International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 4 issue 3 Sept, 2025 Research Article

Antibiotic Resistance Pattern of Bacteria Associated With Wounds from Patients in a Tertiary Health Institution in Port Harcourt

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ABSTRACT
Wound infections remain a significant global healthcare challenge, which contributes to delays in wound healing, increase in morbidity, and rising costs. Hence, this study aimed to evaluate the antibiotic resistance pattern of bacteria associated with wounds from patients in a tertiary health institution in Port Harcourt. A total number of one hundred (100) wound specimens from 45 male and 55 female patients were collected with sterile swab sticks, for a period of 6 months at the University of Port Harcourt Teaching hospital and transported aseptically in ice packs for microbiological analysis in the Department of Microbiology Laboratory, Rivers State University. The bacteria contaminants were isolated using standard bacteriological procedures. The response of the isolates to conventional and crude honey was analyzed using the Kirby-Bauer disc diffusion method. Molecular characterization was achieved using a PCR-dependent technique. The age range of 41-50years had the highest number of population (32 %) while 51-60 and 61-70 years had the lowest number (5 %.). *Staphylococcus* spp had the highest prevalence as it occurred in 85% of the wound cases, while *Pseudomonas* was the least prevalent with 39%. Results of biofilm production showed that, 62% of the isolates produced biofilm, while 38% were negative. The results for honey indicated that it was inhibitory against 69% of the isolates, with no potency observed against 31% of the isolates used in the study. The sensitivity pattern of each isolate showed that *Staphylococcus* spp was resistant to Azithromycin, for Gram negative bacterial isolates, *Pseudomonanas* spp had the highest resistance to ciprofloxican and *Klebsiella* spp was highly resistance to Seprin. Molecular analysis identified the isolates from the specimens as *Staphylococcus aureus* (ON571652.1) *Pseudomonas aeruginosa* (AF440523.1), and *Escherichia coli* (LC595306.1).

Keywords: Wound Infections, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Biofim Antibiotic Resistant Pattern.

Introduction

Wound infections, particularly those caused by antibiotic-resistant bacteria, present significant challenges in clinical settings. Bacteria are one among the most common pathogens isolated from chronic and acute wound infections, and their increasing resistance to antibiotics has made treatment more difficult and costly (Rumbaugh *et al*, 2008). These bacteria are not only difficult to treat but also contribute to longer hospital stays, higher healthcare costs, and increased morbidity and mortality rates (WHO, 2014). In wound infections, pathogenic organisms can form biofilms (complex communities of bacteria that adhere to surfaces and are encased in a protective extracellular matrix). Biofilms make the bacteria more resistant to antibiotics and the host's immune response, complicating treatment and often leading to chronic infection (Hoiby *et al*., 2010). This biofilm formation is particularly problematic in wounds, where it can impede healing and contribute to persistent infection (Otto, 2008).

The isolation of these organisms from wound samples is clinically significant because these bacteria are associated with more severe infections, prolonged healing times, and increased risk of complications. The presence of these pathogens often necessitates aggressive treatment, including the use of broad-spectrum antibiotics, surgical debridement, and, in some cases, advanced wound care techniques such as negative pressure wound therapy (Edwards & Harding, 2004).

Continuous placement of antimicrobial drugs in treating infections has led to the emergence of resistance among the various strains of microorganisms (Flalanga *et al.,* 2000). In addition, several factors including inappropriate infection control practices, overcrowding and use of excessive invasive devices have been snorted to amplify the development of antibiotics resistance among pathogens especially in developing continents including Africa, Asia, and others (Mehrad *et al.,* 2015).

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According to Bansal *et al.,* (2006), multidrug resistance result from the exhibition of resistance to more than one type and class of antibiotics. Not only do these factors influence the development of resistance but also enhance the transmission of these multidrug resistances (MDROs) especially in cases of hospital associated infections (HCAI) (Davis *et al*., 2010).

Among the multidrug-resistant pathogens commonly associated with tertiary hospitals are: multidrug-resistant *Staphylococcus aureus, Acinetobacter* species, P.*auruginosa, E.coli, Serratia marcesens* Proteus species, cephalosporin-resistant *Klebsiella pneumoniae*, methicillin-resistant *Neisseria gonorrhoeae*, and *Rifampicin-resistant Mycobacterium tuberculosis*, among others (Nikaido, 2009; WHO, 2014). Microorganisms have been shown to have the ability to transfer resistant genes (Mbim *et al.,* 2016). The development of resistance by microorganisms in a tertiary hospital has also been attributed to the acquisition of extra-chromosomal elements (plasmids and transposons) (Bansal *et al.,* 2006). Antibiotic resistance in bacteria occurs by the accumulation of resistance (R) plasmids. Plasmids are one of the most common vectors of antibiotic resistance genes in mostly Gram-negative organisms (Collis *et al.,* 2012) or transposons of genes (sequences of DNA that move (or jump) from one location in the genome to another). With each coding for resistance to a specific agent, and by the action of multidrug efflux pumps, each of which can pump out more than one drug type.

