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Research Article: Class 1 Integron (Inti1) Gene In Gram Negative Multi-Drug Resistant Bacteria Isolated From Clinical Samples In Bayelsa State, Nigeria

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ABSTRACT

Background and Objective: Integrons are common features of bacterial genomes that allow efficient capture and expression of new genes which are embedded in gene cassettes. Class 1 integron are mostly identified among clinical Gram-negative bacteria as the major factor responsible for drug resistance. This study was aimed at detecting the presence of the integron-integrase 1 gene (inti1) in Gram negative bacteria.

Materials and Methods: The isolates were determined using Standard Bacteriological methods. Antibiotic susceptibility testing was carried out using Modified Kirby Bauer disc-diffusion method and the genomic DNA was extracted by boiling method. The presence of Inti1 gene was determined using Polymerase Chain Reaction.

Results: A total of 121 clinical samples were collected, of which 99 (81.8%) were urine samples, 5 (4.13%) were Endocervical swabs, 4 (3.31%) were sputum samples, 4 (3.31%) were High vaginal swabs, 6 (4.95%) were wound samples and 3 (2.48%) were ear swabs, 45 (37.2%) samples were collected from males and 76 (62.8%) were collected from females. The findings showed that out of 121 samples analysed, a total of 95 isolates were obtained from the samples with *E.coli* having a high prevalence of 32 (33.7%), followed by *Klebsiella oxytoca* 30 (31.6%), *Klebsiella pneumonia* 21 (22.1%), *Pseudomonas aeruginosa* 6 (6.31%), *Enterobacter aerogenes* 3 (3.2%) and *Citrobacter fruendii* 3 (3.2%). Antibiotics susceptibility pattern showed the resistance percentage of each of the antibiotics used; Gentamicin (50.5%) Pefloxacine (68.4), Narivid (67.4%), Streptomycin (81.1%), Septrin (66.3%) Chloramphenicol (70.5%), Spectinomycin (53.7%), Ciprofloxacin (56.8%), Amoxacillin (53.7%) and Augmentin (88.4%). Out of the 30 isolates selected for molecular analysis, the Inti 1 gene was detected in 26 isolates.

Conclusion: This study showed that there is high prevalence of class 1 integron in multi-drug resistant Gram-negative bacteria. To prevent the development of multi-drug resistance bacteria, the indiscriminate use of antibiotics should be avoided.

Introduction

Antibiotic resistance in bacteria has been a global problem and the genes conferring this resistance have received considerable attention. Particularly, in Gram-negative bacteria many of these genes are associated with mobile genetic elements enabling movement between different DNA molecules (e.g. bacterial chromosome and a plasmid) and thus transfer between cells, including those of different genera. Gene cassettes, also known as integrons, are genetic elements with a site-specific recombination system that allows for the integration, expression, and exchange of particular DNA fragments (Hall *et al.*, 1995). Since it lacks features that allow for self-mobility, the entire integration is not regarded as a mobile element in the traditional sense.

On the other hand, gene cassettes found in integrons are thought to be mobile, even though natural gene cassette exchange is hardly ever shown in experiments (Guerin *et al.*, 2009; Baharoglu*et al.*, 2010). Still, sequence-similar integrons seem to be common across genetic backgrounds and bacterial species, indicating that they are regularly exposed to mechanisms that permit horizontal dissemination through the bacterial population (Stokes *et al.*, 1989).

Commonly found in bacterial genomes, integrons are typically found on chromosomes (Gibson, 1978). An integron-integrase gene (intI), a recombination

site (attI), and a promoter (PC) are their three main characteristics. With the help of these characteristics, external genes can be captured and produced as part of gene cassettes that are then expressed from Pc and recombined into the attI site utilizing the integrase activity provided by intI (Bouches *et al.*, 2007). This makes it possible to acquire and express genes while causing the least amount of disruption to the current genomes (Coillings *et al.*, 2005).

Although they help grab resistance gene cassettes, which then insert into the chromosome, integrons are not mobile in and of themselves. Certain Gram-negative bacteria, *P. aeruginosa*, *Acinetobacter species*, and others have been found to harbor integrons encoding antimicrobial resistance determinants. These integrons are linked to resistance to β-lactams, which include ESBLs, aminoglycosides, trimethoprim, chloramphenicol, and disinfectants. Integrons have the ability to encode for multidrug resistance since they can include numerous gene cassettes; this has also been linked to the release of "Super Bugs" (Kirschner, 1993).

