Antibiotic Susceptibility Profile of Bacteria Isolated from Wound Sepsis Patients from Immanuel Hospital Eket, Akwa Ibom State, Nigeria

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Abstract: Antibiotic Susceptibility Profile of Bacterial Isolated from Wound Sepsis Patients was study. The study aimed at assessing the antibiotic susceptibility of some bacteria isolated from wound sepsis of patients attending Immanuel General Hospital Eket. Random sampling technique was employed in this study. One hundred (100) wound swabs were obtained and cultured on Blood agar and MacConkey agar. Most Bacterial isolates from the wound were susceptible to most of the antibiotics with few of them being resistant. The prevalence of bacteria isolated from wound swabs were *Staphylococcus aureus* (42.8%), *Escherichia coli* (14.3%), *Pseudomonas aeruginosa* (14.3%), *Streptococcus pyogenes* (11.4%), *Klebsiella pneumoniae*, (8.6%) and *Proteus* sp. (8.6%). The percentage susceptibility results showed that *Staphylococcus aureus* was highly susceptible to Rifampicin (96%), Streptomycin (92%) and Levofloxacin (88%). *Streptococcus pyogenes*, exhibited 100% susceptibility to Ciprofloxacin, Chloramphenicol and Levofloxacin but strongly resisted Amoxicillin (75%). *Escherichia coli* showed high susceptibility to Septrin (90%), Augmentin (90%), Tarivid (80%) and Nalidixic acid (70%). *Pseudomonas aeruginosa* and *Proteus* spp. isolates were resistant to Ciprofloxacin, Gentamycin. *Klebsiella pneumoniae* showed resistance to Ciprofloxacin (75%), Augmentin (75%) and Streptomycin (62%). The demographic factor; sex, age, occupation, area of domicile of patients was associated with the occurrence of bacteria from the wound sepsis. The frequency of single and multiple drug resistance is highly alarming and is a growing threat to the control of infectious diseases globally.
Keywords: Antibiotic, Susceptibility, Bacteria, Wound Sepsis

INTRODUCTION

A wound is a type of injury which happens relatively quickly in which skin is torn, cut or punctured (an open wound or where blunt force trauma causes a contusion (a closed wound) (Adegoke *et al.*, 2010; Siddiqui and Bernstein 2010; Ohalete *et al.*, 2012). 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 (Omole and Stephen 2014), (Sahu *et al.*, 2011). Contamination of wound occurs when non-replicating bacteria enters the wound (Sahu *et al.*, 2011; Tom *et al.*, 2018). Contamination can also occur when the bacteria begin replicating and adhere to the wound site but do not cause tissue damage (Goldstein *et al.*, 1996). It can also occur when the number of bacterial is greatly increased and begins to overwhelm the host immune system (Fonder *et al.*, 2008). During this stage, the granulation bed in the wound appears unhealthy example atrophied, deep red or grey discoloration, with increased discharged but there is no sign of invasion of the surrounding tissue (Sani *et al.*, 2012; Fonder *et al.*, 2008; Janet *et al.*, 2005). Wound infections are classified on a continuum; contaminated, colonized, local infection, spreading infection, and systemic infection (sepsis). Infections of the skin and soft tissue either due to trauma, surgery, or burns may result in the generation of exudates composed of dead leucocytes, cellular debris, and necrotic tissues (Dryden, 2010). Chronic wounds can be colonized on the surface by a wide range of organisms (Matsuura and Barg, 2013). Wound infection is characterized by the presence of pus in lesions with pyrexia, pain and in duration. Infection occurs when virulence factors produced by the microorganisms overwhelms the host natural resistance (Bowler *et al.*, 2010). The contamination or mere presence of pathogenic organisms in wound without local or systemic tissue involvement may not result in infection (Sule *et al.*, 2002).