Microorganisms have been shown to have the ability to transfer resistant genes vertically via plasmids and also habour multidrug resistant genes (Mbim *et al.,* 2016). Wound infection occurs as a result of a dynamic interaction between the host and the pathogen, such that the sum of the pathogen‘s burden is greater than the host’s immune defense causing a systematic immunological reaction (White, 2009). Wound infection and wound healing are influenced by several factors. Bacterial colonization and the pathogenic potential of colonizing bacterial agents is one of the factors that determine wound healing. However, the increasing resistance of these bacteria to conventional antibiotics poses a significant challenge in clinical management. This has led to a growing interest in alternative therapies, such as the use of antimicrobial agents derived from natural products like honey, which may offer effective treatment options without contributing to the development of antibiotic resistance (Cooper *et al,* 2010).

Materials and Methods

Description of Study Area and location

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), East West Road Port Harcourt, Rivers State, Nigeria. The hospital lies within 4.8998º North and 6.9292º East. It is a major tertiary healthcare and research facility in Rivers State, which consists of various departments for distinct health cases, and a great number of patients from many geographical regions accessing it.

Study Design and Sample Collection

A cross-sectional (prevalence) study design was implemented to determine the incidence of organisms associated with wound infection at the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria. The study samples made up of one hundred (100) wound cases were collected randomly from patients with different wound types in the hospital using sterile swab sticks. The samples were taken aseptically and transported to the Microbiology Laboratory, Rivers State University, for analysis within two hours of collection.

Collection of Honey

The honey used in this study was purchased from Mile 3 market and the trader assured of the authenticity of the product. The product was collected with carefulness under sterile environmental conditions and kept sterile from microbes at ambient temperature prior to use at Microbiology Laboratory, Rivers State University, Port Harcourt.

Sample Size determination

The sample size for the study was determined by the formula:

N = [Z2 (pq)]/d2 [10] Where: N= the desired sample size Z= Normal standard distribution that corresponds to confidence interval as 1.96 p= Prevalence of *Pseudomonas* species

q = 1-p d= degree of accuracy / precision expected at $p \leq 0.05$.

A minimum of 100 samples were used in the study of which forty-five (45) samples were collected from males and fifty-five (55) samples from females.

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Microbiological Examination

Sterilization of Equipment and media

All glass wares used during the course of this research were carefully washed with detergent, rinsed with tap water, allowed to dry and autoclave at 160ºC in hot air oven Media and diluents were sterilized in an autoclave at 121ºC at 15 psi for 15minutes.

Isolation of Bacteria from Wound Specimens

Wound specimens were plated out on sterile Nutrient Agar, MacConkey Agar, Eosin methylene blue Agar (EMB) and Mannitol Salt Agar plates using streak plate method and incubated at 37°C for 24 hours.

Purification and Preservation of Isolates

Discrete colonies on MSA, MA, EMB and NA were further purified on freshly prepared Nutrient Agar (NA) plates by repeated subculturing until pure isolates were obtained. The obtained pure isolates were inoculated aseptically into nutrient agar slants in Bijou bottles and incubated for 24 hours at 37°C and later preserved in the refrigerator at 4°C.

Identification of isolates

Isolates were identified base on cultural, morphological and characterization of standard microbiological procedures described by Cheesbrough (2006).

Biofilm Production Test

To determine biofilm production using the streak plate method, a control petri dish and a test petri dish containing Brain Heart Infusion (BHI) Agar were prepared. The test dish was streaked with the bacterial suspension in a zig-zag motion, allowing the bacteria to grow in a linear pattern. After incubating at 37°C for 24-48 hours, the dishes were examined for biofilm formation, indicated by a visible film along the streak line in the test dish. A positive result indicates biofilm production, while a negative result indicates no biofilm production.

Antimicrobial Susceptibility Test

Antibiotic susceptibility test was performed using disc diffusion method. A sterile swab stick was dipped into the tube containing the bacterial suspension and its turbidity was equivalent to 0.5m McFarland turbidity. The swab stick was pressed against the tube above the fluid level to remove excess broth. The swab was used to streak over the entire plate surface evenly which contained already prepared Mueller Hinton agar in three dimensions rotating the plate about 60ºC each time. The agar plates were allowed to dry for 5 minutes then the antibiotic disk was placed onto the agar using a sterile forceps on the surface of the inoculated plate 15mm away from the edge of the plate. Using the head of the sterile forceps, the disk is slightly pressed down to ensure good contact with the agar. After applying the disk, the plates were incubated in an inverted position for 16 to 18 hours. After incubation, the test plates were examined to ensure confluence growth or near confluence. The diameter of each zone of inhibition was measured in ml using a ruler on the underside of the plate and recorded for reference purpose (CLSI 2011).

Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index was ascertained for each isolates by using the formula,

$$\text{MAR} = \frac{a}{b}$$

Where a is the number of resistant antibiotics denoted and b is the total number of antibiotics to which the test isolate has been evaluated for susceptibility, s (Krumperman, 2005).

Molecular Identification of the pure culture

The pure bacterial isolates were identified to species level using molecular approaches, these include; Bacteria genomic DNA extraction; DNA Quantification; 16s rRNA Amplification. Deoxyribonucleic corrosive (DNA) Quantification Polymerase Chain Reaction (PCR) Amplification of ITS Gene Sequencing Phylogenetic Analysis Amplification of TEM genes and sequencing. The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X were used for all genetic analysis (Wilson *et al.,* 1990).

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.

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Results

The gender distribution of the patients accessing the hospital due to wound-related syndromes had male and female case with 45% and 55% of the patients, respectively. The age distribution pattern is shown in Figure 1 with age range of 41-50 having the highest percentage 3(32%), while 51-60 and 61-70 had the lowest percentage with (5%) each.

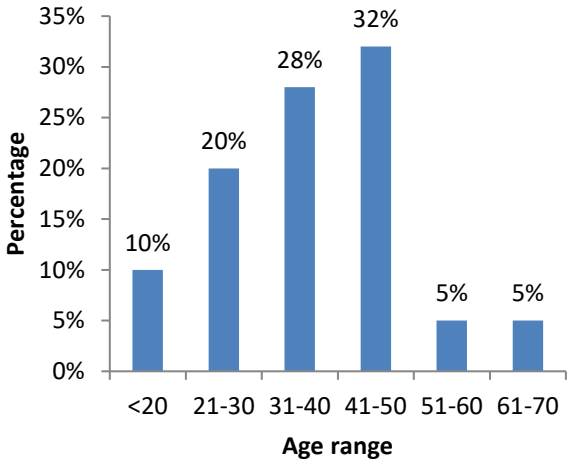

Fig. 1: Age distribution of the patients studied

Data on the types and frequency of wounds in the study is presented in Figure 2.

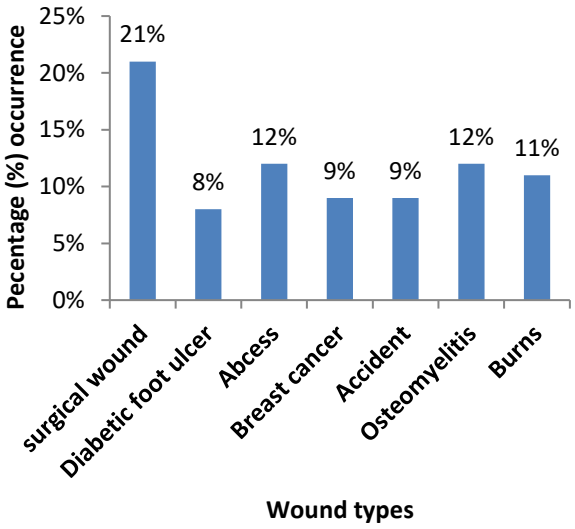

Fig. 2: Types of wound encountered in the study

The surgical wounds and diabetic foot ulcer represented the highest (21%) and lowest (8%), respectively.

The morphological and biochemical characteristics of the bacteria isolated are shown in Table 1. The study encountered *Staphylococcus* species, *Pseudomonas* species, *E. coli* and *Klebsiella* species

The age range distribution of the bacterial species recovered from the wounds is presented in Table 2. It shows that the age bracket 31-40 had the highest Staphylococcal infection with a percentage of 28(100%) while the least Staphylococcal infections was 61-70years with 40%. Also 41-50 had the highest *Escherichia* coli infection, 30(93.7%) while the least *Escherichia c*oli contamination in age bracket was 61-70 with no incidence. The result showed that 51-60 had the highest *Klebsiella* contamination having 5(100%) and the least *Klebsiella* was associated with age range 61-70 with 40%, and 31-40 had the highest *Pseudomonas* contamination , 14(40%) while the least *Pseudomonas* infection was recorded in age group 51-60 and 61-70 with a percentage of 0(0%).