Integrons occur in all environments, are able to move between species and lineages over evolutionary time frames, and they have access to a vast pool of novel genes whose functions are largely yet to be determined. Integrons are related to transposons (a segment of DNA that has a repeat of an insertion sequence element at each end that can migrate from one plasmid to another within the same bacterium, to a bacterial chromosome or to a bacteriophage) but are particularly important for the spread of genes for antibiotic resistance and other properties that give the host a growth advantage in a particular environment (Drew, 1999).

The use of antibiotics has vastly increased the abundance of clinical class 1 integrons, such that they are now present in up to 80% of Gram-negative bacteria in humans and farm animals (Cambray *et al.*, 2010). The complex DNA molecules that now bear class 1 integrons often also carry genes for resistance to diverse antibiotics, disinfectants and other environmental contaminant (Thurston *et al.*, 2000). Resistance genes encoding inactivating enzymes for β- lactam agents (including extended-spectrum β-lactamases (ESBLs) and carbapenemases), macrolides, aminoglycosides, and chloramphenicol; efflux genes for macrolides and tetracyclines; and altered targets for sulfonamides have been found on plasmids (Martins, 2008).

Materials and Methods

Study Area: The study was approved by the departmental ethics and research committee and was conducted in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Ethical Clearance: Ethical clearance was obtained from the ethical committee of the Federal Medical Center Yenagoa, Bayelsa State.

Sample Size: Using Taro Yemane's formula.

$$n = \frac{N}{(1+N(e)^2)}$$

Where n= Sample size N=Population
e=Margin of Error (0.05)
For a population of 173

$$N = \frac{173}{(1+173(0.05)^2)}$$

$$\frac{173}{(1+173(0.0025)}$$

$$\frac{173}{(1+0.4325)}$$

$$\frac{173}{1.4325}$$

n = 121

Sample Collection: One hundred and twenty-one (121) samples were collected from the hospitals. The samples collected consist of urine and sputum. High vaginal swab, endo-cervical swab, ear swab, and wound swab.

Sample Processing

Isolation and Identification: According to Monica (2006), bacterial isolation was carried out by culturing the samples on Mac-Conkey agar and incubating them for 24 hours at 37°C. Following this incubation, the colonies were inspected macroscopically and identified by Gram stain reaction, colonial morphology, biochemical test (KIA, Citrate, Indole, and Oxidase), and molecular analysis following sub-culturing in peptone broth.

Gram staining: Gram staining was done on the bacterial isolates and the results obtained were recorded. With the Gram-positive organisms retaining the primary stain crystal violet due to their thicker peptidoglycan layer and less permeable cell wall while Gram negative organisms have thinner peptidoglycan layer and therefore pick up the counter stain.

Kilger Iron Agar (KIA): Bacterial isolates were streaked and stabbed against the slant and butt of the KIA medium and incubated at 37°C for 24 hours. The color change in the butt and slant and gas and hydrogen sulfide production were recorded.

Citrate Utilization Test: A colony of the bacterial growth was selected, stabbed, and streaked against the slant and butt of Simmon's citrate agar medium using a straight wire loop. The medium was then incubated at 37°C for 24 hours to detect if the organism could utilize citrate by turning the medium from green to blue.

Indole Test: 0.5 ml of Kovac's reagent was added to an overnight culture of test isolates in peptone broth medium. Indole production is indicated by a cherry-red ring on the surface of the broth.

Oxidase Test: Some bacteria possess the ability to produce the enzyme cytochrome oxidase, which oxidizes the oxidase reagent tetramethyl-p-phenylenediamine dihydrochloride to a deep purple color.

Motility Test: On a sanitized cover slip, a loopful of the bacterial isolate suspension was encircled by plasticine. On top of the plasticine, a spotlessly clean glass slide was inverted. The motility of the smear was investigated under a microscope.

