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Antibiotic Susceptibility Profile of Bacteria Isolated from Wounds of Students in Tertiary Institution in Imo State Nigeria

Ohabughiro NB
Department of Microbiology, Imo State University Owerri, P.M.B 2000, Owerri, Imo State, Nigeria

Corresponding Author: **Ohabughiro NB**

Abstract
The antibiotic susceptibility Profile of bacteria isolated from wounds of students attending Imo State University, owerri was studied. Thirty wound samples were aseptically collected, isolated, characterized and identified using standard microbiological methods. Sub-cultured colonies were identified using morphological characteristics and biochemical tests. The total heterotrophic plate bacterial counts ranged from 1.1-4.6 x 10^6 cfu/ml. The total Coliform bacteria plate counts ranged from 1.0-3.2 x 10^6 cfu/ml, and the total Staphylococcal plate counts ranged from 1.0-2.8 x 10^6 cfu/. The Isolates were further subjected to molecular studies using the 16S rRNA sequences. The results of the isolation and identification revealed the following isolates namely, *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. Percentage occurrence showed that 53% were Gram-positive organisms and 47% were Gram-negative organisms. *Staphylococcus aureus* was found to be the most frequent isolate (44%), followed by *Escherichia coli* (22%), *Pseudomonas aerogenosa* (14%), *Klebsiella pneumoniae* (12%) *and Streptococcus pyogenes* (8%). Antibiotic susceptibility profile was determined using the Kirby-Bauer disk diffusion method where the susceptibility of *the* isolates in wounds infections was observed. The findings showed that Ceftazidime was the most sensitive antibiotic especially against *Staphylococcus aureus*. It was also observed that the bacterial isolates had different levels of sensitivity to Ceftazidime, Cefuroxime, imipenem, Cefixime, Ofloxacin, Augmentin and Ciprofloxacin, while the bacterial isolates were resistant to only vancomycin. There is need to have a prior knowledge of the causative organisms of wound infections so that it will be a helpful tool in selecting the empiric antimicrobial therapy to control and treat wound infections.

Keywords: Antibiotic Susceptibility Profile, Bacteria, Owerri, Wounds

Introduction

Wound occurs as a result of a cut, scrape, puncture, burn, or any other injury that breaks the skin or other body tissues. Wounds follow the loss of skin integrity, which provides a moist, warm and nutritive environment. A wound infection occurs when there is a disruption of the skin's integrity or damage to underlying tissues. This results to a conducive environment for microbial colonization, proliferation and growth (Insan *et al.*, 2013) [8]. Wounds are among the leading nosocomial infections and a leading cause of morbidity and mortality in people and leads to increasing medical expense (Gupta, *et al*., 2002) [7].The development of wound infections depends on the integrity and protective function of the skin (Anupurba *et al*., 2010) [2]. Generally, a wound can be considered infected if purulent materials drain from it, even when there is no positive culture confirmation. Wounds are colonized by bacteria, whether infected or not. There are infected wounds which may not yield pathogens by culture because of the fastidious nature of some pathogens, or if the person has received an antimicrobial therapy (Nwachukwu *et al*., 2009) [11].

Bacteria commonly found in wound infections include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* species and *Pseudomonas aeruginosa* (Anupurba *et al*., 2010) [2]. For the treatment of infection, it is ideal to give proper antibiotic and medications after culture and sensitivity of the wound swab, pus or infected tissue. The timing of administration, choice of antimicrobial agent, durations of administration of antibiotics is of much importance in reducing wound infections. However, the severity of complication is largely based on the virulence of the infecting pathogen and the site of infection. Infections due to antibiotic resistant bacteria have increased alarmingly in both developed and developing countries. Unrestrained and rapidly spreading bacterial growth has turned the management of

wound infections into a serious challenge (Lilani *et al*., 2005) [9]. The aim of this study is to determine the Antibiotic susceptibility profile of bacteria isolated from wounds of students in tertiary institution in Imo State Nigeria.