The gender based occurrences of bacteria isolated from the wound swab are presented in Table 3. The results showed that the females had the highest number of Staphylococcal infection with a percentage of 90.9% and the males had the lowest Staphylococcal infection representing 77.7%. The males had the highest number of *Escherichia coli* infection with a percentage of 86.6% and the females had the lowest *Escherichia coli* infection with a percentage of 54.5.7%. The females had the highest number of *Klebsiella* infection with a percentage of 69.0% and the males had the lowest *Klebsiella* incidence of 55.5%. The males also had the highest number of *Pseudomonas* infection with a percentage of 53.3% and the females had the lowest *Pseudomonas* infection with a percentage of 27.3%. The overall prevalence of the bacterial species is recorded in Table 3. The report showed that *Staphylococcus* had the highest prevalence as it occurred in 85% of the wound cases studied, while *Pseudomonas* was the least prevalent bacterial species.

The result of the biofilm forming potentials of the isolates is as shown in Figure 3. The data indicated that 62 percent of the isolates showed in vitro ability to form biofilm, while 38% were negative to biofilm formation.

Response of the isolates to honey presented in the data reported in Figure 4 indicated that honey was inhibitory against 69% of the isolates, with the honey showing no potency against 31% of the isolates used in the study.

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Table 1: Morphological and Biochemical Characteristics of the Bacteria Isolated from different wounds

Isolate code	Colour	Elevation	Opacity	Texture	Shape	GS	CT	OX	MT	CU	MR	VP	GF	LF	MF	XF	CL	SB
SW	Yellow				Cocci	+	+	-	-	+	-	+	A	-	-	-	-	*Staphylococcus capitis*
T	Green	Round	Opague	Smooth	Rod	-	+	+	-	+	-	-	-	-	AG	AG	+	*Pseudomonas sp*
Vw	Clear	Round	Transcucent	Smooth	Rod	-	+	-	+	-	-	-	AG	AG	AG	AG	+	*Klebsiella sp*
Aw	Clear	Round	Opague	Smooth	Rods	-	+	-	+	+	+	+	AG	A	A	AG	-	*Escherichia coli*.

MP: Morphology, GS: Gram stain, CT: Catalase, OX: Oxidase, MT: Motility, IP: Indole production, CU: Citrate utilization, MR: Methyl red, VP: Voges-Proskauer, GF, LF, MF, & XF: Glucose, Lactose, Mannitol, & Xylose fermentation respectively, SH: Starch hydrolysis, CL: Coagulase, SB: Suspected bacteria.

Table 2: Age range distribution of the bacterial species recovered from the wounds

Age Range	Frequency	Percentage (%)	*Staphylococci* No (%)	*E. coli* No (%)	*Klebsiella Spp* No (%)	*Pseudomonas Spp* No (%)
<20	10	10	9(90)	7(70)	4(40)	3(30)
21-30	20	20	17(85)	15(75)	12(60)	9(45)
31-40	28	28	28(100)	16(57.1)	18(64.2)	14(50)
41-50	32	32	26(81.2)	30(93.7)	22(68.7)	13(40.6)
51-60	5	5	3(60)	1(20)	5(100)	0(0)
61-70	5	5	2(40)	0(0)	2(40)	0(0)

Table 3: Gender based distribution of the bacterial isolates in the wound samples

Gender	No (%)	*Staphylococci* No (%)	*E. coli* (No %)	*Klebsiella Spp* (No %)	*Pseudomonas Spp* (No %)
Male	**45(45)**	**35(77.7)**	**39(86.7)**	**25(55.5)**	**24(53.3)**
Female	**55(55)**	**50(90.9)**	**30(54.5)**	**38(69.0)**	**15(27.2)**

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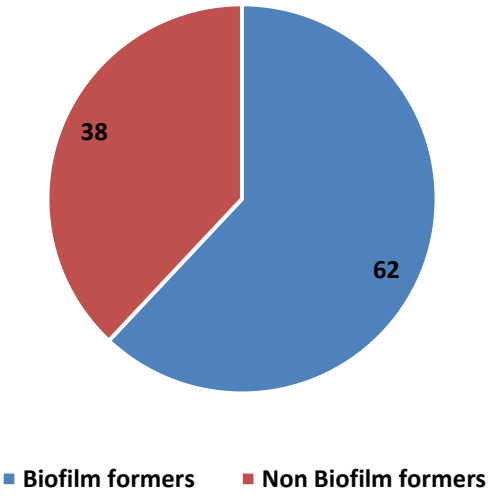