The wound environment is conducive for bacterial growth. The organisms can cause destruction if left untreated (Austrialian Wound Management Association, 2011). Bacterial wound infections are important because they can slow down the healing process, lead to wound breakdown, prolonged hospital stay and increase in the cost of treatment (Pondei *et al.,* 2013; Ducel *et al.,* 2010). Common bacterial pathogens associated with wound infection include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus spp., Bacteriodes spp, Clostridium*, and *Enterococcus spp.* (Dani *et al.,* 2012). These organisms exhibit natural resistance to many antibiotics and antiseptics in which they may survive for long periods, and may even multiply in the presence of minimal nutrients and have the ability to colonize traumatised skin (Richard *et al.,* 1994; Olayinka *et al.,* 2004). Wounds are the third most frequent nosocomial infections (Ducel *et al.,* 2010). In developing and resource-poor countries, traumatic and surgical site infections are reasons for high morbidity and mortality rates (Egbe *et al.,* 2011). The likelihood of a wound becoming infected is related to the number and virulence of the infecting microorganism and the ability of the host to resist infection (ICU, 2016) of these opportunistic pathogens form part of the host's normal microbiota, or are found in damp environmental sites or on hospital equipments and medicament (Olayinka *et al.,* 2004). Once they gain access into the body, they develop mechanisms to exploit the host for continuous survival and dissemination (Tom *et al.,* 2018). Patients with wound complication arising from the dissemination of pathogenic microorganisms tend to be associated with bacteraemia, septicaemia, shock and prolonged hospital stay with an increasing chance of developing drugs resistant infections. Drug resistance leads to prolonged epidemics (Nita *et al.,* 2018), and consequently, an unattended wound-site, being the most vulnerable point of entry of pathogenic bacteria could be difficult to treat if a multi-drug resistant strain is implicated. This development is worrisome with a resultant increase in morbidity, mortality and cost not only to patients and their relatives but including hospital management (Baba *et al.,* 2016).

MATERIALS AND METHODS

Study Area

The samples were processed at Akwa Ibom State University microbiology laboratory, Ikot Akpaden, Mkpat Enin Local Government Area, as they were obtained from patients attending Immanuel General Hospital Eket. Eket local Government occupies the South-Central portion of Akwa Ibom State territorial expanses spanning Northwards between latitude $4^0$33' and $4^0$45' and Eastward between Longitudes $7^0$52' and $5^0$02'. It is bounded on the north by Nsit Ubium Local Government Area, on the east by Esit Eket local Government Area, on the West by Onna Local Government Area and on the South by Ibeno Local Government Area / Bight of Bonny. Immanuel Hospital named after a Lutheran Church is a missionary hospital which during the Civil war gave room to government involvement which` provided enough medical facilities and equipments able to treat patients.

Sample Collection

Random sampling method was used to select the study participants which comprised of 100 patients with various types of wound infections. Data for the study were generated by administering structured questionnaire and by collecting wound swabs. The structured Questionnaires were administered to generate information on the age, gender, occupation, educational qualification and area of domicile (resident). Swab sticks were used to collect different types of wound samples (Trauma, Postoperative wound, Abscess, Ulcer, Burn wounds and Diabetic foot ulcer and location Leg, Abdomen, Hand, Foot, Ankle, Back, Head and neck) from patients with infected wounds.

Isolation of Bacteria from Wound Swabs

A total of 100 wound swab samples were collected. The swab specimens were streaked on blood agar and MacConkey agar and then placed in the incubator. The swab specimens were incubated for 24 hours at 37^0C; the plates were examined for growth and different pattern of hemolysis. The plates were then sub-cultured into MacConkey agar, and pure cultures were obtained and transferred into Nutrient agar slants for further identifications (Cheesbrough, 2010).

Identification of Bacterial Isolates

Identifications were carried out by sub-culturing from the Nutrient agar slants into freshly prepared MacConkey and Blood agar in sterile petri dishes using the streaking method. The pure culture of the isolates were subjected to biochemical tests, namely: Catalase, Coagulase, Indole, Citrate utilization, Urease, Methyl red-Voges Proskauer, Motility and Sugar fermentation for identification after Gram staining which was used to test for the organisms purity (Cheesbrough, 2010).

