated bacteria was performed as described previously [Saha *et al.* 2009 & Mohanta *et al.* 2015]. Different antibiotics namely Ampicillin, Amikacin**,** Amoxyclav, Ampicillin/ Salbactum, Azithromycin, Aztreonam, Bacitracin, Carbenicillin, Cefepime, Cefepime/Tazobactam, Cefotaxime, Choramphenicol, Ciprofloxacin, Co-Trimoxazole, Lomefloxacin, Gatifloxacin, Impenem, Levofloxacin, Rifampicin, Tetreacycline, Vancomycin, Neomycin, Norfloxacin, Nitrofurantoin and Netillin were used to determine the susceptibility against the isolated bacteria.

Molecular characterization

Genomic DNA was extracted from bacteria using the Promega Genomic DNA Purification Kit and purified using phenol-chloroform and 2 microliter column purification protocol. The presence of class 1 integron in all MDR *E. coli* and *Klebsiella* isolates was tested by multiplex polymerase chain reaction (PCR) using primer *IntI1* specific for integrases genes of the integron. The PCR amplification was followed by program: 40 cycles of 10 seceond of denaturing at 95°C, 15 seconds of annealing at 57°C and 20 seconds of elongation at 72°C with a final extension at 72°C for 10 minutes. The size of variable regions of class 1 integron was determined by PCR assay. Primer sequences Forward primer: 5'-ACATGTGATGGCGACGCACGA -3', Reverse primer: 5'- ATTTCTGTCCTGGCTGGCGAA -3' size of amplicon is 559 bp. The PCR assay was performed as follows. 3 µl of template DNA was added to 50 µl of master mixture for PCR amplification.

This mixture also contained 1.5 µl of dNTP mixture (10 mM of each), 10 µl of 5X PCR buffer, 0.25 µl of Taq polymerase (250 U), 2 µl of each primer stock solution (50 pmol/ l), 2.5 µl MgCl (25Mm), and the remaining 28.75 µl volume (Takara Japan). For detection integron integrase specific primer was used. Following PCR, a 1.5% agarose gel with the product applied was examined with a UV transilluminator. PCR products will be sequenced by following Sanger method.

Blast analysis of the sequences and Sequence alignment

The sequence generated from automated sequencing of PCR amplified DNA was analyzed through NCBI BLAST (http:// www.ncbi.nlm.nih.gov) program to find out possible similar organism through alignment of homologous sequences. After blasting all the sequences alignment was done by using T-COFFEE multiple sequence alignment sever.

Result

Isolation of the bacteria from human samples

The isolation and identification process were completed in two separate steps. At first LB agar and later screened in ESBL selective media. The bacteria which were grown on ESBL selective media were selected and the colour of the colony is blue and purple. Total 25 samples were collected among them 15 samples were found to be ESBL positive. We had work using 6 samples, where 2 of them are *Klebsiella* and 4 of them are *E.coli.*

Antibiotic Sensitivity test

Total 25 antibiotics were used for antibiotic susceptibility of 6 samples. The outcome is summarized in Figure 2.