Fig. 3: Occurrence (%) of biofilm producers in the study

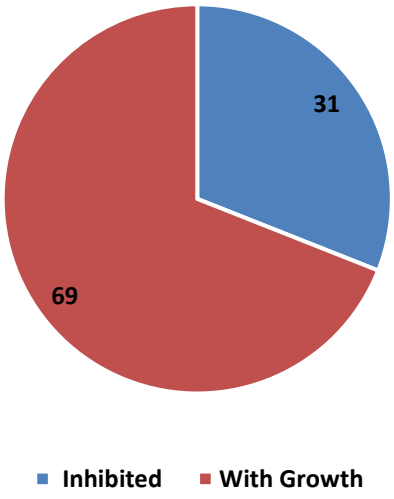

Fig. 4: Growth Inhibitory Effect (%) of honey on the isolates

The antibiotics susceptibility pattern of the isolates is indicated in Tables 4a-d. The study showed that most of the isolates were resistant to conventional antibiotics. The study showed the sensitivity pattern of each of the isolates, and it was observed that for *Staphylococcus* species, 29(89.7%) of the isolates showed, resistance to Azithromycin. For the Gram negative bacterial isolates, *Pseudomonas aeruginosa* had the highest resistance was to Ciprofloxican, as 8 (40%) of the isolates were resistant to the drug (Table 4b) for *Escherichia coli,* the highest resistance was to Ciprofloxican as 11(55%) of the isolates were resistant to the drug and the highest drug resistance for K*lebisella* was seprin as 12(50%) were resistant.

The Multiple antibiotics resistant (MAR) index of bacteria isolated from wounds is presented in Table 5.

Table 4a Antibiotic sensitivity patterns of *Staphylococcus aureus*

Antibiotic	Susceptible	Intermediate	Resistant
Septrin (SXT)	9(31.0)	5(17.3)	15(51.7)
Erythromycin (E)	5(17.2)	3(10.3)	21(72.5)
Pefloxacin (PEF)	3(10.3)	6(20.7)	20(69.0)
Gentamicin(CN)	3(10.3)	1(3.4)	26(86.3)
Ampicillin (APX)	5(17.3)	5(17.3)	19(65.6)
Azithromycin(Z)	2(6.9)	1(3.4)	26(89.7)
Ofloxacin(OFX)	6(20.6)	4(13.8)	19(65.6)
Rifampicin(R)	5(17.3)	5(17.3)	19(65.6)
Ciprofloxacin(CFX)	3(10.3)	1(3.4)	25(86.3)
Streptomycin(S)	3(10.3)	2(6.9)	24(82.8)

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Table 4b: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*

Antibiotic	Antibiotic susceptibility		
	Susceptible	Intermediate	Resistant
Pefloxacin (PEF)	3(15)	4(20)	13(65)
Streptomycin (SP)	7(35)	5(25)	8(40)
Ciprofloxican (CPX)	8(40)	1(5)	11(55)
Amacycilln (AM)	1(5)	3(15)	16(80)
Augmentin (AU)	4(20)	2(10)	14(70)
Cefoxitin(CN)	5(25)	1(5)	14(70)
Reflacine(REF)	4(20)	4(20)	12(60)
Erythromycin (E)	3(15)	2(10)	15(75)
Septrin (SXT)	3(15)	6(30)	11(55)
Streptomycin(S)	4(20)	4(20)	12(60)

Table 4c: Antibiotic sensitivity pattern of *Escherichia coli*

Antibiotic	Antibiotic susceptibility		
	Susceptibility	Intermediate	Resistant
Pefloxacin (PEF)	5(18.5)	5(18.5)	17(63)
Streptomycin (SP)	8(40)	1(5)	11(55)
Ciprofloxican (CPX)	9(33.3)	2(7.4)	16(59.3)
Amacycilln (AM)	4(14.8)	3(11.1)	20(74.1)
Augmentin (AU)	5(18.5)	4(14.8)	18(66.7)
Cefoxitin(CN)	5(18.5)	1(3.7)	21(77.8)
Reflacine(REF)	3(11.1)	6(22.2)	18(66.7)
Erythromycin (E)	7(25.9)	1(3.7)	19(70.4)
Septrin (SXT)	5(18.5)	4(14.8)	18(66.7)
Streptomycin(S)	6(22.2)	7(25.9)	14(51.9)

Table 4d: Antibiotics sensitivity pattern of *Klebsiella* spp

Antibiotic	Antibiotic susceptibility		
	Susceptible	Intermediate	Resistant
Gentamicin(GEN)	5(20.8)	5(20.8)	24(58.4)
Ampicilin (SP)	8(33.3)	4(16.7)	12(50)
Ciprofloxacin (CPX)	6(25)	2(8.2)	16(66.7)
Amacycilln(AM)	5(20.8)	3(12.5)	16(66.7)
Augmentin (AU)	5(20.8)	4(16.7)	15(62.5)
Cefoxitin(CN)	6(25)	1(4.2)	17(70.8)
Reflacine (PEF)	3(12.5)	2(8.3)	19(79.2)
Tetracycline(T)	6(25)	2(8.3)	16(66.7)
Streptomycin (S)	4(16.7)	0(0)	20(83.3)
Seprin(SXT)	7(29.2)	5(20.8)	12(50)

