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Detection of Integrons in Multidrug Resistant Wound Isolates

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ABSTRACT

Integrons are mobile genetic structures that carry genes responsible for resistance to different classes of antibiotics. These genetic platforms are disseminated easily among bacteria through horizontal transfer. This makes it possible for bacteria infecting parts of the body including wounds to harbor integrons resulting to poor therapeutic outcomes. This study was conducted to detect the presence of integrons in multidrug resistance isolates from wounds. Three hundred and sixty chronic wound patients were sampled using sterile cotton- tipped swab sticks. The specimens were cultured according to standard microbiological procedures. The isolates were characterized by standard biochemical tests. The genomic DNA of the isolates was extracted by boiling method and was sequenced using the Big Dye kit on 3510 ABI sequencer. Antimicrobial susceptibility test was done using disc diffusion method. Multiplex Polymerase Chain Reaction was carried out on The DNA extracts using Class 1 and Class 11 Integron primers. The result shows that all 360 wound swab specimens yielded single bacteria isolate each. Pseudomonas aeruginosa was the most prevalent isolate (44.2%). The antimicrobial susceptibility test indicates that 42 isolates (11.7%) were multidrug resistant (MDR). Streptomycin attracted the highest resistance of 88.89%. The least resistance was to Imipenem (35.71%). The gel electrophoresis of the Multiplex PCR product indicates that 90.5% of the MDR isolates possess Class 1 Integron, 33.33% possess Class 11 Integron and 23.8% possess both Integron 1 and Integron 11. In conclusion, this study reports high prevalence of Pseudomonas aeruginosa in chronic wound swabs and 11.7% multidrug resistance among all isolates. The study also reports high prevalence of Class 1 Integron in multidrug resistance isolates. It is therefore recommended that stringent infection control measures be adopted to prevent the spread of bacteria harbouring antibiotic resistance genetic structures. Also rational antibiotic policy is recommended to avoid selection of drug resistance under antibiotic pressure.

KEYWORDS: *genes, Integrons, multidrug, resistance, wound*

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INTRODUCTION

Wound refers to an injury that results when the local barrier against the entry of infectious agents, the skin, is compromised. This crack in the local physical barrier predisposes the wound to contamination and infection by different pathogenic bacteria [1][2][3] from both endogenous and exogenous sources [4]. Empirical treatment of infected wounds without proper microbiological investigations (to identify the aetiologic agents, determine the antimicrobial susceptibility profile of the aetiologic agents) as well as self-medication and other forms of drug abuse have the tendency to select for drug resistance leading to poor therapeutic outcomes and increased morbidity and mortality [5][6]. The drug resistance is mediated by a number of factors like drug resistance genes which may be innate or arise from mutations or

acquired from other bacteria or from the environment [7]. Such genes include those carried in gene cassettes also called integron cassettes [8]. The role of integrons in carrying multidrug resistance genes and promotion of virulence of pathogens have variously been reported [9][10]. This study was conducted to detect the presence of integrons in multidrug resistance wound isolates.

MATERIALS AND METHODS

Wound specimens were collected from patients with chronic wounds using sterile cotton tipped swabs. The specimens were inoculated on MacConkey Agar, Blood Agar and Chocolate Agar. These were incubated aerobically at 37^0C. The isolates were identified by standard biochemical tests according to Cheesbrough [11].

Antimicrobial susceptibility testing was carried out using disc diffusion method with the following antibiotics: ciprofloxacin, ofloxacin, levofloxacin, streptomycin, gentamycin, ceftazidime, imipenem, aztreonam, amoxicillin + clavulanic acid and ampicillin. The susceptibility test was interpreted as Sensitive, Intermediate or Resistant according to CLSI interpretation criteria [12].

The isolated bacteria were further sub-cultured into Luria Bertani broth for deoxyribonucleic acid (DNA) extraction using boiling method and quantified using the Nanodrop 1000 spectrophotometer [13].

Polymerase Chain Reaction for characterization of integrons was carried out using Multiplex PCR. The Class I and Class II Integron genes were amplified using IntII: 5^1-ACA TGT GAT GGC GAC GCA CGA-3^1 on an ABI 9700 Applied Biosystems thermal cycler. DNA ladder digest of 1000bp and 500bp were employed as molecular weight markers for Class 1 Integron and class 11 Integron respectively.

