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Research Article

Antibiogram and Molecular Characterization of Bacteria isolated from Wound Specimens from a Teaching Hospital in Nigeria

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ABSTRACT

Wound infection remains a common and widespread problem contributing to significant morbidity and mortality. It prolongs hospital stay and increases the cost of hospitalization. Morbidity and mortality is partly attributed to increase in infections due to antimicrobial resistant bacteria which make the choice of empirical therapy more difficult. This study evaluates the antibiogram and molecular characterization of bacteria isolated from wound specimens. One hundred and twenty (120) wound specimens from 70 females and 50 males attending clinic in Rivers State University Teaching Hospital, Port Harcourt were collected with sterile swab sticks, for a period of six (6) months. Specimens were aseptically transported to the Department of Microbiology Laboratory, Rivers State University for bacteriological analyses using standard microbiological techniques and molecular approaches. Antimicrobial susceptibility testing was performed using the disc diffusion method, following the Clinical and Laboratory Standards Institute Guidelines. Bacterial species identified from the specimens were: *Staphylococcus aureus* (L37597.1), *Bacillus cereus* (CM000715.1), *Pseudomonas aeruginosa* (CP000438.1) and *Escherichia coli* (LC654898.1). Multiple antibiotic resistant bacteria were *Pseudomonas aeruginosa* recording 26% and *Escherichia coli* 18% being gram-negatives and *Staphylococcus aureus* had 39% and *Bacillus cereus,* 17%, being gram-positives. Gram-negatives were resistant to Cloxacillin, Ceftazidime, Cefuroxime, Augmentin and Gentamycin. While Gram positives were resistance to Augmentin, Ceftazidime, Gentamycin Erythromycin, Cloxacillin, and Cefuroxime. All the bacteria isolated recorded multiple antibiotic-resistant (MAR) index > 0.2 which indicates high level of drug abuse among the patients. Results of antimicrobial susceptibility testing identified a high prevalence of resistance among the isolates, particularly to commonly used antibiotics like Penicillin, Erythromycin, and Augmentin. These findings highlight the need for regular monitoring and surveillance of antimicrobial resistance patterns to guide empirical treatment strategies and infection control measures in wound management at Rivers State University Teaching Hospital.

Keyword: Wounds, antibiograms, *Pseudomonas aeruginosa*, *Bacillus cereus*, molecular, characterization, teaching hospital.

Introduction

Wound is the disruption in the continuity of soft parts of the body structures. Development of wound infection depends on the many factors including preexisting illness, length of operation, wound class and contamination (Akubuenyi *et al.,* 2011). Wound can be infected by a variety of microorganisms ranging from bacteria to fungi and parasites. The common organisms that have been associated with wound infection include: *Staphylococcus aureus* which from various studies have been found to account for 20-40%. Infections with *Pseudomonas aeruginosa* mainly following surgery and burns account for 5 -15%.

Other pathogens such as Enterococci, *Escherichia coli*, *Klebsiella* species and *Proteus* species have been implicated especially in immunocompromised patients and following abdominal surgery (Akon *et al.,* 2013).

The skin represents a defense barrier against the colonization of pathogens. Therefore, the disruption of the normal anatomical structure by surgical operations or by chemical, physical, mechanical and thermal events, with an alteration of skin functions, results in a wound (Ching *et al.,* 2018). Skin is exposed to injuries, scratches and it is in contact with the external environment, thus it is more susceptible to colonization by pathogens.

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Wounds are divided into two categories: acute and chronic. Acute wounds, like cuts, burns, abrasions and surgical wounds heal through the regular phases of wound repair and they are caused by external factors (Wolcott *et al.,* 2004).

The risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected (Sahu *et al.,* 2011). Hsiao *et al.,* (2011) observed that *Staphylococcus aureus* accounts for 20-40% of hospital -acquired wound infection. While Sahu *et al. (*2011) reported that *Pseudomonas aeruginosa* accounts for 5 -15% of nosocomial wound infections. Other pathogens associated with nosocomial wound infections include *Escherichia coli, Staphylococcus, Klebsiella, Pseudomonas* and *Proteus* species (Giacometti *et al.,* 2000). Nosocomial wound infection tends to be associated with bacteremia and septicemia, shock and prolonged hospital stay in some patients.