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Table 5: Multiple antibiotics resistant (MAR) index of bacteria isolated from wounds

MAR Index	Bacteria isolated from wounds and Percentage			
	S. aureus (*n*=29)	*Pseudomonas* (*n*=20)	*E. coli* (*n*=27)	*Klebsiella* sp (*n* = 24)
0.1	0(0)	0(0)	0(0)	0(0)
0.2	0(0)	0(0)	0(0)	0(0)
0.3	0(0)	0(0)	0(0)	1(4.2)
0.4	0(0)	2(10)	2(7.4)	0(0)
0.5	2(6.7)	3(15)	3(11.1)	3(12.5)
0.6	5(17.2)	7(35)	10(37)	4(16.6)
0.7	10(34.5)	3(15)	7(30)	13(54.2)
0.8	7(24.1)	4(20)	4(14.8)	2(8.3)
0.9	4(13.8)	1(5)	1(3.7)	1(4.2)
1.0	1 (3.4)	0(0)	0(0)	0(0)

The phylogenetic tree showing the evolutionary distance between the bacteria isolated from the wounds and percentage relatedness with their close relatives in the gene bank is presented in Figure 4.7. The phylogenic tree obtained from the 16SrRNA sequence of the isolates gave a similar match during the mega blast search for similar sequences, and showed that the isolates identified showed a 100% similarity in their 16SrRNA to their other neighbours in the gene bank.

The calculated values obtained using the Jukes-Cantor method were consistent with the positioning of the 16SrRNA of the isolates within the *Staphylococcus aureus* (ON571652.1), at 100%, *Pseudomonas* sp, with *Pseudomonas aeruginosa* (AF440523.1)at 98.6% and *Escherichia coli* (LC595306.1) at 100%. The bacterial isolate T9 has 98.6% pairwise identity with *Pseudomonas aeruginosa strain C* which has NCBI accession number AF440523.1. The e value is 0. Sequences of the isolates from wound are as shown in the phylogenetic tree below.

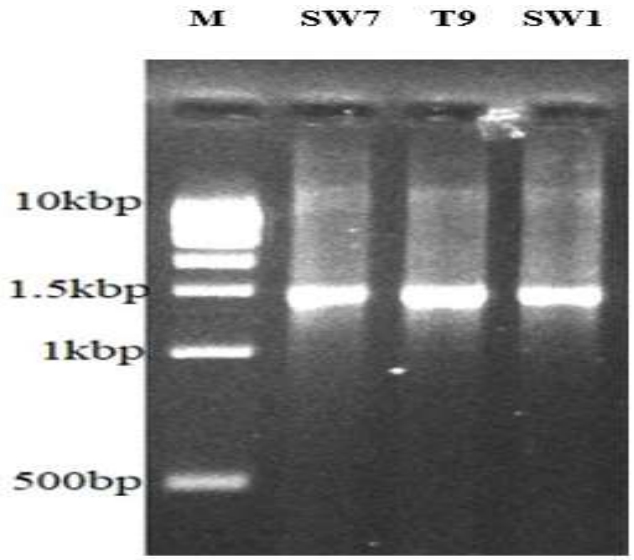

Plate 1: Agarose gel electrophoresis of amplified 16S rRNA gene. Lane SW7 to SW1 represents 16S rRNA gene bands (11.5kbp). Lane M represents the 10kbp DNA ladder

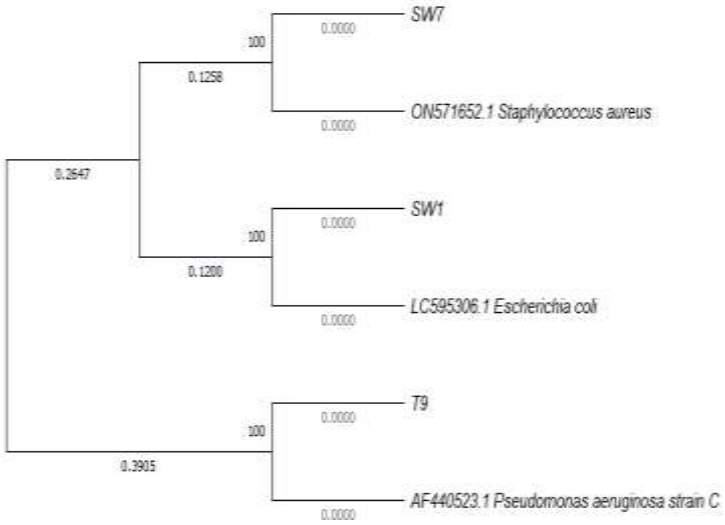

Figure 5: Phylogeny of the bacterial isolates sequenced The bacterial isolate T9 has 98.6% pairwise identity with *Pseudomonas aeruginosa strain C* which has NCBI accession number AF440523.1. The e value is 0.