Antibacterial Susceptibility testing: To ascertain the organism's susceptibility pattern, antibiotic susceptibility testing was conducted using commercially available discs and

the agar disc diffusion method (also known as the Kirby Bauer method). This was accomplished by pouring the organism's peptone broth culture onto the Mueller Hinton Agar plate and then draining the excess. Mueller Hinton Agar was covered with the sensitivity disc, which was then incubated for 24 hours at 37°C.Following that, the plates were checked for zones of inhibition, and the diameter of the zones was calculated using a meter rule in millimeters. The medications that are susceptible to or resistant to the bacteria were then identified by comparing the readings with a standard. The antibiotics that are employed are Chloramphenicol (CH), Ofloxacin (OFX), Ciprofloxacin (CPX), Amoxacillin (AM), Gentamycin (CN), Pefloxacin (PEF), Streptomycin (S), Spectinomycin (SP), Augmentin (AU), and Chloramphenicol (CH).

DNA Extraction (Boiling Method): A 1.5-ml Eppendorf tube was filled with a pure isolate culture that had been grown overnight in Luria bertani broth; the volume transferred depended on how turbid the growth was in the broth. The soup in the Eependorf tube was filled to the 1.5 ml mark by adding 0.5% normal saline. After that, it was put in a Denville 260D brushless microcentrifuge and spun for five minutes at 1200 rpm. The sediment was vortexed using an Eltech XH-B vortex after the supernatant was decanted and 500 µl of 0.5% normal saline was added. After that, it was put inside the centrifuge and spun for three minutes at 1200 rpm. After decanting the supernatant, 500 µl of regular saline was added, and it was vortexed. To make sure the cells were thoroughly cleaned, the process was carried out once more. The supernatant was decanted after the third spin cycle, and 400 µl of DNA elution buffer was added before vortexing. After being heated to 95°C for 20 minutes, the tube was taken out of the block and allowed to cool quickly for a further 10 minutes. After that, the tube was spun once more for five minutes at 1200 rpm. 200 µl of the supernatant was then taken out and placed in a new 1.5 ml Eppendorf tube, which was then placed in the freezer at -2°C for additional examination and preservation.

DNA Quantification: The extracted bacterial DNA quantity and purity were estimated using a tabletop Nanodrop 1000 Spectrophotometer with its corresponding software installed on a computer system. 2µl was put onto the lower pedestal, and the purity was read.

Amplification of Class 1 Integron Integrase Gene: Using single-plex PCR and a heat cycler, the IntI 1 gene was amplified to a final volume of 25 µl. An ice rack held the PCR tubes in place. The cocktail was then created by pipetting 412.5µl of the master mix, 16.5µl of each of the forward and reverse primers, and 346.5µl of nuclease-free water into a 2 ml PCR tube. Subsequently, 1µl of the extracted bacterial DNA template was added to the 24µl of cocktail that had been pipetted into the PCR tubes that had been labeled, and the tubes were then loaded into the heat cycler.
The initial denaturation for the Inti 1 amplification was set for 5 minutes at 95°C. Thereafter, there were 34 cycles of 30 seconds at 95°C, 30 seconds at 55°C, 30 seconds of elongation at 68°C, and 3 minutes and 30 seconds of final extension at 68°C.

The sequence of the Primer Forward:
5'-GGCATCCAAGCAGCAAG-3'
Reverse: 5'-AAGCAGACTTGACCTGA-3'

Agarose Gel Electrophoresis: Using 1.5% agarose and Tris EDTA buffer, the resultant PCR product (amplicon) was subjected to gel electrophoresis and stained with ethidium bromide at 150 volts for 30 minutes. With the use of a UV transilluminator, the bands became visible. A 1 kb molecular DNA ladder with a fast load was used to measure the band diameters.