The product was resolved (gel electrophoresis) on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator in a photo documentation system.

The 16S rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler. The sequencing of the amplified gene was done using the Big Dye Terminator kit on a 3510 ABI sequencer (Inqaba Biotechnological, Pretoria South Africa).

RESULTS

The 360 wound swab specimens from both male and female patients aged 10 – 50 years yielded 360 single isolates comprising Gram positive and Gram negative bacteria. *Pseudomonas aeruginosa* was the most prevalent isolate (44.2%) followed by *Staphylococcus aureus* (21.9%). *Neisseria sica, Alcaligenes faecalis, Paenalcaligenes* sp, *Enterobacter asburie, Staphylococcus sciuri* and *Pantoea dispersa* were the least prevalent isolates (0.3% each) (Table 1). The result of the presumptive biochemical identification had only 3.6% variation from the sequencing result. *Alcaligenes, sp, Providentia Stuartii* and *Pantoea dispersa* were misidentified as *Pseudomonas aeruginosa* while *Enterobacter* spp were misidentified as *Escherichia coli* etc.

Table 1: Identity of isolates

Name of isolate	No of isolates	%
Pseudomonas aeruginosa	159	44.2
Alcaligenes faecalis	1	0.3
Paenalcaligenes sp UN24	1	0.3
Providencia stuartii	3	0.8
Pantoea dispersa	1	0.3
Escherichia coli	29	8.1
Enterpbacter hormaechei	4	1.1
Enterobacter asburiae	1	0.3
Klebsiella aerogenes	30	8.3
Proteus vulgaris	10	2.8
Proteus mirabilis	10	2.8
Neisseria sica	1	0.3
Staphyloccus aureus	79	21.9
Staphyloccus sciuri	1	0.3
Enterococcus faecalis	30	8.3
Total	360	100

The antimicrobial susceptibility test indicates that 42 isolates (11.7%) were multidrug resistant (resistant to at least one antibiotic from up to 3 different classes according to the definition of Magiorakos et al, [14]2012) (Table 2). Fifteen percent of the *Proteus vulgaris*, 15.7% of *Pseudomonas aeruginosa* and 5.1% *Staphylococcus aureus* were multidrug resistant.

Table 2: Multidrug Resistance Isolates (MDR)

Name of isolate	No of isolates	No of MDR ISOLATES	%
Pseudomonas aeruginosa	159	25	15.7
Alcaligenes faecalis	1	0	0
Paenalcaligenes sp UN24	1	0	0
Providencia stuartii	3	0	0
Pantoea dispersa	1	0	0
Escherichia coli	29	3	10.3
Enterpbacter hormaechei	4	0	0
Enterpbacter asburiae	1	0	0
Klebsiella aerogenes	30	3	10
Proteus vulgaris	10	3	15
Proteus mirabilis	10	2	10
Neisseria sica	1	1	100
Staphylococcus aureus	79	4	5.1
Staphylococcus sciuri	1	0	0
Enterococcus faecalis	30	1	3.3
Total	360	42	11.7

Streptomycin attracted the highest resistance of 88.89% among the MDR isolates followed by Ampicillin (88.10%) Levofloxacin (88.10%) and Ciprofloxacin (83.3%). The least resistance was to Imipenem (35.71%) Figure 1).

