Original Research Article

Molecular detection of Integrons I and II in some clinical isolates from Niger Delta University Teaching Hospital, Okolobiri, Nigeria

ABSTRACT

Aim: This study aimed at detecting the integron-integrase I and II gene in Gram negative bacteria.
Study Design: Cross-sectional and descriptive study design were utilized in this study.
Place and Duration of Study: NigerDelta University Teaching Hospital OkolobiriandNiger Delta University Molecular Laboratory CHS, WilberforceIsland, Amassoma, between February 2023 and April 2023.
Methodology: This study was specifically carried out on clinical isolates with a population figure obtained from 6 different samples collected from Niger Delta University Teaching Hospital, okolobiri in Bayelsa state. Of 500 different clinical samples, 100 clinical isolates were isolated and identified using Standard Bacteriological methods. Genomic DNA was extracted by boiling method. The Integrons I and II genes were detected using Polymerase Chain Reaction.
Results: Of the 100 isolates used for molecular analysis, the Int I1 gene was detected in 54 isolates of which 8(44.4%) were*Escherichia coli,14*(63.63%) were *Pseudomonas aeruginosa* and 14(70%) were *Klebsiella oxytoca.* IntI2 gene was detected in 78 isolates of which16(88.88%) were *Escherichia coli,*20(90.90%) was*Pseudomonas aeruginosa* and 10(50%) were *Klebsiella oxytoca.* IntI1 and IntI2 genes were detected in 38 isolates of which9(50%) were *Escherichia coli*,12(54.54%) was*Pseudomonas aeruginosa* and 6(30%) were *Klebsiella oxytoca.*
Conclusion: This study showed that there was a high prevalence of class I and II integrons in multi-drug-resistant Gram-negative bacteria, therefore justifying the extent of resistance observed.

Keywords: Integrons, Clinical isolates, Niger Delta UniversityTeaching Hospital, Okolobiri, Nigeria

1. INTRODUCTION

One of the huge public health threats globally today is the rise in the number of resistant clinical isolates (bacteria). This creates a treatment conundrum and may be caused by mutation or the acquisition of new genetic components that provide a resistance mechanism. Integrons are responsible for the spread of antibiotic resistance among clinical isolates, as evidenced by the connection between integrons and drug resistance[12]. To evaluate the contribution of integrons in bacterial species, however, a thorough understanding of their types, contents, and distribution is required. Molecular methods have also rarely been used in Bayelsa state to assess how antibiotics affect the transfer of genes encoding resistance determinants.

There is a limited understanding by the wider research community of the role of integrons in the broader environment; as drug resistances caused by these genetic elements are the major concern in public health. To help combat the growing epidemic of antibiotic resistance, especially in the 3rd world countries where we have out-of-date methods or no have modern tools, if we device simple tools to help detect, predict, or determine this genetic element causing drug resistance, this has the capacity to be counterproductive in solving or improving the antibiotic resistance problem in the future. This will in turn help to reduce costs, waste of materials, inappropriate prescription, improve understanding, control and reduce antimicrobial resistance. Furthermore, help in design of better drugs that are not affected by current known, predicted or unknown mechanism of resistance[13].

It is crucial to have a complete awareness of the different types, makeup, and distribution of the different bacterial species. Integrons have been discovered in clinical isolates. However, as more metagenomic investigations uncover integrons in a range of clinical specimens, it

has grown more challenging to fully recover integrons from metagenomic data. The initial barrier to discovering integrons is the absence of a well-designed process for doing so and a well-maintained database. Additionally, primers biases could lead to incomplete knowledge of it. Furthermore, because there is a shortage of diagnostic tools, not all kinds of organisms can be cultured because some can't be. Although it is a serious health issue even in industrialized nations, Bayelsa state only has access to a scant amount of information about the detection. The mechanism of integrons transported by plasmids or contained within transposons, as well as their function in the dispersion of resistance genes in microorganisms, has all been well established and documented[14]. Integrons were also believed to have a part in the release of "Super Bugs." Pathogen resistance and immune system development are both profoundly influenced by the human gut flora [15]. On the other side, the usage of antibiotics may lead to the spread of antibiotic resistance genes within the local gut flora. Integrons are significant vectors for this [1].