Results

Out of the 121 clinical samples that were collected, 45 (37.2%) came from men and 76 (62.8%) from women. Urine samples were taken from 99 (81.8%) people, 35 (35.4%) of whom were men and 64 (64.6%) of whom were women. Four (100%) male sputum samples were taken in total. Five endo-cervical swabs (100%) were taken from female subjects. Four (100%) high vaginal swabs were taken from female subjects. Six (100%) of the wound swabs from the male subjects were obtained. Table 1 shows that 3 (100%) of the females' ear swabs were taken.
From the clinical specimens, a total of 95 isolates were collected, of which 32 (33.7%) were recovered from males and 63 (66.3%) from females. Of the cases of *Klebsiella pneumonia*, 10 (or 31.25%) were found in men and 11 (17.4%) in women. Of the *Escherichia coli* isolates, 10 (31.25%) came from males and 22 (34.9%) from females. Male *Klebsiella oxytoca* samples accounted for 9 (28.1%) and female samples for 21 (33.3%). Of *Pseudomonas aeruginosa*, 3 (0.9%) from males and 3 (4.8) from females were isolated. Three (4.8%) isolates of *Enterobacter aerogenes* were found in females. Table 2a & b shows that 3 (4.8%) of the *Citrobacter freundii* isolates were from females.
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Of the 95 bacterial isolates that were identified, 81 (86.7%) were found in urine samples, 4 (3.33%) in endocervical swabs, 4 (33.3%) in sputum, and 6 (6.67%) in wound specimens. Neither the ear nor the high vaginal swab had any germs (Table 3).
The antimicrobial susceptibility pattern of the bacterial isolates is shown in Table 4. Of the bacterial isolates, 48 (50.5%) were resistant to Gentamicin, 65 (68.4%) to Pefloxacine, 64 (67.4%) to Narivid, 77 (81.1%) to Streptomycin, 63 (66.3%) to Septrin, 67 (70.5%) to Chloramphenicol, 51 (53.7%) to Spectinomycin, 54 (56.8%) to Ciprofloxacin, 51 (53.7%) and 84 (88.4%) were resistant to Amoxacillin and Augmentin, respectively.
The distribution of the samples is depicted in the pie chart, with urine samples accounting for 81%, high vaginal swabs for 4%, endocervical swabs for 4%, sputum for 3%, wounds for 5%, and ears for 3%. (Figure 1).
The Agarose gel electrophoresis image displays positive bands corresponding to the inti1 gene. The positive bands of the inti1 gene in the bacterial isolates are represented by lanes 1 through 10. The 1500 bp molecular ladder is represented by Lane M. (Plate 1).

Table1: Distribution of Specimen by Gender

SPECIMENS	MALE (%)	FEMALE (%)	TOTAL (%)
Urine	35(35.4)	64(64.6)	99 (81.8)
Sputum	4 (100%)	—	4 (3.3)
Wound	6(100)	—	6(5.0)
Endocervical Swab	—	5(100)	5(4.13)
Ear	—	3(100)	3(2.5)
High vaginal Swab	—	4(100)	3(3.3)
TOTAL	**45(37.2)**	**76(62.8)**	**121**

Table 2a: Distribution of Bacterial Isolates by Age and Gender (Male %)

Age range No. Examined	*K.pneumoniae* (%)	*E.coli* (%)	*K. oxytoca* (%)	*E. aerogenes* (%)	*P.aeruginosa* (%)	*C. freundii* (%)	TOTAL (%)
<20	-	-	6(100)	-	-	-	6(18.8)
21-30	4(100)	-	-	-	-	-	4(12.5)
31-40	-	1(100)	-	-	-	-	1(3.1)
41-50	6(100)	-	-	-	-	-	6(18.75)
51-60	-	6(50)	3(25)	-	3(25)	-	12(37.5)
>60	-	3(100)	-	-	-	-	3(9.3)
Total	**10(31.25)**	**10(31.25)**	**9(28.1)**	**-**	**3(0.9)**	**-**	**32**

Table 2b: Distribution of Bacterial Isolates by Age and Gender (Female %)

Age range No. Examined	*K.pneumoniae* (%)	*E.coli* (%)	*K. oxytoca* (%)	*E. aerogenes* (%)	*P.aeruginosa* (%)	*C. freundii* (%)	TOTAL (%)
<20	-	3(100)	-	-	-	-	3(4.7)
21-30	-	6(22.2)	12(44.4)	3(11.1)	3(11.1)	3(11.1)	27(42.8)
31-40	6(25)	12(50)	6(25)	-		-	24(38.1)
41-50	3(50)	-	3(50)	-	-	-	6(9.5)
51-60	2(100)	-	-	-	-	-	2(3.17)
>60	-	1(100)	-	-	-	-	1(1.6)
Total	**11(17.4)**	**22(34.9)**	**21(33.3)**	**3(4.8)**	**3(4.8)**	**3(4.8)**	**63**