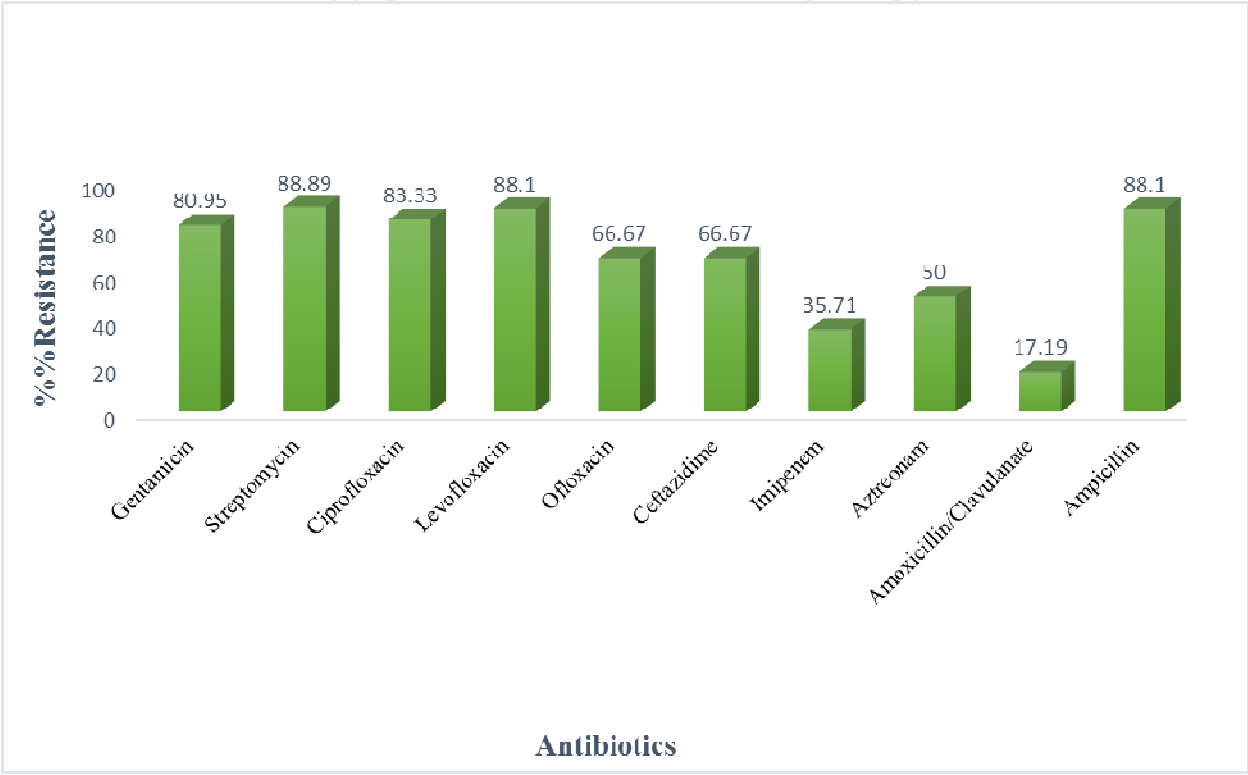

Figure 1: Resistance Pattern of MDR isolates

All *Proteus* sp were resistant to Gentamicin, Streptomycin, Ciprofloxacin and Ofloxacin; all *Staphylococcus* sp were resistant to Streptomycin, Ofloxacin and Aztreonam. All species show less resistance to Imipenem (Figure 2).