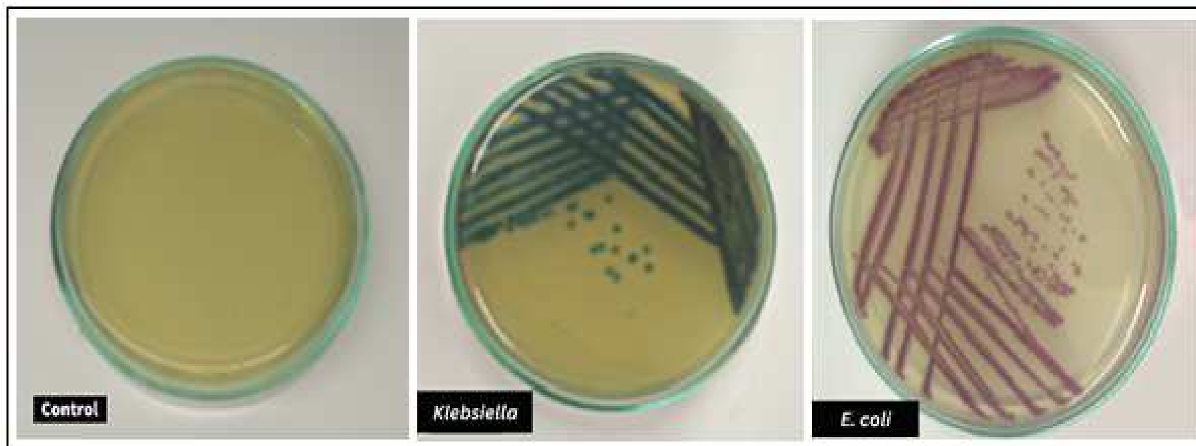

Fig. 1. ESBL Samples on Selective Media.

Blast analysis of the sequences and Sequence alignment

Through blast analysis we had found four *E.coli* designate by A, B, C and D and *Klebsiella pneumoniae* designate by E and F. A, B, C, D, E and F containing *Escherichia coli* strain LA133 plasmid pLAO63 (Sequence ID: OP242283.1), *Escherichia coli* strain E6474 plasmid pE6474.1(Sequence ID: CP116982.1), *Escherichia coli* strain E6474 plasmid pE6474.1(Sequence ID: CP116982.1), *Escherichia coli* strain E6474 plasmid pE6474.1(Sequence ID: CP116982.1), *Klebsiella pneumoniae* strain SC-KP169 plasmid pSC-KP169-2 (Sequence ID: CP119185.1) and *Klebsiella pneumoniae* strain SC-KP169 plasmid pSC-KP169-2 (Sequence ID: CP119185.1) synchronously. Here is the alignment of all the sequences. T-Coffee is a multiple sequence alignment tool that can be used to align bacterial sequences and it is a powerful tool for aligning bacterial sequences and can provide valuable insights into the evolutionary relationships between different bacteria.