Materials and Methods

The study area is in Owerri, Imo State. Imo state is located in the south eastern part of Nigeria and is bounded to the North by Anambra State, to the East by Abia State, to the South by Rivers State and to the West by Delta State. Imo State is made up of 3 Senatorial zones, Orlu, Owerri and Okigwe. The State lies between latitude 5° 30' and 6° 15' North. Longitude 6° 38' and 7° 18' East. (Federal Republic of Nigeria official gazette, 2007) [5]. The site is Imo State University, Owerri.

Collection of samples: A total of 30 swab stick samples of wound infections were collected from both male and female students of Imo State University, Owerri. The swab stick samples were collected aseptically to avoid contamination by normal flora of the surrounding skin. Then the specimens were taken immediately to the Microbiology laboratory of Imo State University, Owerri.

Cultural Identification of Bacterial Isolates.

Sterilization

All glass wares used were washed with detergent and and dried with hot air oven, they were then sterilized. Media were also sterilized by autoclaving at 121^0 C, 15Psi. All wire loops were flamed to red hot using Bunsen burner. Laboratory benches were cleaned before and after work with 75% alcohol (Ohazuruike *et al*., 2017) [12].

Media preparations

The powder components of the media, Nutrient agar, MacConkey, Muller Hinton agar, Simmon's citrate agar, were dissolved in a conical flasks according to the manufacturer's instruction, the conical flasks was closed with cotton plug and covered with Aluminium foil, placed into an autoclave and sterilized at 121^0C for 15mins. The medium were cooled to 45^0C after sterilization, the cotton plug was removed and the mouth of the flask flamed over a Bunsen burner in other to ensure sterility, and the medium was poured into sterile, empty petri dishes (15-20ml into each petri dish).The petridishes were kept horizontally until the media were completely solidified, then they were turned upside down and stacked for storage.

The plates were labeled according to the medium and also a sterility test was performed on them by incubating some plates at 37^0C for 24hrs and after which they were examined (Ohazuruike *et al*., 2017) [12].

Culturing/Sub-culturing of the cultures

After the Culturing and incubation period, the plates were observed and colonies were counted, and the discrete colonies were sub-cultured into a freshly prepared Nutrient agar and other media plate to get a pure culture. The sub-cultured plates were incubated for 24hrs at $37^{\circ C}$, and examined for pure culture. The pure culture growth was used for gram staining, motility test and biochemical characterization of the organisms like Catalase, Oxidase tests, Coagulase, Citrate utilization test, Indole test, Methyl-red test, Voges proskaeur test and Sugar fermentation. A stock culture was prepared using a bijou bottle: this stock culture was used in storing the organisms for further biochemical characterization (Ohazuruike *et al*., 2017) [12].

Biochemical tests and Identification of isolates

Gram Staining, Motility test and biochemical properties; Catalase, Oxidase tests, Coagulase, Citrate utilization test, Indole test, Methyl-red test, Voges proskaeur test and Sugar fermentation were carried out (Ohazuruike *et al*., 2017) [12].

Gram Reaction

This technique is used to differentiate microorganisms into Gram-positive and Gram-negative as a result of their gram reaction (Cheesbrough, 2011) [3]. Gram staining method described by (Cheesbrough, 2011) [3] was adopted. With the aid of a sterile inoculating wire loop; smears of the isolates was made on clean, grease-free microscope glass slides, air-dried and heat-fixed by passing the slides 2-3 times over a Bunsen burner flame. Afterwards, each smear was covered with a Crystal violet (primary stain) for 60 seconds, after which it was quickly washed off with clean water. The smear was flooded with lugol's iodine (Mordant) for 30 seconds. After, they were decolorized with 75% alcohol for 30seconds, which was washed off quickly with clean water and counter stained with safranin for 30 seconds. The safranin stain was washed off quickly with clean water. Back of the slides were then wiped and placed in a draining rack to air-dry. The smear was then examined microscopically using the oil immersion objective (X100). Gram positive cells showed purple while gram negative cells showed red colour (Cheesbrough, 2011) [3].