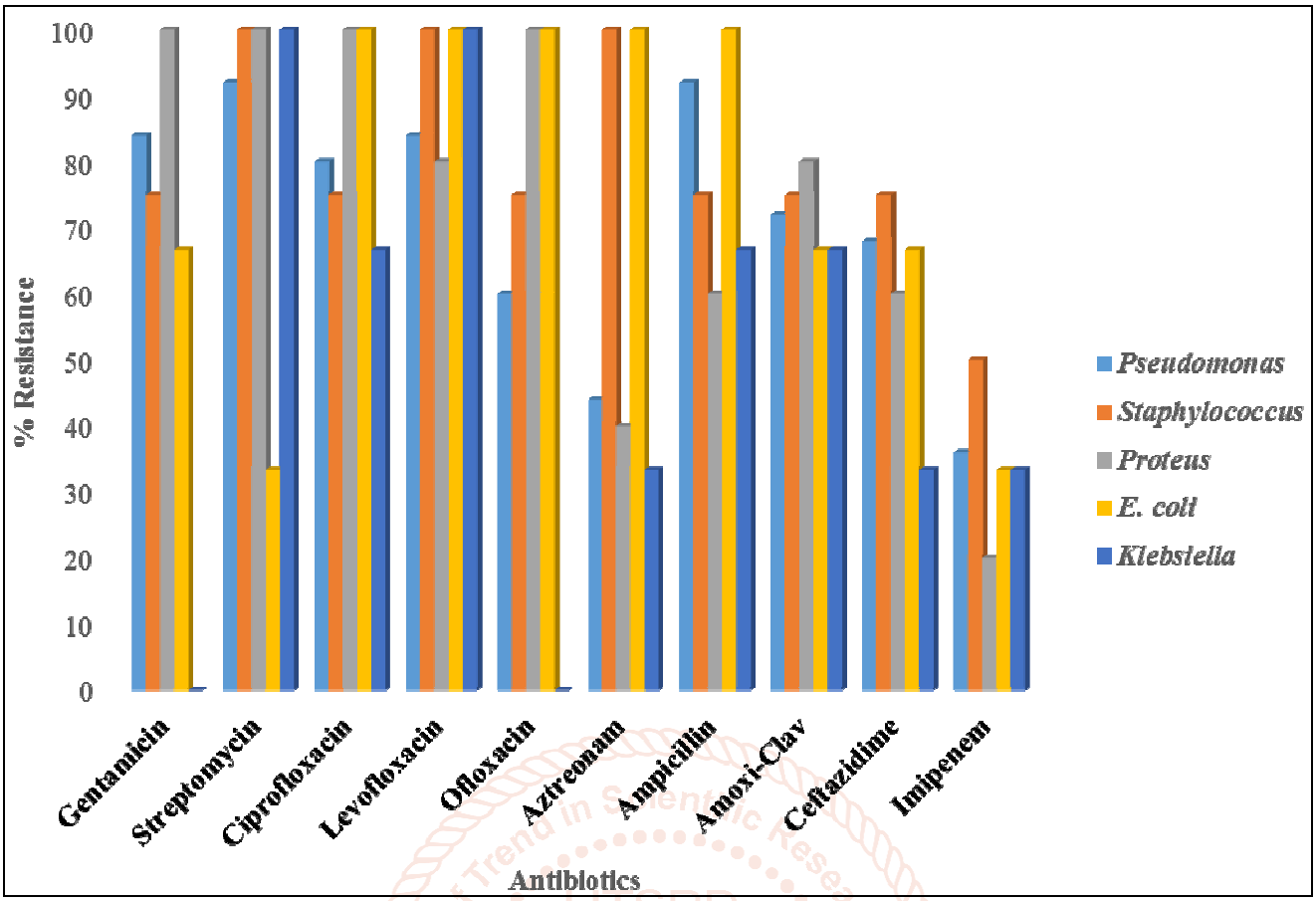

Figure 2: Resistance of each bacteria species to each antibiotics

The Multiplex PCR of the genes indicates that 90.5% of the MDR isolates show positive amplification at the corresponding band size for Intl 1 gene representing Class 1 Integron (plate 1) while 33.33% show positive amplification at the corresponding band size for Intl 11 gene representing class 11 Integron (plate 11). Up to 23.8% of the MDR isolates possess both Intl 1 and Intl 11 genes for both Integron 1 and Integron 11.

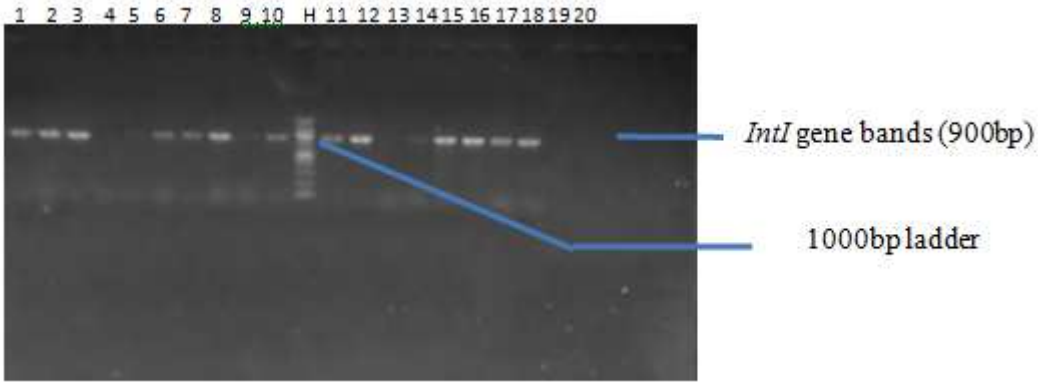

Plate 1: Image of Gel Electrophoresis of Integron I gene (900bp) of the bacteria isolates. Lane H represents a 1000bp ladder (Molecular weight marker).