Integrons are discovered to be prehistoric genetic structures that play a significant part in the acquisition, storage, expression, and disposal of various antimicrobial resistance genes. As a result, exogenous genes appear as mobile gene cassettes [2].Intergrons are original replica and expression structures that guarantee a prompt and on-demand reaction to external forces. The spread of multidrug resistance (MDR) in bacteria because of site-specific recombination sparked by their integrase is documented. Open reading frames (ORFs) included in gene cassette units can be acquired and recreated, and by ensuring their proper expression, they can also turn them into functional genes. Around the end of the 1980s, the concept of integrons was initially introduced [3]. A bioinformatics-based analysis of partially or fully sequenced bacterial genomes showed the presence of about 10% and 17% of integrons and integrase genes, respectively. Though this structure can also be found in a variety of non-clinical environments, such as the forest, desert soils, aquatic environment, Antarctic soils, hot springs, biofilms, plant surfaces, marine sediments, and deep-sea sediments, the majority of integron explanations have focused on their occurrence in human clinical isolates. Genetically mobile elements like transposons or plasmids are linked to an action plan in resistant integrons, which increases transmission across and within species[3].

All integrons are structurally made up of three basic parts: a central variable region between the 5' and 3' zone, a conserved 5' and 3' segment, and the exogenous genes that make up the gene cassette that are captured and expressed by integrons. All integrons' 5' and 3' zone essential components comprise. The integrase gene (intI), a member of the tyrosine recombinase family, encodes a particular recombination site [3].Integrase discovered the attI receptor location next to the intI gene, which is upstream of intI. Additionally, the integrase protein can catalyze a recombinant between the input gene cassette and the attI. [4]. The Pc and Pint promoter sequence, which is positioned inside intI or between intI and attI, induces expression of the integrated gene cassette's existing genes in integrons and integrases. Integron classes differ in the architecture of the 3' conserved segment. Between the 3' and 5' zones lie the gene cassettes, which are how integrons acquire new genes. The integrin system offers two key advantages for genetic innovation: first, the new genetic material is integrated into the bacterial genome at attI, so they do not create anomalies in the existing genes; second, the new integrated genes are expressed by the integronspromoter[3,4].

The class 1 integron is the one with the highest prevalence among the integrons and is found in a variety of bacteria, particularly clinical isolates. Three different types of recombination sites can be recognized by intI. (attI, attc, and secondary sites). As a result, they have access to gene cassettes via recombination sites. They share a direct ancestor with the Tn402 and Tn3 transposons. The promoters Pc and P2 allow gene cassettes in this type of integrons to be expressed further. Because it guarantees that the gene cassettes are expressed properly, PC is crucial for integronfunction[5].It is present in 22–50% of Gram-negative bacteria isolates and carries more than 40 resistance genes for substances like aminoglycosides, beta-lactams, chloramphenicol, macrolides, sulphonamides, etc. The qacE1 gene, which codes for resistance to quaternary ammonium salts, and the sul gene, which codes for resistance to sulfonamide, makes up the 3'conserved segment. The second major kinds discovered from clinical isolates are class 2 integrons. They belong to the Tn7 transposon families (derived Tn1825, Tn1826, and Tn4132), which carry the Pc and attI2 recombination sites. The 5ns (tnsA, tnsB, tnsC, tnsD, and tnsE) genes that are involved in transposon movement are present in the 3'CS. Dihydrofolate reductase (dfrA1), streptomycin-acetyl transferase (sat1),