A myriad of substances such as antibiotics, synthetic drugs are used in the hospitals for treatment (Akubuenyi *et al.,* 2011), in addition to formulated drugs. Ruseel, (2001), is of the opinion that acquired resistance to antibiotics may arise by cellular mutation or by acquisition of genetic elements in the form of plasmids. According to Akubuenyi *et al.* (2011), the occurrence of strongly selective environments such as hospitals, promotes not only the growth of resistant bacteria but also leads to an increase in the frequency of resistance bacterial genes and genetic elements such as plasmids.

The resistance of the hospital strains of *S. aureus* to methicillin remains a global problem so the control of wound infections has become more challenging. As a result of indiscriminate use of antimicrobial agents, significant changes occur in microbial genetic ecology. So spread of antimicrobial resistance is now a global problem (Ching *et al.,* 2018).

An infected wound affects the quality of life and compromises the wound's healing rate. Wound infections are associated with morbidity and mortality in patients, especially in developing countries, regardless of the type of wound (Akubuenyi *et al.,* 2011). Failure in the treatment implies an increase in the healthcare costs, since they involve a prolonged hospitalization due to diagnostic tests, a huge administration of antibiotics and sometimes, invasive surgery (Boucher *et al.,* 2009).

In particular, the detection of the different microbial species colonizing a wound, as well as their susceptibility to the antimicrobials, can provide an indication for a more appropriate therapy to be administered to patients, significantly reducing the health care costs. On the other hand, chronic wounds, like arterial or leg ulcers, take a longer time to heal and they are caused by internal factors that can be associated with diseases like diabetes or immune deficiency diseases. Therefore, this study was aimed to determine the antibiogram and molecularly characterize the bacterial isolates associated with Wound Specimens in Rivers State University Teaching Hospital, Port Harcourt.

Materials and Methods

Description of Study area

This study was carried out in the Rivers State University Teaching Hospital, Port Harcourt. Rivers State University Teaching Hospital formerly known as Braithwaite Memorial Specialist Hospital (BMSH), is a Government owned Hospital, named after Eldred Curwen Braithwaite, a British doctor and a pioneer of surgery. It is located in Old GRA, Port Harcourt and is operated by Rivers State Hospital Management Board. It was established in March, 1925 as Braithwaite Memorial Hospital and originally served as a Medical Facility for Senior Civil Servants. It later became a General Hospital and has since gained status as a "Specialist Health Institution". In 2018, it was renamed to serve as a Teaching Hospital for Rivers State University, a State owned University following the establishment of College of Medical Sciences.

Study Population and Sample Collection

A total of one hundred and twenty (120) patients of different ages both male (50) and female (70) with wound infections that attended clinic of the Rivers State University Teaching Hospital, Port Harcourt and consented to the study were included for this study. Wound specimens were collected with the aid of sterile swab sticks from the patients. Specimens were collected twice weekly (Mondays and Thursdays) over a period of six (6) months (March to August) in the year 2022 and immediately transported aseptically in ice packs to the Department of Microbiology Laboratory, Rivers State University, Port Harcourt for bacteriological analyses.

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Bacteriological Examination

Cultivation and isolation of bacteria in wound specimens

Standard microbiological procedures according to Cheesbrough (2006) were adopted for the microbiological examination of the wound swabs. The following media were used; Nutrient agar was used for total heterotrophic bacteria, Cetrimide agar for *Pseudomonas* sp., Mannitol salt agar for Staphylococci and Eosin methylene blue agar for *Escherichia coli*. The swab sticks containing the specimens were streaked on the respective culture media and incubated at 37^{o} C for 24hours except for Eosin methylene blue agar that was incubated at 44.5^{o} C and 28^{o} C for 24 hours. After incubation, pure isolates were obtained by picking (with sterile inoculating loop) distinct culturally and morphologically different colonies from the various plates. These were subjected to streaking on sterile nutrient agar plates to obtain pure distinct colonies of the isolates (Cheesbrough, 2006).