Table 3: Distribution of Bacterial Isolates by Specimens

Specimens	*K.pneumoniae (%)*	*E.coli* (%)	*K. oxytoca* (%)	*E. aerogenes* (%)	*P.aeruginosa* (%)	*C. freundii* (%)	Total (%)
Urine	14(15.38)	31(38.5)	27(34.6)	3(3.85)	3(3.85)	3(3.85)	81 (86.7)
High vaginal Swab	-	-	-	-	-	-	-
Endocervical Swab	4(100)	-	-	-	-	-	4(3.33)
Sputum	3(100)	1	-	-	-	-	4(3.33)
Wound	3 (50)	-	-	-	3(50)	-	6(6.67)
Ear	-	-	-	-	-	-	-
Total	**24(23.3)**	**32(33.3)**	**27(30)**	**3(3.33)**	**6(3.33)**	**3(3.33)**	**95**

Fig 1: Pie chart showing distribution of sample

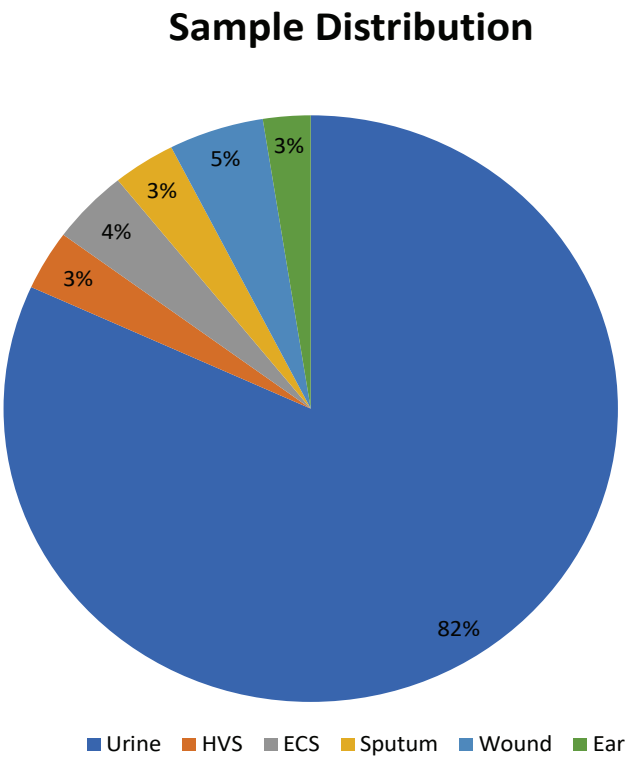

Table 4: Antimicrobial susceptibility pattern of bacterial isolates

ISOLATES	CN (R%)	PEF (R%)	OFX (R%)	S (R%)	SXT (R%)	CH (R%)	SP (R%)	CPX (R%)	AM (R%)	AU (R%)
***K.pneumoniae*(24)**	12(50)	8(33.3)	13(54.2)	16(66.7)	12(50)	15(62.5)	14(58.3)	12(50)	9(37.5)	15(62.5)
***E.coli* (32)**	17(53.1)	25(78.1)	23(71.9)	27(84.4)	24(75)	21(65.6)	16(50)	15(46.9)	18(56.3)	30(93.8)
***K.oxytoca*(27)**	14(51.9)	23(85.2)	21(77.8)	25(92.6)	18(66.7)	24(88.9)	15(55.6)	21(77.7)	18(66.7)	27(100)
***E.aerogenes*(3)**	2(66.7)	3(100)	3(100)	3(100)	3(100)	1(33.3)	3(100)	3(100)	3(100)	3(100)
***P.aeruginosa*(6)**	3(50)	6(100)	4(66.7)	6(100)	6(100)	6(100)	3(50)	3(50)	3(50)	6(100)
***C.fruendii* (3)**	0	0	0	0	0	0	0	0	0	3(100)
Total (95)	**48(50.5)**	**65(68.4)**	**64(67.4)**	**77(81.1)**	**63(66.3)**	**67(70.5)**	**51(53.7)**	**54(56.8)**	**51(53.7)**	**84(88.4)**