Motility test

The method also described by (Cheesbrough, 2011) [3] was adopted. It is used to differentiate between motile and non-motile organisms due to the presence of locomotory structures like flagella and cilia. This test was carried out using the stab method. Test tubes of semi-solid motility medium were inoculated by stabbing a sterile straight wire loop charged with inoculums from the isolated pure culture vertically into the media and it was incubated at 37^0C for 24hours. Non-motile bacteria produced growths that were un-diffused from the line of stab while motile bacteria produced diffused growth away from the line of stab into the medium and rendered it opaque (Cheesbrough, 2011) [3].

Other biochemical tests like Catalase test, Citrate utilization test, Coagulase test, Indole test, Oxidase test, Methyl red/ voges-proskauer (mr/vp), Sugar fermentation test were performed as described by (Cheesbrough, 2011) [3].

Molecular identification of bacterial isolates

The deoxyribonucleic acid (DNA) was extracted using Zymo research bacterial DNA mini prep extraction kit. The qualitative estimation of genomic DNA was done using agarose gel electrophoresis. The extracted DNA was amplified using Polymerase chain reaction amplification protocol. 16S rRNA sequencing protocol was used to characterize bacterial isolate. PCR products were cleaned using ExoSAP Protocol. Fragments were sequenced using the Nimagen brilliant dye terminator cycle sequencing kit according to manufacturer's instructions (Platt *et al*., 2007) [13]. The sequenced data were subjected to Basic Local Alignment Search Tool Nucleotide (BLASTn) to identify corresponding organisms from National center for

bioinformatics information (NCBI) as described by (Altschul *et al*., 1990) [1].

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was carried out using the Kirby-Bauer disk diffusion susceptibility test on Muller Hinton agar medium. The following antibiotics were employed for sensitivity analysis; Ceftazidime (30 µg), Cefuroxime (30 µg), imipenem (10 µg), Cefixime (5 µg), Ofloxacin (2 µg), Augmentin (30µg), vancomycin (30 µg) and Ciprofloxacin (5 µg). The growth was standardized by diluting the culture with normal Saline to match the turbidity of 1.0×10^6 cfu/ml (0.5 McFarland standards).Then, 0.1ml was collected and spread on the surface of Muller Hinton agar using sterile glass rod. The antibiotic disc was placed carefully to make good contact with the agar surface using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30mins to allow for diffusion of the antibiotics and were incubated at 37°C for 24hrs and results were interpreted as sensitive and resistant. The inhibition zone diameters were measured using meter rule after 24 hours incubation and recorded in millimeter (mm). It was further interpreted according to Clinical Laboratory Standards Institute (CLSI, 2006) [4].

Results

The result from this study shows that the total heterotrophic bacterial plate counts ranged from 1.1-4.6 x 10^6 cfu/ml. The total Coliform bacteria plate counts ranged from 1.0-3.2 x 10^6 cfu/ml, and the total Staphylococcal plate counts ranged from 1.0-2.8 x 10^6 cfu/. The Isolates were further subjected to molecular studies using the 16S rRNA sequences. Antibiotic susceptibility pattern was determined using the Kirby-Bauer disk diffusion method. The results of the isolation and identification revealed the following isolates namely, *Staphylococcus aureus, Streptococcus* pyogenes, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Percentage occurrence showed that showed that 53% were Gram-positive organisms and 47% were Gram-negative organisms. *Staphylococcus aureus* was found to be the most frequent isolate (44%), followed by *Escherichia coli* (22%), *Pseudomonas aerogenosa* (14%), *Klebsiella pneumoniae* (12%) *and Streptococcus pyogenes* (8%). Among the gram positive isolate, *Staphylococcus aureus* (44%), was predominant than *Streptococcus pyogenes* (8%). Among the gram negative bacteria, *Escherichia coli* (22%), was the predominate isolate followed by *Pseudomonas aeruginosa* (14%), then *Klebsiella pneumoniae* (12%).

The Percentage of wound sample according to gender of students showed that male students (63%) had more wound infections that female students (37%).

The antibiotic susceptibility profile of different isolates was observed. The highest sensitivity for all the isolates was observed for Ceftazidime. The bacterial isolates showed different level of sensitivity to Ceftazidime, Cefuroxime, imipenem, Cefixime, Ofloxacin, Augmentin and Ciprofloxacin, while the bacterial isolates were resistant to only vancomycin.