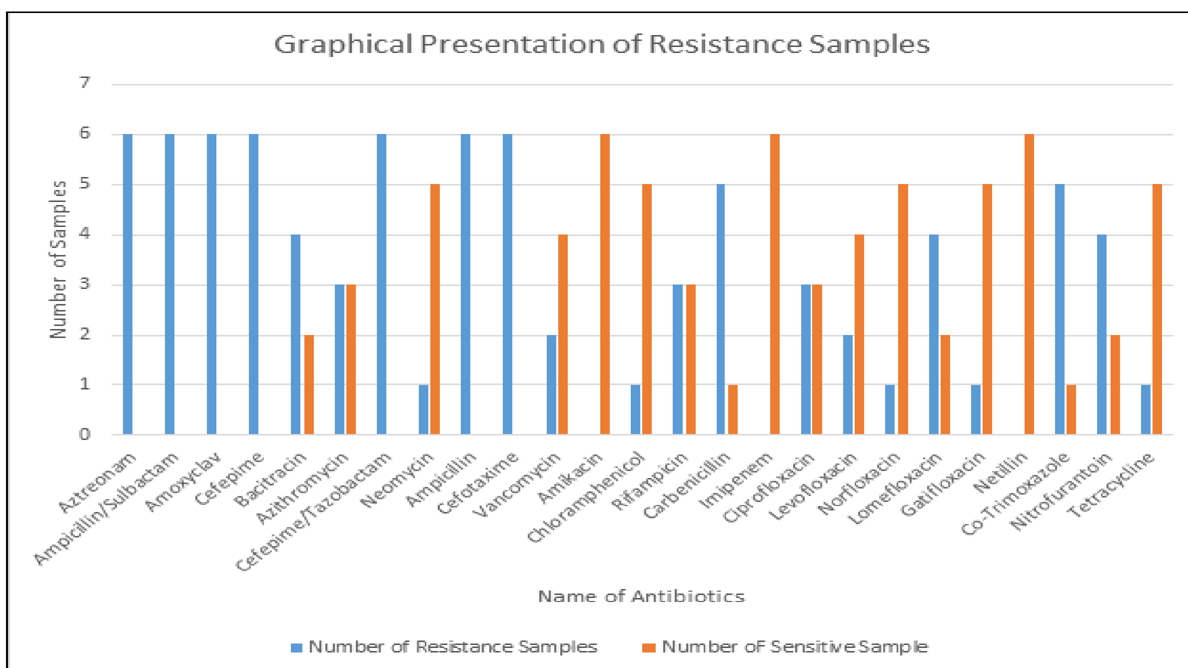

Fig. 2. Antibiotic Test of Resistance Samples of *E. coli* & *Klebsiella strain*. This graphically presents the results the antibiotic sensitivity tests performed among 25 antibiotics. The results revealed that ESBL behaves completely resistant against Aztreonam, Ampicillin/Sulbactam, Amoxyclav, Cefepime, Cefepime/Tazobactam, Ampicillin and Cefotaxime due to presence of antibiotic resistance gene.

Discussion

Antimicrobial resistance is a significant and increasing danger to human health. It undermines the effectiveness of medical treatments and leads to the emergence of multidrug-resistant infections in healthcare settings. Prompt treatment is essential, especially for vulnerable individuals who are at a high risk of bacterial infections. Antimicrobial resistance in bacteria involves various mechanisms. Susceptible bacteria can develop resistance through mutations or acquiring resistance genes from mobile DNA elements like integron (Barlow *et al.* 2004, Normak & Normak 2002) and this integron plays a vital role for antibiotic resistance. In this research work samples were collected from the laboratory of Rajshahi Medical College.