Identification of Bacterial Isolates by Cultural and Molecular Methods

Pure bacterial isolates were identified by the method as described by Collins *et al*. (1989) and Cheesbrough (2006). Pure bacterial isolates were subjected to Biochemical tests which include oxidase test, Catalase test, Indole test, methyl red test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate test, Sugars fermentation test and Triple sugar iron agar test. Bacterial isolates were identified with reference to the Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The pure bacterial isolates were further identified to species level using molecular approaches. First step was the DNA extraction, DNA quantification, amplification, sequencing, phylogenic analysis (Wilson *et al*., 1990)

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility patterns of isolated bacterial were performed by Kirby-Bauer disc diffusion method according to the Guidelines of the Clinical and Laboratory Standards Institute (2011).

Eight (8) different antibiotics viz;- Augmentin (30µg), Ceftriaxone (30µg), Gentamycine (10 µg), Cefuroxine 30ug (5µg), Ofloxacin (5µg), Erythromycin (5µg), Cloxacillin (30µg) and Ceptazidine (30µg) were used.

The density of suspension to be inoculated was determined by comparison with the opacity standard on McFarland 0.5 Barium sulphate. Sterile swab was dipped into the suspension of the isolate in the peptone water, squeezed free from excess fluid against the side of bottle and spread over the Mueller-Hinton agar plates. Sensitivity discs for appropriate drugs were placed onto the media and incubated at 37°C for 24hours. After 24 hours each plate was examined and growth zones were measured to the nearest millimeter, using sliding caliper which was held at the back of the inverted media plate. Results were interpreted according to CLSI guidelines (CLSI, 2011).

Multiple antibiotic resistant (MAR) Index

Multiple antibiotic resistant index of the various bacterial isolates were calculated using the formula below:

$$MAR\ INDEX = \frac{a}{b} \quad \text{..... Equation 1}$$

Where 'a' = the number of antibiotics that the isolated bacteria were resistant to. 'b' = the total number of antibiotics tested (Osunduya, 2000).

Statistical analysis

Statistical analyses were carried out on the data generated during the study. Analysis of Variance and Duncan Multiple Ranged Test were used to test for significance and means separation respectively.

Results

The results of the demographic and clinical characteristics of the patients are presented in Figures 1- 4. Majority of the study populations were females 70 (58.33%) while male population was 50 (41.67%). The age range with the highest prevalence of wound infection was between the ages of 21-26 years while the least prevalence with wound infection was in the age range of 33 – 38 years.

Results of the sources of wound infections showed that accident and emergency had the highest prevalence (50%) while surgical wounds had the lowest prevalence (20%). Results showed that 91.6% of the patients had records of one month exposure to antibiotics while 9.4% were not exposed. Moreover, 62.5% of the patients had 6 months exposure to antibiotics while 37.5% have not been exposed.

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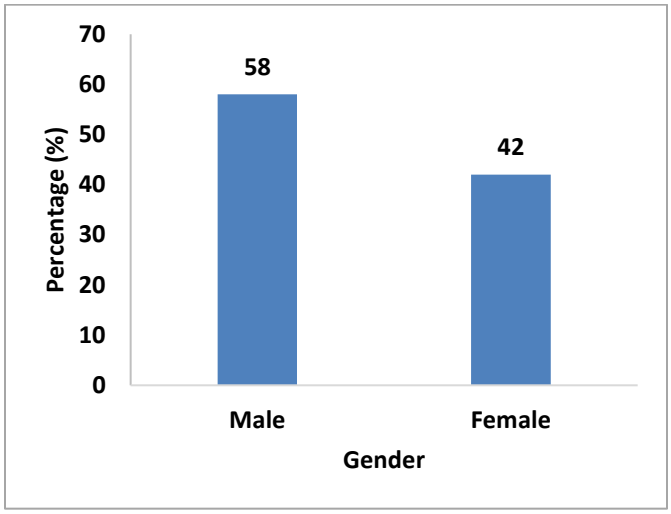