There is the need for routine antibiotic sensitivity check. There is also need to have a prior knowledge of the causative organisms of wound infections so that it will be a helpful tool in selecting the empiric antimicrobial therapy to control and treat wound infections.

Table 1: Bacterial load of wound samples

	Total viable count (x 10^6 Cfu/ml)		
Samples	**THBC**	**TCC**	**TSC**
1-5	1.1-3.5	1.0-2.5	1.0-2.4
6-10	2.4- 4.6	1.5-3.2	1.4-2.2
11-15	2.1-3.8	1.7-2.6	1.3-2.5
16-20	2.2-3.7	2.1-2.7	1.2-2.7
21-25	1.7-3.6	1.7-2.6	2.0-2.8
26-30	2.0-3.2	1.5-2.9	1.5-2.3

Keys:
THBC = Total Heterotrophic Bacteria count
TCC = Total Coliform count
TSC = Total Staphylococcal count

Table 2: Biochemical characterization of bacterial isolates

Isolates	Bacteriological tests			Biochemical tests										Probable organism
	Gram reaction	Cellular arrangement	Motility	Catalase	Citrate	Indole	Oxidase	Coagulase	Voges- Proskauer test	Methyl red test	Glucose	Lactose	Sucrose	
1	+	Cocci	-	+	+	-	-	+	+	+	***A***	***A***	***A***	*Staphylococcus aureus*
2	+	Cocci	-	-	-	-	-	-	-	-	***A***	***A***	***A***	*Streptococcus spp.*
3	-	Rod	+	+	-	+	-	-	-	+	***A/G***	***A/G***	***A/G***	*Escherichia coli*
4	-	Rod	+	+	-	-	-	-	-	-	**-**	**-**	**-**	*Pseudomonas aeruginosa*
5	-	Rod	+	+	+	-	-	-	+	-	***A/G***	***A/G***	***A/G***	*Klebsiella spp.*

Key: A - Acid G - Gas

Table 3: Molecular sequence 16S rRNA identity of various Bacteria

S. No	Biochemical Isolates	Percentage (%)	Molecular Sequence
1	*Staphylococcus aureus*	97	*Staphylococcus aureus MH401415*
2	*Streptococcus* spp.	96	*Streptococcus pyogenes* NC018936
3	*Escherichia coli*	99	*Escherichia coli M75029*
4	*Pseudomonas aeruginosa*	96	*Pseudomonas aeruginosa WE 41437*
5	*Klebsiella spp.*	97	*Klebsiella pneumoniae* MH003688

Table 4: Prevalence of wound isolates

Isolates	Number	Percentage (%)
Staphylococcus aureus	42	44
Streptococcus pyogenes	8	8
Escherichia coli	21	22
Pseudomonas aeruginosa	13	14
Klebsiella pneumoniae	11	12
	95	100

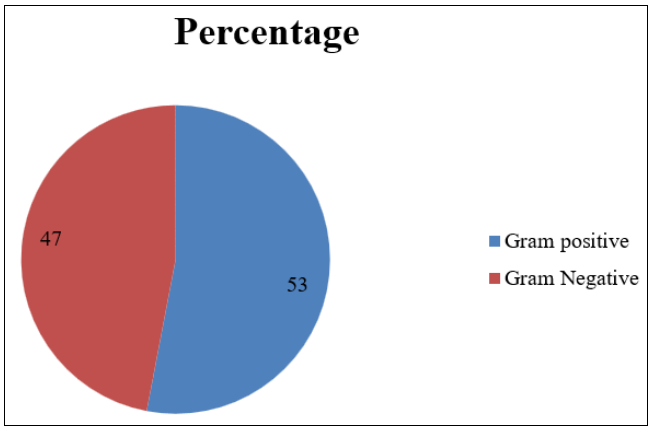

Fig 1: Percentage occurrence of gram positive and gram negative bacteria

Table 5: Percentage of wound samples according to gender of students

Students	Number of wound samples	Percentage
Males	19	63
Females	11	37
Total	30	100