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Discussion

The demographic and clinical characteristics of patients surveyed in this study revealed that 55% were female and 45% male. The research focused on wound cases amongst patients accessing the University of Port Harcourt Teaching Hospital, Rivers State with various types of wounds (Surgical wounds, Diabetic foot ulcers, venous leg ulcers Burns, Accident, Abscess, Breast cancer and Osteomyelitis. Findings revealed surgical wounds had the highest prevalence (21%) while diabetic foot ulcer had the lowest prevalence (8%). According to Mawalla *et al.* (2011), age, sex, sources of wounds and other such as hygiene practices and severity of the wound can play significant role in determining the likelihood of infection as well as microbial diversity Fromartin *et al.* (2014).

Survey of exposure to antibiotics by patients showed that 89.8% of the patients had records of one month exposure to antibiotics while 10.2% were not exposed. Moreover 67% of the patients had 6 months of exposure to antibiotics while 22% have not been exposed. Pre-exposure to antibiotics can have several effects. Firstly it can limit the effectiveness of antibiotics in treating infections. Antibiotic resistance occurs when bacteria adapt and become resistant to the drugs used to kill or inhibit their growth (Han & Ceilley, 2017). This can lead to prolonged illness, increased healthcare costs, and in severe cases, potential complications or even death. The difference in the occurrence of bacterial contamination patterns may be attributed to variations in environmental exposure, epithelial barrier disruption, and immune system dysfunction (Klein et al., 2018).

In the recent study, Three hundred and fitfty (350) isolates belonging to *Staphylococcus, Escherichia coli, Pseudomonas* and *Klebsiella* were isolated. The predominance of gram positive bacterial isolates in the wound swabbed was observed in this study, *Staphylococcus aureus* being the most isolated organism followed by *Escherichia coli, Pseudomonas and Klebsiella* species this pattern of organisms causing wound infection observed in this study disagrees with previous studies reported by Lateef *et al.* (2001). Jobater *et al.* (2022), according to studies conducted, *Pseudomonas spp* was the most predominance isolates.

However, in the present study *Staphylococcus* spp recorded the highest percentage. The observed variation among these studies may be attributed to differences in the environment conditions, the population investigated, the diversity of surgical procedures performed on the study participants, as well as timing of specimen collection (Lateef *et al.,*2001). In another study by Dettenkoter *et al.* (2009) on the pattern of aerobic bacteria with their drug susceptibility of surgical patients carried out in Mymensingh showed rate of wound infection of 61.6% positive which is less compared to the percentage recorded in the study, never less the prevalence organisms isolated were *Pseudomonas* spp*, Staphylococcus* spp*, Salmonella* spp*, Acinetobacter* and *Klebsiella* spp. Which are similar to those obtained in this study except *Acinetobacter* and *Salmonella* species which were not isolated in this study. Multiple factors may have contributed to the high percentage of Gram-positive bacterial infections in this study. A recent review has reported that the hands of health care workers and patients can play a role in the transfer of Gram negative bacteria during cross-infection (Thanni *et al.* (2003).

A similar findings by Stephen *et al*. (2013) reported that Gram positive bacteria as the most frequent species isolated in Orthopedics unit followed by *P. auruginosa.* These findings suggest that the aetiologic agents of wound infection depend on where the procedures are performed and whether skin was incised or gastrointestinal tract was opened Thanni *et al.,* (2003). When gastrointestinal tract is opened, organisms usually include aerobic Gram negative rods. In the present study majority of surgery operations involved colon operation, likely explaining the increased isolation of Enterobacteriaceae in surgical wards (Yasmeen *et al.,* 2014).

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 break in the skin. Here are some implications of *Star31phylococcus aureus* in wound infections; *Staphylococcus aureus* can impair the natural wound healing process by causing inflammation and prolong the recovery. It can lead to the formation of chronic wounds that longer to heal (Yasmeen *et al.,* 2014).