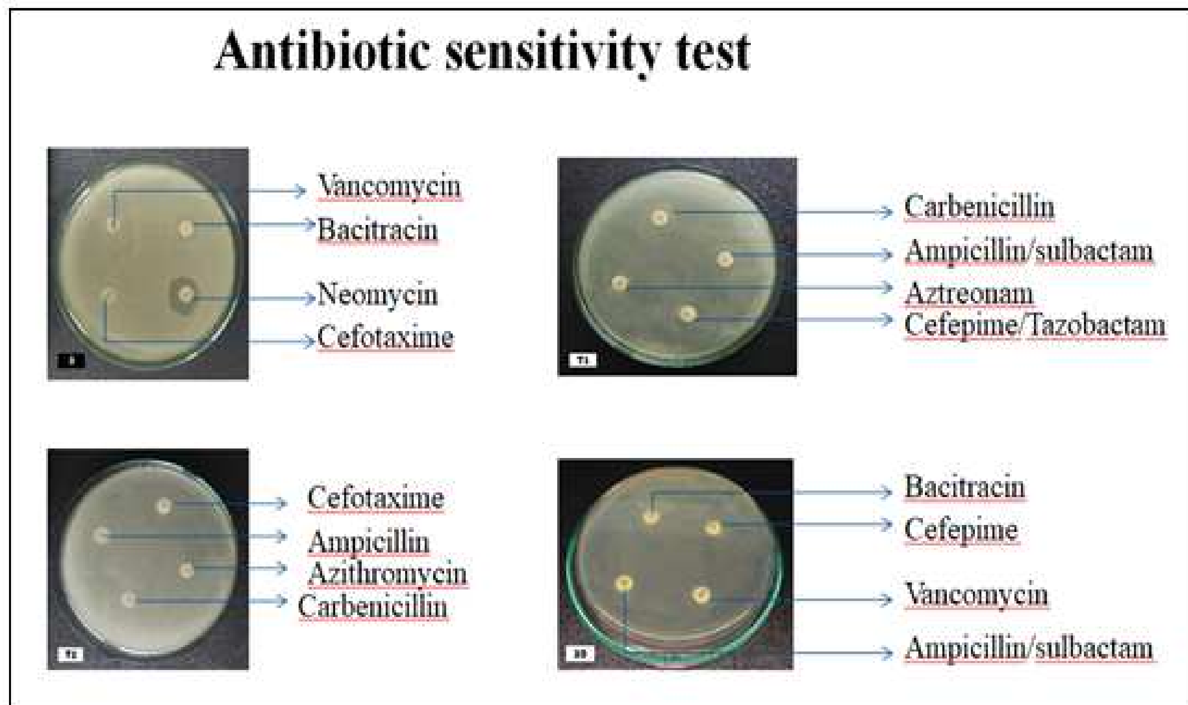

Fig. 3. The antibiotic sensitivity test was conducted on ESBL strains, and the results revealed that all of them exhibited resistance, as evidenced by the absence of zone formation.

In this study, samples grown in ESBL media were selected, and we had found 2 *Klebsiella* and 4 E. coli. In this research, a comprehensive analysis was carried out to assess the susceptibility of E. coli and Klebsiella bacteria to a total of 25 different antibiotics. The results revealed that both E. coli and Klebsiella strains displayed resistance towards the following antibiotics are Aztreonam,

Ampicillin/Sulbactam, Amoxyclav, Cefepime, Cefepime/Tazobactam, Ampicillin, and Cefotaxime. Amikacin, Imipenem, and Nitillin were found to be effective against all tested bacteria strains, including E. coli and Klebsiella. These antibiotics demonstrated sensitivity, indicating their potential as treatment options for infections caused by these bacteria.

Through blast analysis, we identified six bacterial strains: A, B, C, D (Escherichia coli) and E, F (Klebsiella pneumoniae). All six strains contain specific plasmids: strain A has plasmid pLAO63 (Sequence ID: OP242283.1), strains B, C, and D have plasmid pE6474.1 (Sequence ID: CP116982.1), and strains E and F have plasmid pSC-KP169-2 (Sequence ID: CP119185.1).

The presence of the integron integrase 1 gene indicates that antibiotic resistance in these bacteria is due to the horizontal transfer of plasmids carrying resistance genes. This horizontal transfer mechanism facilitates the movement of plasmids between bacteria and can contribute to the emergence of multidrug-resistant strains.