and aminoglycoside adenyltransferase (aadA1) resistance to trimethoprim, streptomycin, and spectomycin are among the gene cassettes found in class 2 integrons. EreA, which codes for erythromycin resistance [5]. The integrase gene in class 2 integrons is halted early by the end codons (TTA), which results in the deactivation of the 178 amino acid protein synthesis. This is one of the most significant differences between int1 and int2. As a result, class 2 integrons are less effective at moving gene cassettes than class 1 integrons. However, end codon mutations cause these amino acids to be reactive, which activate the integrase gene [5].The aim of the study was to use molecularmethod to detect Integrons I and II in some clinical isolates from Niger Delta University Teaching Hospital, Okolobiri, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Bayelsa state. Bayelsa state was created out of the former old Rivers state in 1996 during the administration of the late General Sani Abacha. The state of Bayelsa is bordered by Delta state to the north, Rivers state to the east, and the Atlantic Ocean to the west and south. It is located between 4015' North and 50 and 23' South. Bayelsa state has the largest wet land in West Africa, which was also the first to discover commercial quantities of crude oil. According to census data from 2006, there are about 1.7 million people living in the state of Bayelsa.

2.2 Experimental Design

Cross sectional and descriptive study design were utilized in this study.

2.3 Study Population

This study was specifically carried out on clinical isolates with a population figure obtained from 6 different samples collected from Niger Delta University Teaching Hospital, Okolobiri in Bayelsa state.

2.4Sampling Technique

Simple random sampling technique was used to collect isolates for this research work.

2.5Sample Size Determination

Taro Yamane statistical formula was applied to determine the sample size mathematically expressed as follow[6].

n = N/ 1 + N (e) 2
In the formula above.
n ----- Is the required sample size from the population under study.
N ----- Is the whole population that is under study (134).
e. ----- Is the precision or sampling error (0.05).
Substituting:

n=134/〖1+134(0.05)〗^2
n = 134/(1+134(0.0025))
n = 134/(1+0.335)
n = 162/1.355
n = 100
Therefore, sample size 100respondents out of the entire 134 respondents was the lowest accepted number of responses to maintain a 95% confidence level.

2.6Inclusion and Exclusion Criteria

Inclusion: All isolates that were obtained and are multidrug resistant, were included in this research work.

Exclusion:All isolates that were obtained and are not multidrug resistant, were excluded in this research work.

2.7Sample Collection

Clinical isolates were collected from Niger Delta University Teaching Hospital (NDUTH) Okolobiri in Bayelsa State. They were immediately transported to Niger Delta University Microbiology Laboratory for analysis.

2.8Molecular Analysis(11)

2.8.1 DNA Extraction

The boiling method was used for the extraction of DNA from clinical isolates, which was inoculated into LuriaBertani (LB) medium in a small tube (2ml Eppendorf tube) and centrifuged in a Denville 260D brushless micro centrifuges at 1400 rpm for 4minutes in a micro centrifuge. The supernatant was discarded using a micropipette and 1ml of 0.5 normal saline was added to the sediments and the tube was vortexon eltech XH-B Vortex. The tube was incubated in the heating block at 95□c for 20minutes; the tube was then taken to the freezer for fast cooling for 4minutes. The tube was further centrifuged at 14000 rpm for 3minutes and 500µl of the supernatant was transferred into 1.5ml eppendorf tube and stored in the freezer at -2□c for further analysis.

2.8.2 DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2µl of sterile distilled water and blanked using normal saline.2 µl of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the measure “measure” button. DNA concentration was measured in ng/µl (5 – 100ng/ µl) while purity level was determined at260/280, and absorbance (1.5 – 2.0).

2.8.3 Integron I and II Gene Amplification

Integron I and II resistant gene allelic variants were amplified respectively using ABI 9700 Applied Biosystems thermal cycler at a final volume of 30µL for 35 cycles. The PCR mix include: the X2 Dream Taq polymerase master mix supplied by Inquababiotec, South Africa (Taq polymerase, DNTPs, $MgCl_2$), the primers at a concentration of 0.5M and 80ng of the extract DNA as a template. Touch-down PCR (Applied Biosystem Thermal Cycler) was used to detect the Integrons genes of the isolates. It was performed in a 20ml reaction mixture containing 2X master mix (Taq polymerase, dNTPs, $MgCl_2$, and Buffer). The forward and reverse primers at a concentration of 0.4N molar supplied by Inquaba Biotech in South Africa, Water and DNA extract as template were added. The PCR tube was arranged on ice rack, 19µl of the cocktail was pipette into each of the labeled tubes and 1µl of the DNA template from isolates were pipette into the labeled tubes containing the cocktail respectively.