Fig. 1: Gender of the Patients with wound infection

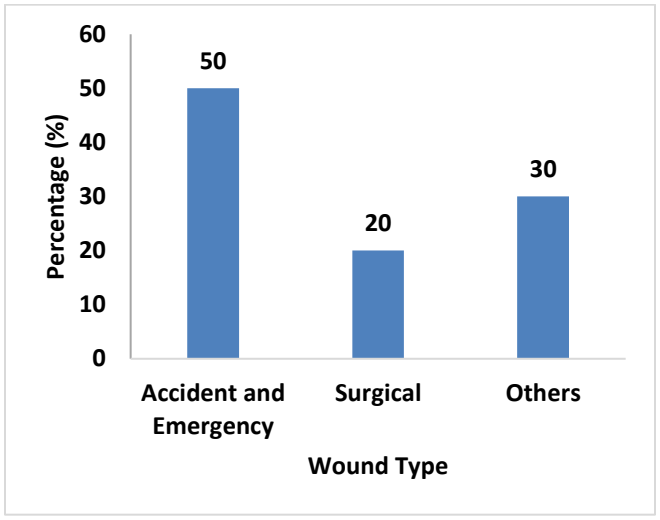

Fig. 3: Prevalence of sources of wound in patients

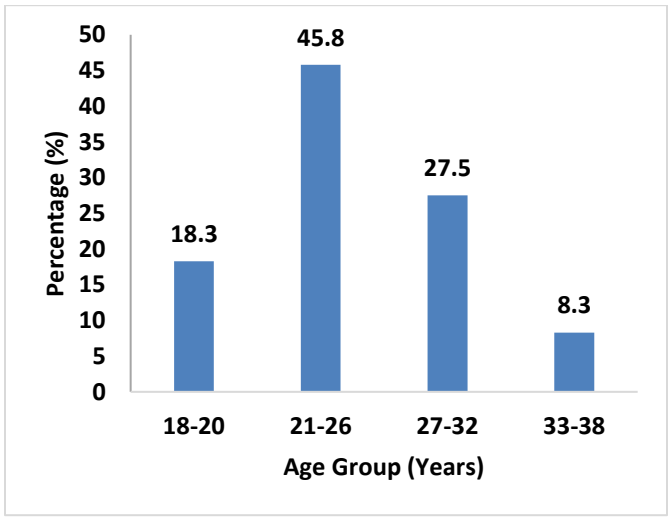

Fig. 2: Age group of patients with wound infection in the Teaching Hospital

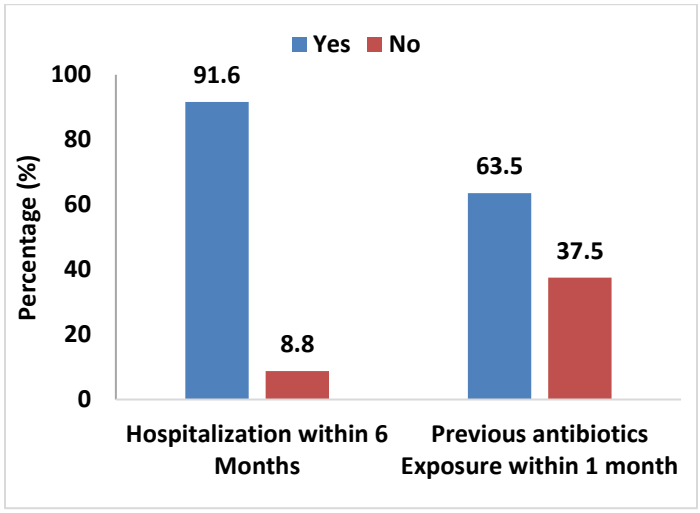

Fig. 4: Exposure of patients with wound infection to antibiotic and hospitalization

The bacteria that were isolated from the wound specimens of patients of the Rivers State University Teaching Hospital during the period of this study were identified as; *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli*.

The result of the antibiotic sensitivity profile of the bacterial isolated from the wound specimens from the Teaching hospital is presented in Table 1.

Results revealed that out of the eight antibiotics used, all the Gram positive isolates were 100% susceptible to only Ceftriaxone and Ofloxacin. *Pseudomonas aeruginosa* was 100% susceptible to Ofloxacin and Nitrofurantoin and 100% resistant to Cloxacillin. *E. coli* was 100% susceptible to Ofloxacin, Ceftriaxone and Nitrofurantoin and 100% resistant Ceftazidine, Cefuroxine and Cloxacillin.