Antibiotics Susceptibility Test

The antibacterial susceptibility testing was done using Kirby-Bauer NICCS modified disc agar diffusion technique: all procedure was done under aseptic technique. Isolated bacteria were subculture in normal saline for 3 hours interval to obtain a solution with turbidity equal to 0.5 McFarland standards. Sterile swab stick was used to evenly spread the organism across the Muller Hinton agar plate and allowed to dry. The impregnated disks were carefully picked with sterile forceps and carefully placed on the inoculated Muller Hinton agar plate. The plates were incubated at 37^0C for 24 hours. The diameters of the zones of inhibition were measured and recorded for the urine samples that showed zones of inhibition. The zones of inhibition were measured using a meter rule. All measurements were recorded in millimeters. The Sensitivity pattern of the isolates to Tarivid, Reflacine, Ciprofloxacin, Augumentin, Gentamycin, Streptomycin, Ceporex, Nalidixic acid, Septrin, Norfloxacin, Amoxil, Rifampin Erthromycin, Chloramphenicol, Ampiclox, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing (2007).

Statistical Analysis

This was analyzed using Chi- Square test of association between the socio - demographic factors and the occurrence of bacteria in the wound sepsis of the patients.

RESULTS

Table 1 showed the percentage occurrence of the different bacteria isolated where *Staphylococcus aureus* was the most predominant with 15 (42.8%) isolates. This is followed by *Escherichia coli* and *Pseudomonas aeroginosa* with 5 (14.3%) isolates each, *Streptococcocus pyogenes* with 4 (11.4%) and the least were *Klebsiella pneumoniae* and Proteus spp with 3(8.6%) isolates each.

Table 2 shows Susceptibility of Gram negative isolates to some antibiotics *Escherichia coli* had high susceptibility to Septrin (90%), Augmentin (90%), Tarivid (80%) and Nalidixic acid (70%). *Klebsiella pneumoniae* showed susceptibility to most of the antibiotics, but resisted Ciprofloxacin, Augmentin and Streptomycin. *Pseudomonas aeruginosa* and *Proteus spp* isolates showed susceptibility to all the antibiotics, but resisted Ciprofloxacin, Gentamycin and Septrin (Table 2).

Table 3 showed the percentage susceptibility of Gram positive isolates to some antibiotics were *Staphylococcus aureus* isolates were highly susceptible to Rifampicin (96%), Streptomycin (92%), and Levofloxacin (88%). In the case of *Streptococcus pyogenes*, the isolates exhibited 100% susceptibility to Ciprofloxacin, Chloramphenicol, and Levofloxacin and strongly resisted Amoxil (75%). Table 4

showed the Biochemical Characteristics of Bacteria Isolated from Wound Sepsis in Patient. The demographic factor; sex, age, occupation, area of domicile, site of infection and types of wound of patients are presented in Table 5.

Table 1: Percentage Occurrence of the Bacterial Isolates from Wound Sepsis of Patients attending Immanuel General Hospital Eket

Bacteria	Total number	Percentage (%)
Staphylococcus aureus	15	42.8
Escherichia coli	5	14.3
Psuedomonasaeroginosa	5	14.3
Streptococcus pyogenes	4	11.4
Klebsiellapneumonia	3	8.6
Proteusspp	3	8.6
Total	35	100.0

Table 2: Percentage Susceptibility of Gram-Negative Isolates to some Antibiotics

ANTIBIOTICS	*Escherichia coli* (n=5)%	*Klebsiella pneumoniae* (n=3)%	*Pseudomonas aeroginosa* (n=5)%	*Proteus spp* (n=3)%
Tarivid	80	88	66	66
Reflacine	70	75	100	100
Ciprofloxacin	40	25	0	0
Augmentin	90	25	66	100
Gentamycin	30	50	0	33
Streptomycin	60	38	100	66
Ceporex	50	88	66	100
Nalidixic acid	70	75	100	66
Septrin	90	50	33	33
Amplicin	50	75	66	100

Table 3: Percentage Susceptibility of Gram-Positive Isolates to some Antibiotics

Antibiotics	*Staphylococcus aureus* (n=15)%	*Streptococcus* pyogenes (n=4)%
Ciprofloxacin	65	100
Norfloxacin	69	50
Gentamycin	19	75
Amoxil	38	25
Streptomycin	92	50
Rifampicfin	96	75
Erythromycin	18	75
Chloramphenicol	57	100
Ampiclox	23	75
Levofloxacin	88	100

Table 4: Biochemical Characteristics of Bacteria Isolated from Wound Sepsis in Patient Attending Immanuel General Hospital Eket, Akwa Ibom State, Nigeria