MSA The multiple sequence alignment result as produced by T-coffee.

```
T-COFFEE, Version_11.00 (Version_11.00)
Cedric Notredame
SCORE=1000
*
 BAD AVG GOOD
*
23C102_145_A_1n    :  100
23C102_147_B_1n    :  100
23C102_147_C_1n    :  100
23C102_150_D_1n    :  100
23C102_151_E_1n    :  100
23C102_146_F_1n    :  100
cons               :  100

23C102_145_A_1n    AACCACAGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGCGCAACG
23C102_147_B_1n    AACCACGGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGCGCAACG
23C102_147_C_1n    AACCACGGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGCGCAACG
23C102_150_D_1n    AACCACGGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGCGCAACG
23C102_151_E_1n    AACCACGGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGCGCAACG
23C102_146_F_1n    AACCACGGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCGGGAAGCGCAACG
cons               ****** ********************************************************

23C102_145_A_1n    CCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGCTGCTCGCGC
23C102_147_B_1n    CCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGCTGCTCGCGC
23C102_147_C_1n    CCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGCTGCTCGCGC
23C102_150_D_1n    CCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGCTGCTCGCGC
23C102_151_E_1n    CCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGCTGCTCGCGC
23C102_146_F_1n    CCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGCTGCTCGCGC
cons               ***************************************************************

23C102_145_A_1n    AGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGCCCTCCCGC
23C102_147_B_1n    AGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGCCCTCCCGC
23C102_147_C_1n    AGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGCCCTCCCGC
23C102_150_D_1n    AGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGCCCTCCCGC
23C102_151_E_1n    AGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGCCCTCCCGC
23C102_146_F_1n    AGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGCCCTCCCGC
cons               ***************************************************************

23C102_145_A_1n    ACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGCAAACCCTCACTGATC
23C102_147_B_1n    ACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGCAAACCCTCACTGATC
23C102_147_C_1n    ACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGCAAACCCTCACTGATC
23C102_150_D_1n    ACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGCAAACCCTCACTGATC
23C102_151_E_1n    ACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGCAAACCCTCACTGATC
23C102_146_F_1n    ACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGCAAACCCTCACTGATC
cons               ***************************************************************

23C102_145_A_1n    CGCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAGAAAACCGAGG
23C102_147_B_1n    CGCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAGAAAACCGAGG
23C102_147_C_1n    CGCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAGAAAACCGAGG
23C102_150_D_1n    CGCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAGAAAACCGAGG
23C102_151_E_1n    CGCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAGAAAACCGAGG
23C102_146_F_1n    CGCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAGAAAACCGAGG
cons               ***************************************************************

23C102_145_A_1n    ATGCGAACCACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCG
23C102_147_B_1n    ATGCGAACCACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCG
23C102_147_C_1n    ATGCGAACCACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCG
23C102_150_D_1n    ATGCGAACCACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCG
23C102_151_E_1n    ATGCGAACCACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCG
23C102_146_F_1n    ATGCGAACCACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCG
cons               ***************************************************************

23C102_145_A_1n    ATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGTAG
23C102_147_B_1n    ATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGTAG
23C102_147_C_1n    ATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGTAG
23C102_150_D_1n    ATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGTAG
23C102_151_E_1n    ATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGTAG

23C102_146_F_1n    ATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGTAG
cons               *********************************************
```

Fig. 4. Detection of Mutation Using T-Coffee is a multiple sequence alignment tool.

Following the sequencing process, the T-Coffee multiple sequence alignment tool was employed to perform sequence alignment. This analysis enabled the detection of specific point mutations within the aligned sequences, which are believed to be responsible for the observed antibiotic resistance. These mutations are thought to play a significant role in conferring resistance to the tested antibiotics in the studied bacteria.

Conclusion

Six bacteria sequences were aligned and mutation were discovered. The presence of integron integrase gene is therefore inferred to be the cause of the

antibiotic resistance in all samples. Multidrug-resistant strains are produced as a result of these genes facilitation of the assembly and integration of antibiotic resistance gene into the bacterial genome. This highlights the critical requirement for effective antibiotic resistance management techniques, such as the creation of novel medications and improved antibiotic stewardship procedures.

Reference:

Barlow RS, Pemberton JM, Desmarchelier PM, Gobius KS. 2004. Isolation and characterization of integron-containing bacteria without antibiotic selection. Antimicrobial agents and chemotherapy **48(3),** 838-842.

Davies, Julian, Dorothy Davies. 2010."Origins and evolution of antibiotic resistance." Microbiology and molecular biology reviews **74(3),** 417-433.

Gillings MR, Stokes HW. 2012. Are humans increasing bacterial evolvability?. Trends in ecology & evolution **27(6),** 346-352.

Mazel D. 2006. Integrons: agents of bacterial evolution. Nature Reviews Microbiology **4(8),** 608-620.

Murray AE, Chambers JJ, Van Saene HK. 1998. Infections in patients requiring ventilation in intensive care: application of a new classification. *Clinical* microbiology and infection **4(2),** 94-99.

Normark BH, Normark S. 2002. Evolution and spread of antibiotic resistance. Journal of internal medicine **252(2),** 91-106.

Saha AK, Haque MF, Karmaker S, Mohanta MK. 2009. Antibacterial effects of some antiseptics and disinfectants. Journal of Life and Earth Science, 19-21.

Yu G, Li Y, Liu X. 2013. Role of integrons in antimicrobial resistance: A review. African Journal of Microbiology Research Apr; **7(15),** 1301–1310.