The genes were amplified using the set PCR condition as follows: initial denaturation (98□c for 30sec), followed by 35 cycles of denaturation (98□c for 10sec). Annealing (63□c for 30 seconds); initial extension (72□c for 6 min) and final extension (72□c for 10min).

2.8.4 The sequence of the Primers

INT1F GGCATCCAAGCAGCAAG, INT1_2R AAGCAGACTTGACCTGA, INT2F TCTGGGTAACATCAAGG

2.8.5 Agarose Gel Electrophoresis

One gram of Agarose powder was weighed on a weighing balance and poured into a conical flask with 100ml of already prepared 1X TAE buffer. It was mixed to dissolve very well for few seconds and then placed on microwave at 80 - 95 □c for 3minutes to dissolve properly. The dissolved gel powder in a conical flask and allowed it to cool for few seconds. Combs were placed in the casting tray on one end to enable the shape of the gel. The gel was poured into the casting tray having combs placed on and allowed them to solidify within 30 minutes. The Agarose polymerizes into a gel as it cools, the combs were removed from the gel to form wells for sample. The DNA ampliconcoloured with tracking dye (ethidium bromide) was pipette into the wells. The tray was placed into a chamber that generates electric current through the gel. The negative electrode was placed on the side nearest to the sample, the positive electrode placed on the other side. DNA has a negative charge and was drown to the

positive electrode at 125volt, 500miliamps, for 25minutes, small DNA molecule was able to travel faster through the gel and visualized with a UV transilluminator. One well called the DNA ladder contains DNA fragments of known sizes. This ladder was used alongside the plasmid samples to determine the size.

3. RESULTS AND DISCUSSION

Table 1: Distributions of Integrons by Isolates

Name of isolates	NE	INTI	INTII	INTI AND INTII
Escherichia coli	18	8 (44.44%)	16 (88.88%)	9 (50%)
Proteus vulgaris	6	2 (33.33%)	6 (100%)	2 (33.33%)
Proteus mirabilis	8	4 (50%)	6 (75%)	4 (50%)
Pseudomonas aeruginosa	22	14 (63.63%)	20 (90.90%)	12 (54.54%)
Klebsiella pneumoniae	10	4 (40%)	8 (80%)	2 (20%)
Klebsiella oxytoca	20	14 (70%)	10 (50%)	6 (30%)
Citrobacter freundii	14	6 (42.87%)	10 (71.42%)	4 (28.57%)
Citrobacter koseri	2	2 (100%)	2 (100%)	2 (100%)
Total	**100**	**54 (54%)**	**78 (78%)**	**38 (38%)**

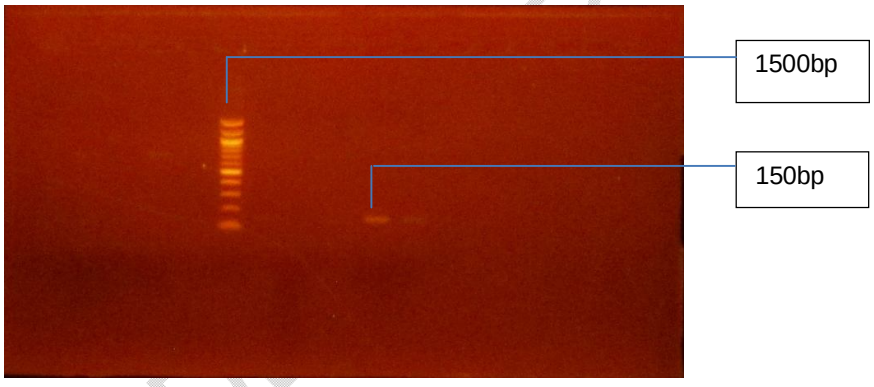

Plate 1: Agarose gel electrophoresis of INTI I gene of some selected bacteria isolates. Lane 8 represents the INTI I gene band (150bp).