KEY: CN- Gentamicin, PEF- Pefloxacin, OFX-Tarivid, S-Streptomycin, SXT-Septrin, CH- Chloramphenicol, SP-Spectinomycin, CPX-Ciprofloxacin, AM, Amoxacillin, AU-Augmentin., R%- Resistance in percentage

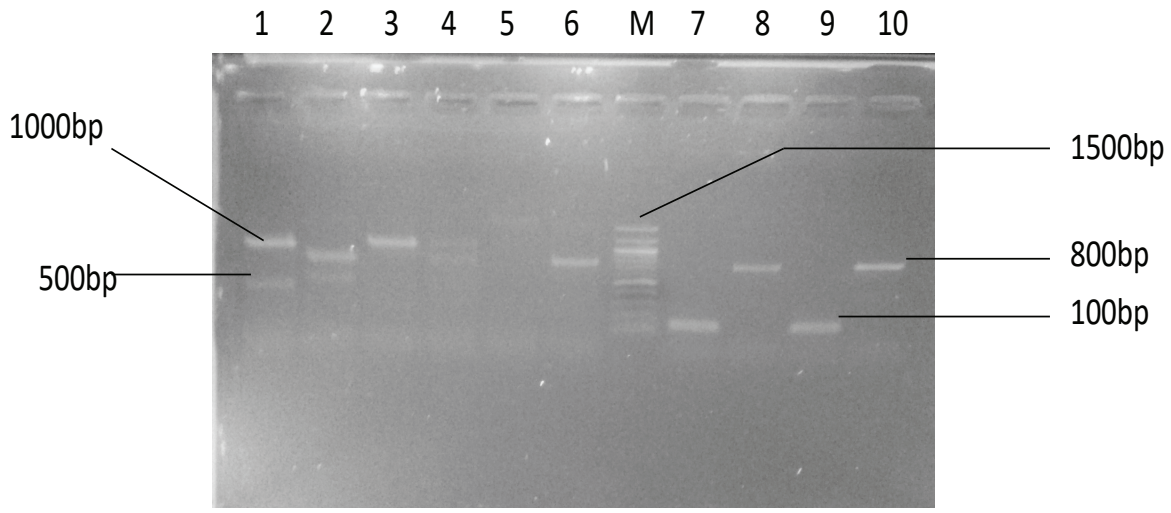

Plate 1: Agarose gel showing class 1 integron of polymorphic genes. Lane 1, 3 and 4 at 1000bp; lane 2, 4, 6, 8 and 10 at 800bp; lane 2 at 550bp; lane 1 at 500bp; and lane 7 and 9 at 100bp. Lane M represent molecular ladder of 100bp