Table 6: Antibiogram zone of inhibition for isolated organisms in mm

Antibiotics	*Staphylococcus aureus* (mm)	Streptococcus *pyogene* (mm)	*Escherichia coli* spp (mm)	*Pseudomonas aeruginosa* (mm)	*Klebsiella Pneumonia* (mm)
Ceftazidime (30µg)	28 ± 1.000	26 ± 1.000	27 ± 1.527	26 ± 1.527	26 ± 1.527
Cefuroxime (30 µg)	27 ± 1.527	25 ± 1.000	24 ± 1.000	23 ± 1.000	23 ± 1.527
Imipenem (10 µg)	26 ± 1.527	23 ± 1.527	22 ± 1.000	21 ± 1.000	20 ± 0.577
Cefixime (5 µg),	24 ± 1.527	23 ± 1.000	21 ± 1.000	18 ± 1.000	18 ± 1.000
Ofloxacin (2 µg),	26 ± 1.527	18 ± 1.000	16 ± 1.000	16 ± 0.577	15 ± 1.000
Augmentin (30µg),	27 ± 1.000	26 ± 1.527	26 ± 1.527	25 ± 1.000	27 ± 1.000
Vancomycin (30µg)	8 ± 1.154	10 ± 0.577	6 ± 1.000	8 ± 1.527	8 ± 1.527
Ciprofloxacin (5 µg)	23 ± 1.000	21 ± 1.527	25 ± 0.577	23 ± 1.000	24 ± 1.000

Key: Breakpoint table for *Staphylococcus aureus* Susceptible (S) ≥ 25 mm, Intermediate (I) 18-24 mm, Resistance (R) ≤ 19mm. Enterobacteriaceae Susceptible (S) ≥ 23mm, Intermediate (I) 15-22 mm, Resistance (R) ≤ 14mm (CLSI, 2006) [4].

Discussion

This study showed that Gram positive organisms accounted for 53% of isolates while gram negative organisms accounted for 47% of the isolates. The work of (Nwachukwu *et al.,* 2009) [11] showed that Gram-positive organisms accounted for 62.9% of isolates, compared to Gram-negative isolates that accounted for 37.1% which showed similarities among the two studies. *Staphylococcus aureus* was the major isolate responsible for wound infection. This study also concurred with the work of (Nwachukwu *et al*., 2009) [11] where 42.3% of infections were found to be caused by *Staphylococcus aureus* which was the most frequent isolate.

The work of (Garba *et al*., 2012) [6] on wound infections observed that while *Staphylococcus aureus* was the predominant Gram-positive organism, which concurred with this research work. The work of (Garba *et al*., 2012) [6] also observed that 55(55%) of the isolates were Gram-negative organisms and 44 (44%) were Gram-positive. The isolates he isolated and identified were *Klebsiella* species and

Pseudomonas aeruginosa which accounted for 25% of the Gram negative organisms, followed by *Proteus* species 19%, *Klebsiella* species 14% and *Escherichia coli* accounts for 11%. Similar isolates were also observed from this work. In this study, it was observed that among both gram positive and gram-negative bacteria, namely; *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae* were found to be sensitive to Ceftazidime, Cefuroxime, imipenem, Cefixime, Ofloxacin, Augmentin, and Ciprofloxacin. While all of the isolates were resistant to only vancomycin. This concurred with the study of (Mengesha *et al*., 2019) which found out that Gram positive bacteria like *Staphylococcus aureus* and gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa and Klebsiella* spp. were sensitive to cefixime, ceftazidime, and cefuroxime.

Conclusion

Wound infections among students are common and are usually caused by *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Streptococcus pyogenes*. Wound infections can increases the rate of morbidity and mortality of people. There is the need for routine antibiotic sensitivity check as well as the need to have a prior knowledge of the causative organisms of wound infections so that it will be a helpful tool in selecting the empiric antimicrobial therapy to control and treat wound infections. The need to control and treat wound infections therefore cannot be overemphasized.

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