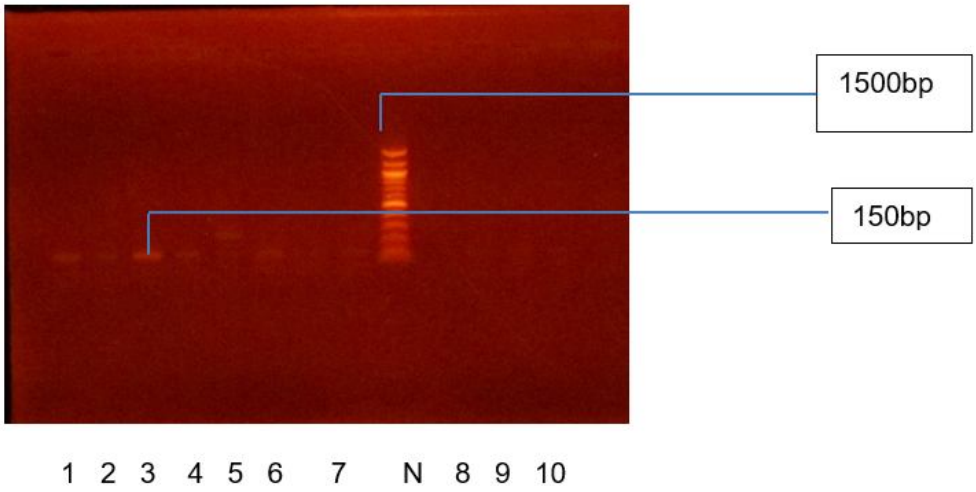

Plate 2 : Agarose gel electrophoresis of INT II gene of some selected bacteria isolates. Lane 1,2,3,4,5,6 and 7 represents the INT II gene band(150bp).Lane N represents the 100bp molecular ladder of 1500bp

The current study revealed a high prevalence of multidrug resistance among integron-bearing organisms, including *Escherichia coli,Klebseillaoxytoca*, *Klebseilla pneumoniae,* and Pseudomonas aeruginosa, among others, isolated from clinical samples. There is strong evidence that class 1 and class 2 integrons and growing multidrug resistance in gram negative bacteria are related. Numerous gene cassettes that code for resistance to beta-lactamase, sulphonamides, and aminoglycosides, among other things, can be found in the variable region of Int1 and Int2. A similar phenomenon of elevated gene expression for drug resistance to medications like cephalosporins, fluoroquinolones, sulfamethoxazole, and trimethoprim was observed in our study. These findings are consistent with those of other studies that showed cassettes for beta-lactamase, trimothoprim, and aminoglycoside. According to a study by Odetoyinet al. [7], there is a high prevalence of drug resistance to quinolones, aminoglycosides, and beta-lactam drugs. aadA (aminoglycoside adenyltransferase) encodes a type of aminoglycoside modification enzyme against aminoglycoside. Other genes associated with drug resistance include blaTEM,blaSHV, blaCT The usage of these medications as first-line therapies for the treatment of clinical disorders or selective pressure placed on them due to their extensive use in our institutions may be responsible for these findings. Most of the bacterial isolates in this investigation that were drug-resistant to various medicines had both the Int1 and Int2 genes, however they were polymorpic genes since certain class 1 and class 2 integrons had several gene cassettes. This is in accordiance with a study by Hosein, [8,9] that found integrons encoding antimicrobial resistance determinants in some gram-negative bacteria, as well as in Pseudomonas aeruginosa and Acinetobacter species, and that they are linked to drug resistance to beta-lactamases (including ESBL), Ofloxacin, Chloramphenicol, Trimethoprim, etc. Integrons frequently encode for various resistances because they can include numerous gene cassettes[10].

4. CONCLUSION

The current study's findings point to an expanding spread of integrons in line with the movement of antibiotic gene cassettes. Integrons were identified as having genes that strongly correlate with multidrug resistance in our isolates and resistance to various drug families. A high risk of resistance transfer and the dissemination of multidrug-resistant isolates in hospitals is indicated by our high prevalence of class 1 and 2 integrons and the presence of several resistant genes. To decrease resistance in clinical isolates in the area, antibiotic stewardship and consumption control are essential.

Ethical Approval

Ethical clearance was duly obtained from hospital ethical clearance committees of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa state.

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