Table 5: Distribution of inti1 gene in the bacterial isolates tested

Specimen	CN	PEF	OFX	S	SXT	CH	SP	CPX	AM	AU	Inti1 gene
ECS 1	R	R	R	R	R	R	R	R	R	R	Inti1
Ur1	-	R	R	R	R	R	S	R	S	R	Inti1
Ur2	S	R	R	R	S	S	S	R	R	R	Inti1
Ur3	R	R	R	R	S	R	R	R	R	R	Inti1
Ur4	R	R	R	R	R	R	R	R	R	R	-
Ur5	R	R	R	R	R	S	S	R	S	R	Inti1
Sp1	S	S	S	R	R	R	S	S	S	R	Inti1
Ur6	R	R	R	R	R	R	R	R	R	R	Inti1
Ur7	-	R	R	R	R	R	S	R	R	R	Inti1
Ur8	R	R	R	R	S	R	S	R	S	R	Inti1
Ur9	S	R	R	R	R	R	R	S	R	R	Inti1
Wd1	R	R	R	R	R	R	R	R	R	R	Inti1
Ur10	R	R	R	R	R	R	R	R	R	R	Inti1
Ur11	R	R	R	R	R	R	R	R	R	R	Inti1
Ur14	R	R	S	R	S	S	S	S	S	R	Inti1
Ur15	S	S	S	S	S	R	S	R	S	R	-
Ur16	S	R	R	S	S	S	S	S	S	R	-
Ur17	R	R	R	R	R	R	R	R	R	R	Inti1
Ur18	-	S	S	R	R	S	S	S	S	R	Inti1
Ur19	R	R	R	R	R	R	S	S	S	R	Inti1
Ur20	R	S	R	R	R	R	R	R	R	R	Inti1
Ur21	S	R	R	R	S	S	R	R	S	R	Inti1
Ur22	S	S	S	S	S	S	S	S	S	R	Inti1
Ur23	R	R	R	R	R	R	R	R	R	R	Inti1
Ur24	R	R	S	R	R	R	S	S	S	R	Inti1
Ur25	S	R	R	R	R	R	R	R	S	R	Inti1
Ur26	S	R	S	R	R	R	S	S	S	R	Inti1
Ur28	R	R	R	R	S	R	R	S	R	R	Inti1
Ur31	S	S	S	R	R	R	S	S	S	S	-
Wd2	R	S	R	R	R	R	R	R	R	R	Inti1
Total=30	17(56.7%)	23(76.7%)	22(73.3%)	27(90%)	21(70%)	23(76.7%)	15(50%)	19(63.3%)	15(50%)	29(96.7%)	

Table 6: Chi-square analysis of the prevalence of Inti1 among the bacterial isolate obtained from various sources

Category	**Observed**	**Expected**	**(Obs-Exp)**	**$(Obs-Exp)^2$**	**$(Obs-Exp)^2/Exp$**
Urine	26	7.5	18.5	342.25	45.63
Wound	2	7.5	-5.5	30.25	4.03
Sputum	1	7.5	-6.5	42.25	5.63
ECS	1	7.5	-6.5	42.25	5.63
					$\sum$=60.92
					X^2=60.92

Degree of freedom = no of categories – 1 = 4-1 =3
P-value = 7.81, p-value > 0.05, therefore it is not statistically significant
$Xc2 = \sum (Obs-Exp)^2/Exp$
Where C=Degree of Freedom
Oxp= Observed value
Exp=Expected value

Discussion

According to the study, females had a higher frequency of the specimen distribution based on gender (76.8%) than did males (45.2%). This demonstrates that women seek medical care and visit hospitals more frequently than men. The results of this investigation are consistent with a study by Jumbo *et al.* (2006), which found that hospital attendance was more common among women than men.

The specimen distribution for urine showed the highest number of specimens collected, with a higher proportion of female specimens. This may be supported by the finding that, in line with Nejad *et al.* (2011), urinary tract infections are the most frequent reason for hospital visits. Women are more vulnerable to these infections because of the near proximity of their urethra to the genital tract and the anus.

Urine specimen distribution revealed the greatest number of specimens obtained, with a greater percentage of specimens belonging to the female gender. According to Nejad *et al.* (2011), the most common reason for hospital visits is urinary tract infections, which may lend credence to this. The urethra of women is located close to the vaginal tract and the anus, making them more susceptible to these illnesses.

In contrast to a recent study by Azimi *et al.* (2019), where the highest bacterial isolate was acquired from the age group of 10–19 and >50 years, the highest bacterial isolate in our study was obtained from the middle-aged group (21–30). This age group includes sexually active people who are more likely to get urinary tract infections, which could explain the increased frequency of bacterial isolates in this age range.

The majority of the bacterial isolates in this investigation were resistant to the actions of antibiotics, indicating a low prevalence of antimicrobial susceptibility overall. Considered a multidrug-resistant organism, *Klebsiella species* and *E. coli* were resistant to Gentamycin (50.5%), Pefloxacin (68.4%), Tarivid (67.4%), Streptomycin (81.1%), Septrin (66.3%), Chloramphenicol (70.5%), Spectinomycin (53.7%), Ciprofloxacin (56.8%), Amoxacillin (53.7%), and Augmentin (88.4%). This is consistent with the findings of Azimi*et al.* (2019), who found that *E. coli* and *Klesiella spp.* have significant levels of antibiotic resistance. The overuse of antibiotics, the ease with which medications are given in hospitals, the existence of drug-resistant genes and mechanisms like efflux pumps, and other factors are all responsible for this high prevalence of resistance.