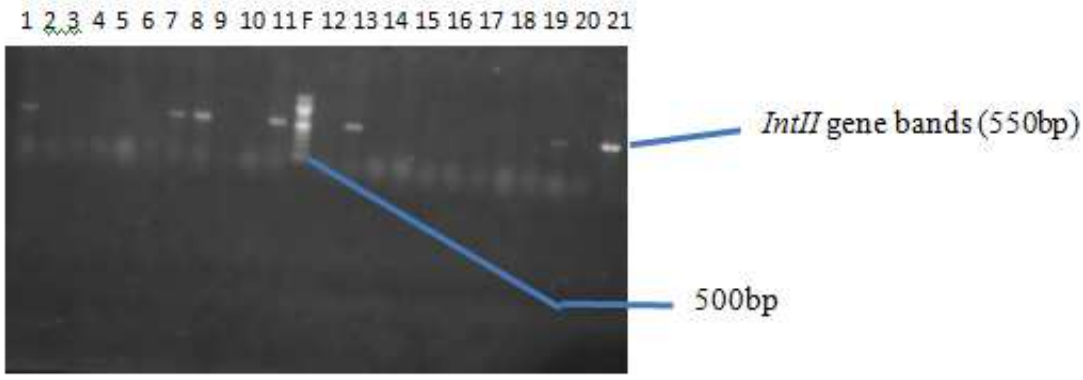

Plate 2: Image of Gel Electrophoresis of Integron II gene (550bp) of the bacteria isolates. Lane F represents a 500bp DNA ladder (molecular weight marker).

DISCUSSION

All the wound swab specimens yielded single bacterial isolates (100%). Similar studies reported varying high level of infection of wounds. Farrag et al [15] reported 82% while Pondei et al [16] reported 86.13% and Kassam et al [17] reported 91.4%. However, the little differences in level of contamination of wounds could be attributed to differences in level of hygiene, socioeconomic status, nutritional styles and presence of comorbidities. The most prevalent isolate was *Pseudomonas aeruginosa* (44.2%) followed by *Staphylococcus aureus* (21.9%), *Enterococcus faecalis* (8.3%), *Klebsiella aerogenes* (8.3%), *Escherichia coli* (8.1%), ete., in that order. Pondei et al [16] and Enwuru et al [6] reported same *Pseudomonas* sp as the most prevalent isolate whereas Kassam et al [17] Ayub et al [5] and Mohammed et al [18] reported *Staphylococcus aureus* as the most prevalent isolate. Sule et al [19] on the other hand reported *Klebsiella* sp. as the most prevalent isolate from obstetrics and Gynaecology wounds and *Pseudomonas aeruginosa* as the most prevalent in orthopaedic wounds in western Nigeria. In all these, even though the prevalent rates of the isolates vary from one study to the other, it is evident that the aerobic aetiologic agents of wound infections are similar. The bacteria so implicated such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* etc. are ubiquitous and very readily contaminates and infects wounds.