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Wound sepsis is a serious medical emergency that requires immediate treatment. *Staphylococcus aureus* can spread from an infected wound to others through close contact or contaminated surfaces. This highlights the importance of proper wound care, hand hygiene, and infection control measures (Yasmeen *et al.,* 2014). *Pseudomonas aeruginosa* is a gram-negative bacterium commonly associated with opportunistic infections, include FBJng 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, increased risk of complications (Akon *et al.,* 2013).

Escherichia coli 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. When *E. coli* enters a wound, it can grow and multiply, leading to infection. Wound infections can cause pain, redness, swelling, discharge and ay impede the healing process (Estahbansti *et al.,* 2002).

The significance of honey as antibacterial agent showed that 69 of the wound samples were positive, and honey exhibited significant antibacterial activity against most isolates. The antibacterial effect of honey on organisms isolated from wound samples can be attributed to multiple mechanisms. Firstly, honey possesses high osmolarity due to its sugar content, which creates a hypertonic environment that dehydrates bacterial cells, inhibiting their growth and survival (Irish *et al.,* 2011). Honey demonstrated potent antibacterial activity against Organisms isolated from wound samples, indicating its potential as a natural antimicrobial agent for wound care. Further clinical trials are warranted to explore the therapeutic efficacy of honey in managing wound infections caused by antibiotic-resistant Bacteria.

The study has shown that most of the wound samples were observed and recorded to produce biofilms, which were the surgical wounds, diabetic ulcer and burns, thereby subjecting them to treatment measures. Antibiotics have over the decades been used for both human and animal disease treatment.

However, the extensive and indiscriminate use of both antibiotics, especially the β-lactamase therapeutic and sub-therapeutic doses for treatment of infections, growth promotion and prophylaxis has led to an increased rate of antibiotics resistance among food producing animals Kirketep *et al.* (2008).

Several studies have reported lack of tangible relationships between anthropogenic activities and antibiotics resistance in bacteria and many believe that the elements that select for resistance are naturally present within microbial genome Stephens, (2003). On the other hand, evidence abounds that increased bacteria resistance to antibiotics and the transfer of resistance elements is a modern phenomenon having a strong link with anthropogenic activities (Howell-Jones *et al*., 2005; Hakim and Gress, 2007). The susceptibility profile observed in this study indicated the isolates showed different patterns of resistance from Ampicillin, Azithromycin, and Streptomycin, thereby rendering them ineffective (Bowler *et al.,* 2018). In contrast, Septrin, Erythromycin, Amoxicillin, Pefloxacin, Gentamicin, Rifampicin, and Ciprofloxacin showed susceptibility, indicating potential therapeutic utility (Kumar et al., 2018). This resistance pattern raises concerns about the efficacy of traditional treatments for bacterial infections. The emergence of antibiotic-resistant bacteria is facilitated by multiple factors, including contamination, drug factors (antibiotic stability, concentration, and class), physical factors (temperature, pH, humidity), environmental factors (water, soil, air pollution, climate change), virulence and pathogenicity factors (biofilm formation, quorum sensing), and genetic factors (plasmid-borne resistance genes, chromosomal mutations, horizontal gene transfer) (World Health Organization, 2020).The increasing prevalence of antibiotic-resistant bacteria in wound infections poses a significant challenge to healthcare, necessitating alternative antimicrobial therapies. The extract similarity from the obtained 16srRNA sequence of the isolates produced during the mega blast search were very similar to the sequences from the non- redundant nucleoitide NCBI databae the 16SrRNA classification of 1,2,and 3 have been identified . Isolated 1has 100% pair wise with *Staphylococcus aureus* which has NCBI accession number ON5716521.1, isolate 2 has 98.6% pairwise identity with *Pseudomonas aeruginosa* which has NCBI accession number (AF440523.1) and isolate 3 has100% pairwise identity with *Escherichia coli* which has NCBI accession number LC654898.1.

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Conclusion

This study recovered one hundred (100) bacterial isolates belonging to the genera *Escherichia coli, Staphylococcus, Klebsiella,* and *Pseudomonas* associated with wounds from patients in a tertiary health institution in Port Harcourt. Most of the isolates have the potential to produce biofilm with 62% having the highest potential, thereby potentiating some virulence attributes. The study revealed the efficacy of honey in the control of wound pathogens as higher percentage of the isolates were inhibited by the honey tested which is a promising finding regarding the application of honey in wound management.

The isolates were resistant to multiple antibiotics most especially *Staphylococcus* spp. The partial nucleotide sequences of 16SrRNA gene led to identification of these isolates as different strains of *Pseudomonas aeruginosa* AF440523.1; *Staphylococcus aureus ON5716521.1; Escherichia coli* LC595306.1. The presence of bacteria in this study may be attributed to poor hygienic practices, patients' health status which could result to wound infections, multi-drugs resistance, mortality and morbidity complication.

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