Despite being polymorphic genes because certain class 1 integrons have numerous gene cassettes, the majority of the bacterial isolates in this investigation that were resistant to multiple medications carried the inti1 gene. This is in line with the research done by Kelvin (2018), which found that some species of *P. aeruginosa, Acinetobacter* and Gram-negative bacteria have integrons encoding antimicrobial resistance determinants. These integrons are linked to resistance to β-lactams, including ESBLs, ofloxacin, chloramphenicol, trimethoprim, and other antibiotics. Integrons frequently encode for multidrug resistance because they may hold several gene cassettes.

Conclusion

Multiple antibiotic-resistant bacteria exhibit a high incidence of class 1 integron. *Escherichia coli, Klebsiells species, Pseudomonas aeruginosa,* and other bacteria are examples of this type. The Class 1 integron's many gene cassettes are what give them their resistance to antibiotics. A specific antibiotic resistance is imparted by each gene cassette. Therefore, the bacteria will be resistant to several antibiotics if they have several gene instances.

Reference

Azimi, T., Maham, S., Fallah, F., Azimi, L. and Gholinejad, Z. (2019). Evaluating the antimicrobial resitance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran. *Infection and Drug Resistance.* **12**:2089-2102.

Baharoglu Z, Bikard D, Mazel D. Conjugative DNA transfer induces the bacterial SOS response and promotes antibiotic resistance development through integron activation. *PLoS Genet.* 2010; 6

Boucher, Y., Labbate, M., Koenig, J and Stokes, H. (2007). Integrons: Mobilizable platforms that promote genetic diversity in bacteria. *Trends Microbiology.* **15**:301-309.

Cambray, G., Guerout, A and Mazel, D. (2010). Integrons. *Annual Review of Genetics* **44**:141-166.

Collins, C., Grammaticopoulos, G., Briton, J., Stokes, H and Hall, R. (1993). Site-specific insertion of gene cassettes into integrons. *Molecular Microbiology.* 9:41-52.

Drews and Gerhart. (1999). Ferdinard Cohn, a founder of modern microbiology. *America Society for Microbiology News.* **65**(8): 547-552.

Gibbons, N. and Murray, R. (1978). Proposals concerning the higher taxonomy of Bacteria. *International Journal of Systematic Bacteriology.* **28** (1): 1-6.

Guerin E, Cambray G, Sanchez-Alberola N, Campoy S, Erill I, Da Res et al. The SOS response controls in integron recombination. *Science.* 2009; 324:1034

Hall, R., Collis, C. (1995). Mobile gene cassettes and integrons: Capture and spread of genes by site-specific recombination. *Molecular Microbiology.* **15** (4): 593-600.

Jumbo, G., Egah, D., Banwat, E. and Ayeni, J. (2006). Nosocomial and community acquired urinary tract infections at a teaching hospital in north central Nigeria: findings from a study of 12,458 urine samples. *Nigerian Journal of Medicine.* **15**(1): 230-236.

Kirschner, P., Springer, B., Vogel, U., Meier, A., Wrede, A., Kiekenbeck, M., Bange, F and Bottger, E. (1993). Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experienced in a clinical laboratory. *Journal of Clinical Microbiology.* **31**:2882-2889.

Martins, B., Lapierre, L., Cornejo, J., Bucarey, S., (2008). Characterization of antibiotic resistance genes linked to class 1 and class 2 integrons in strains of Salmonella spp. Isolated from swine. *Canadian Journal of Microbiology.* **54**:569-576.

Nejad, S., Allegranzi S., Syed, S., Ellis, B. and Pittet, D. (2011). Health care associated infection in Africa: a systematic review. *Bulletin of the World Health Organization.* **89**(10): 757-765.

Stokes, H., O'Gorman, D, Recchia, G., Parsekhian, M and Hall, R. (1997). Structure and function of 59-base element recombination sites associated with mobile gene casettes. *Molecular Microbiology.* **26**:731-745.

Thurston, A. (2000). Of blood, inflammation and gunshot wounds: the history of the control of sepsis. *The Australian and New Zealand Journal of Surgery.* **70** (12): 855-861.