The antibiotic susceptibility profile reveals that only 11.7% of the isolates were Multidrug resistant (MDR). This implies resistance to at least one antibiotic in at least 3 different classes of antibiotics [14]. This finding contrasts several other studies that have reported higher MDR rates. Godebo et al [20] reported 77% and 59.3% MDR in Gram positive and Gram negative isolates respectively. Mohammed et al [18] and Enwuru et al [6] reported 95.5% and 64% MDR rates respectively. Patients wounds may become infected directly with organisms possessing drug resistance genes already or organisms that are induced to acquire resistance while in the wounds owing to antibiotic abuse such as self-medication, under dosing, indiscriminate or unwarranted use [21][22][23]. These activities unleash antibiotic pressure which help select for MDR bacteria, thus making treatment of chronic wounds a nightmare. Among the MDR isolates, the highest resistance was noted with the aminoglycoside Streptomycin (88.89%) followed closely by Ampicillin (Penicillin) and Levofloxacin (Fluoroquinolone) at 88.10% each. Ciprofloxacin and Gentamicin equally attracted high resistance of 83.3% and 80.9% respective whereas the less commonly used antibiotics, Aztreonam (a Monobactam) and Imipenem, (a Carbapenem) attracted less resistance of 50% and 35.71% respectively. This pattern of resistance among the MDR isolates goes to show that the less prescribed antibiotics, hence less used and abused Monobactams, Carbapenems and Cephalosporins attract less resistance [24][25][26]. This is because frequent exposure to a particular antimicrobial agent helps to select for resistance to that particular agent [27][28].

Up to 90.5% of the entire MDR isolates possess Intl 1 gene which codes for Class 1 Integron and 33.3% possess Intl 11 gene for Class 11 Integron, while 23.8% possess both Intl 1 and Intl 11 genes which implies presence of both Class 1 Integron and Class 11 Integron in the same isolate. Previous studies have associated integron structures with resistance to some antibiotics in addition to co-presence of several other genes and mobile elements such as plasmids that play significant roles in antibiotic resistance [10], especially to beta-lactam antibiotics [29] [30]. These genes are efficiently shared through horizontal gene transfer among several species of bacteria, rendering commonly used antibiotics ineffective. The scenario is worsened by the capacity to recruit different arrays of drug resistance genes in a single bacteria and effectively transfer same to other bacteria that do not possess them [7].

Our result shows that the integron- bearing isolates were resistant to fluoroquinolones (represented by Ciprofloxacin, Ofloxacin and Levofloxacin), Aminoglycosides (represented by Gentamicin and Streptomycin), Cephalosporins (represented by Ceftazidime), Carbapenems (represented by Imipenem), Monobactams (represented by Aztreonam) and penicillins (represented by Ampicillin). This finding corroborates previous reports that variously detected drug resistance genes to these different classes of antibiotics located in integrons such as aminoglycoside modifying enzymes, beta-lactamases, carbapenemases etc [8] [31]. The vigorous spread of mobile genetic elements such as integrons in virtually all bacteria including enterobacteriaceae, *Pseudomonas* sp and some Gram positive isolates such as *Staphylococcus aureus* is reportedly aided by antibiotic pressure occasioned by misuse and over use of antibiotics [32] [33]. Specifically, these genetic platforms (integrons) have been associated with resistance to specific antibiotics such as beta- lactam antibiotics [34] [35] for instance, VIM (Verona- Integron encoded Metallo- beta-lactamase), tetracycline and sulphamethoxazole-trimethoprim (*tetA, TetB*), quinolones (*qnrB*) etc. [10]. The unmitigated spread of integrons harbouring these array of multidrug resistance genes portends

serious danger as this will result to more therapeutic failures while treating bacterial infections of not only wound but of all other parts of the body. The ease with which these genetic platforms are transmitted through horizontal transfer (by conjugation and transformation) is indeed worrisome [34].

CONCLUSION

This study reports high prevalence of *Pseudomonas aeruginosa* (44.7%) followed by *Staphylococcus aureus* (22.22%) in wound swabs with 11.7% multidrug resistance among all isolates.

The study also reports 90.5% prevalence of Intl 1 gene representing Class 1 Integron, 33.33% prevalence of Intl 11 gene representing Class 11 Integron and 23.8% prevalence of Intl 1 and Intl 11 genes for both Class 1 Integron and Class 11 Integron in the same isolate. The 16S rRNA sequencing more properly identifies the bacteria isolates compared to conventional biochemical tests.

RECOMMENDATIONS

Microorganisms causing wound infections carry drug resistance gene cassettes making it difficult to adequately treat wound infections leading to poor therapeutic outcomes. It is therefore recommended that stringent infection control measures be adopted to prevent spread of these organisms. Also rational antibiotic policy is recommended to avoid selection of drug resistance under antibiotic pressure.

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