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Table 1: Antibiotic Sensitivity Patterns of Bacteria Isolated from wound of patients in the Teaching Hospital

Antibiotics	***Pseudomonas* sp. (N=56)**			***E. coli* (N=64)**			***S. aureus* (n=32)**			***Bacillus* sp. (n=88)**		
	S	**I**	**R**	**S**	**I**	**R**	**R**	**I**	**S**	**R**	**I**	**S**
Ceftazidime (30µg)	0(0)	18(32.1)	38(67.9)	0(0)	0(0)	64(100)	32(100)	0(0)	0(0)	88 (100)	0(0)	0(0)
Cefuroxime (30µg)	16(28.6)	8(14.3)	32(57.1)	0(0)	0(0)	64(100)	32(100)	0(0)	0(0)	88 (100)	0(0)	0(0)
Gentamicin (10µg)	16(28.6)	10(17.8)	30(53.6)	4(6.3)	22(34.4)	36(56.3)	0(0)	8(25)	24(75)	88 (100)	0(0)	0(0)
Cloxacillin (5µg)	0(0)	0(0)	56(100)	0(0)	0(0)	64(100)	32(100)	0(0)	0(0)	27(30.7)	14(15.9)	47(53.4)
Ofloxacin (5µg)	56(100)	0(0)	0(0)	64(100)	0(0)	0(0)	0(0)	0(0)	32(100)	60(68.2)	28(31.8)	0(0)
Augmentin (30µg)	6(10.7)	10(17.8)	40(71.4)	10(15.6)	10(15.6)	46(71.9)	32(100)	0(0)	0(0)	60(68.2)	13(15)	15(17)
Ceftriaxone (30µg)	46(82)	5(8.9)	5(8.9)	64(100)	0(0)	0(0)	32(100)	0(0)	0(0)	60(68.2)	0(0)	28(31.2)
Nitrofurantoin (30µg)	56(100)	0(0)	0(0)	64(100)	0(0)	0(0)	ND	ND	ND	ND	ND	ND
Erythromycin (5µg)	ND	ND	ND	ND	ND	ND	8(25)	0(0)	24(75)	0(0)	0(0)	88 (100)

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Table 2: Multiple Antibiotics Resistant (MAR) Index of bacteria isolated from wound specimens

MAR Index	Bacteria isolated from wound specimens			
	E. coli **(n=64)**	***S. aureus*** **(n=32)**	***Bacillus*** **sp. (n=88)**	***Pseudomonas*** **sp (n=56)**
0.1	0(0)	0(0)	0(0)	0(0)
0.2	0(0)	0(0)	0(0)	0(0)
0.3	0(0)	12(37.5)	15(17.0)	12(3.6)
0.4	21(32.8)	8(25)	25(24.4)	19(33.9)
0.5	33(51.6)	10(31.3)	48(54.5)	30(53.5)
0.6	10(15)	2(15.6)	0(0)	5(8.9)

Note: MAR > 0.2= Multiple antibiotic resistant

Figure 7 shows the Phylogenetic tree of the evolutionary distance between the bacteria isolated from wounds of patients of the Rivers State University Teaching Hospital. The phylogenic tree obtained from 16S rRNA sequence from the isolates gave a similar match during the megablast search for similar sequence shows that the isolates identified showed a 100% similarity in their 16S rRNA to their other neighbours in the gene bank.

The calculated values obtained using the Jukes-Cantor method were consistent with the positioning of the 16S rRNA of the isolates within the *Bacillus* sp. showed a close relationship to *Bacillus cereus*(CM000715.1), *Pseudomonas* sp., with *Pseudomonas aeruginosa (*CP000438.1), *Staphylococcus aureus* with *Staphylococus aureus* (L37597.1) and *Escherichia coli* (LC654898.1), all at 100% (Fig. 7).

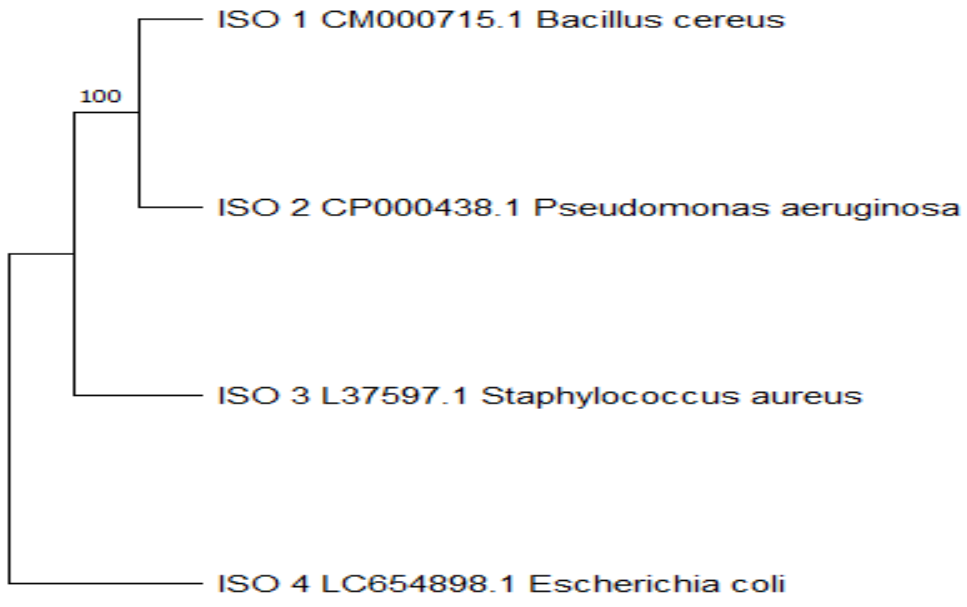

Fig 7: Phylogenetic tree of the evolutionary distance between the wound Bacterial Isolates

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Discussion

Demographic and clinical characteristics of patients were directly obtained using a structured questionnaire in order to avoid incomplete information. The data obtained showed that 58% of the patients were female and 42% male while their ages ranged from 10 to 80years. Fifty percent (50%) of the patients were in accident and emergency unity, 20% from surgical unit and 30% from other units (Figures 1-4). Investigation carried out in this study also revealed that 91.6% of the patients had had, previous exposed to antibiotics within one month while 62.5% were previously hospitalized within the last six months. These observations are capable of influencing the diversity of bacterial population in the wounds as well as antibiotic sensitivity profile of the bacteria.

The bacteria isolated in this study are presented in the phylogenic tree, which shows the diagrammatic representation of the evolutionary relationships among different species. It is a visual tool that shows how different species are related to each other through common ancestors (Figure 7). These isolates, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli* are the same with those isolated by Giacometti *et al.* (2000), whom worked on Epidemiology and Microbiology of Surgical wound infections. The organisms identified from the wounds in this study is in line with previous studies reported by Mawalla *et al.* (2011) where *S. aureus* was the most common wound infection bacteria. *Staphylococcus aureus* is a type of bacteria commonly found on the skin and mucous membranes of humans. While it is usually harmless in these locations, it can cause infections when it enters wounds or breaks in the skin. *Staphylococcus aureus* can impair the natural wound healing process by causing inflammation and prolonging the recovery (Mawalla *et al.*, 2011). It can lead to the formation of chronic wounds that take longer time to heal. *Staphylococcus aureus* wound infections can cause cellulitis, which is a bacterial skin infection characterized by redness, warmth, swelling, and pain in the affected area (Mawalla *et al.*, 2011). Cellulitis can spread rapidly and may require antibiotic treatment (Mawalla *et al.*, 2011). *Staphylococcus aureus* can also lead to the formation of abscesses, which are localized pockets of pus within the wound. Abscesses may necessitate drainage and antibiotic therapy.

In severe cases, *Staphylococcus aureus* can enter the bloodstream from a wound, causing a condition known as wound sepsis. This can lead to systemic symptoms such as fever, chills, low blood pressure, and organ dysfunction Dryden *et al.* (2009).

Pseudomonas aeruginosa was also identified from this study. It is a gram-negative bacterium commonly associated with opportunistic infections, including wound infections. Here are some key implications of *Pseudomonas aeruginosa* in wound infections: delayed wound healing, biofilm formation which can enhance their resistant to antibiotics and increased risk of complications (Akon *et al.*, 2013). *Pseudomonas aeruginosa* is often associated with healthcare-associated infections, especially in hospital settings. It can be transmitted through contaminated medical equipment, unclean wound dressings, or inadequately sterilized surgical instruments (Ching *et al.*, 2018).

Bacillus cereus is identified in this study too, which is a gram-positive, spore-forming bacterium that is commonly found in the environment, including soil, water, and food. While it is generally considered an opportunistic pathogen that primarily causes foodborne illnesses, it can also be associated with wound infections under certain circumstances (Kramer *et al.*, 1989). Here are some implications of *Bacillus cereus* in wound infections: *Bacillus cereus* can infect wounds, especially those resulting from trauma, burns, surgery, or intravenous drug use according to Estahbanati *et al.* (2002). These infections are typically associated with contaminated soil, dust, or other environmental sources (Dhar *et al.*, 2007). Individuals with compromised immune systems, such as those with diabetes, cancer, or chronic diseases, as well as those on immunosuppressive medications, are at a higher risk of developing *Bacillus cereus* wound infections (Ching *et al.*, 2018).

Escherichia coli (*E. coli*) observed in this research, is a type of bacteria that is commonly found in the human intestines. While most strains of *E. coli* are harmless and even beneficial to humans, certain strains can cause infections, including wound infections (Lautenbach *et al.*, 2001). Here are some implications of *E. coli* in wound infections: When *E. coli* enters a wound, it can grow and multiply, leading to infection. Wound infections can cause pain, redness, swelling, discharge, and may impede the healing process (Estahbanati *et al.*, 2002).

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Antibiotic sensitivity profile of *Staphylococcus aureus* and *Bacillus cereus* revealed 100% resistant to Cloxacillin, Ceftazidine and Cefuroxime, and 100% susceptibility to ofloxacin (Figures 5 and 6). While antibiotic sensitivity profile of *Pseudomonas aeruginosa*, and *Escherichia coli* shown 100% resistant to Cloxacillin, Cloxacillin, Ceftazidime and 100% susceptibility to ofloxacin Ceftriazone and Nitrofurantoin (Tables 1 and 2).The high percentage susceptibility of the isolates to Ofloxacin Ceftriazone and Nitrofurantoin is in consonance with a study by Ogbonna and Inana, (2018). This study recorded high resistance to Cloxacillin, Ceftazidine, Cefuroxime, Augmentin and Gentamycin. Drug resistance could lead to the emergence of resistant bacteria that may be transferred to consumers, leading to difficulty to treat infections (Onuoha, 2018), which may in turn lead to increased cost of treatment, mortality and morbidity rates (Igbinosa and Obuekwe, 2014).

It has been suggested that resistance to multiple antibiotics coded for by genes could be carried on plasmid DNA and in other chromosomal DNA. It is established that antibiotics pressured resistant strains and eliminates sensitive strains. The more antibiotics used, the more the elimination of the sensitive strains thereby allowing resistant strains to dominate. It is also true that resistant strains are outcompeted by sensitive strains when antibiotic pressure is removed from the environment (Ogbonna and Azuonwu, 2019).

Multiple Antibiotics Resistant Index of the various bacterial isolates revealed that all isolates, both Gram negative and Gram Positive recorded MAR index greater than 0.2. MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to. A MAR index greater than 0.2 means high risk source of contamination is where antibiotics are frequently used which is a clear indication of drugs abuse among the patients (Akualing *et al.*, 2018).

In Conclusion, the antibiogram and molecular characterization of the bacteria isolated from wound specimens from the Rivers State University Teaching Hospital identified the following isolates: *Staphylococcus aureus* (L37597.1), *Bacillus cereus* (CM000715.1), *Pseudomonas aeruginosa* (CP000438.1) and *Escherichia coli* (LC654898.1) through molecular characterization.

The antibiogram revealed the resistance profiles of the *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli* isolated. This study recorded resistance of *Pseudomonas aeruginosa* and *Escherichia coli* to Cloxacillin, Ceftazidine, Cefuroxime, Augmentin and Gentamycin. *Staphylococcus aureus* and *Bacillus cereus* recorded resistance to Augmentin, Ceftazidime, Gentamincin, Erythromycin, Cloxacillin, and Cefuroxin. This information is crucial for guiding appropriate antibiotic therapy for wound infections.

The antibiogram analyses indicated the prevalence of multidrug-resistant bacteria in wound infections and this would help in monitoring the emergence and spread of antibiotic resistance in hospital settings. It aids in identifying the specific organisms responsible for wound infections and enables targeted treatment strategies. The findings from both antibiogram and molecular characterization may have important implications for treatment decisions and infection control practices in the hospital. It highlights the need for judicious use of antibiotics and the development of new treatment options to combat antibiotic-resistant bacteria.

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