Isolates	Catalase	Coagulase	Indole	Methyl	Voges proskauer	Urease	Motility	Sugar fermentation				Gram staining	Most probable micro-organism
								Glucose	Sucrose	lactose	Gas		
1	+	+	-	+	+	+	-	+	+	+	-	Positive cocci in chains	*Staphyloccocus aureus*
2	+	-	+	+	-	-	+	+	Variable	+	+	Negative rods	*Escherichia coli*
3	+	-	-	-	-	-	+	+	-	-	-	Negative rods	*Pseudomonas aeruginosa*
4	-	-	-	-	-	-	-	+	+	+	-	Positive cocci	*Streptococcus pyogenes*
5	+	-	-	-	+	+	-	+	+	+	+	Negative rods	*Klebsiella pneumoniae*
6	+	-	-	+	-	+	+	+	-	-	+	Negative rods	*Proteus* spp

Keywords: + (positive), - (negative)
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TABLE 5: The Demographic Factor of Patients with the Wound Sepsis

Variable	No Studied	No Infected	% Infected	X^2	P-value
Age					
1-10	7	7	100		
11-20	19	19	100		
21-30	24	24	100		
31-40	17	17	100		
41-50	15	15	100		
51-60	10	10	100		
61-70	8	8	100	16.4751	0.033
Sex					
Male	65	65	100		
Female	35	35	100	9.0000	0.083
Occupation					
Farmer	30	30	100		
Cyclist	18	18	100		
Business owner	8	8	100		
Non-employed student	16	16	100		
Student	14	14	100		
Employed	14	14	100	16.1566	0.075
Area of Domicile					
Urban	68	68	100		
Rural	32	32	100	12.9600	0.083
Site of Infection					
Foot	28	28	100		
Leg	25	25	100		
Hand	25	25	100		
Shoulder	8	8	100		
Ankle	5	5	100		
Knee	6	6	100		
Finger	3	3	100	51.7445	0.017
Types of Wounds					
Diabetic	28	28	100		
Ulcer	20	20	100		
Burn	13	13	100		
Post Operation	20	20	100		
Abscess	15	15	100		
Trauma ulcer	4	4	100	19.6361	0.205

X^2=Chi-square

DISCUSSION

The high prevalence of *Staphylococcus aureus* as revealed by the study could be related to the fact that this organism is an endogenous microbial flora. Infections with these bacteria may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of the natural skin barrier, *Staphylococcus aureus*, which is a common bacterium on surfaces, easily find their way into wounds. Similarly, high percentage of microbial growth was reported in Minna (Baba *et al.*, 2016), Ekpoma (Emele *et al.*, 1999), India (86-100%) and Pakistan (98%) (Basu *et al.*, 2009). Most of the isolates identified in this study had mixed with other bacterial species and some of these have shown to be resistant to many antibiotics, and this indicates the high contamination of wounds in our hospitals. In the present study, the most commonly isolated bacteria from wounded patients were *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa*. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients on long admission, thus complicating illnesses, prior to the administration of antimicrobial agents. The result of percentage prevalence of the bacteria isolates from this study also indicated that the rate of isolation of Gram-positive bacteria was more than that of Gram negative bacteria. This finding negates the report of Obritsch *et al.* (2004), who reported that the rate of Gram Negative bacteria isolation from wound was more than twice that of Gram Positive, and it was noticed that *Klebsiella spp*. was the pathogen less isolated followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Pseudomonas aeruginosa and *Proteus* spp were found to be highly resistant to Gentamicin and Ciprofloxacin. High resistance of the isolates to antibiotics may be due to practicing self-medication, lack of diagnostic laboratory services or unavailability of guideline regarding the selection of drugs thereby leading to inappropriate use of antibiotics. This finding is supported by the investigation of Obritsch *et al.* (2004), who reported that more than 72% of the Gram negative isolates of wound were resistant to Gentamycin, a commonly used antibiotic against Gram positive infections. The study findings indicated that *Staphylococcus aureus* isolates are highly susceptible to Rifampicin (96%), Streptomycin (92%), and Levofloxacin (88%). In the case of *Streptococcus pyogenes*, the isolates exhibited 100% susceptibility to Ciprofloxacin, Chloramphenicol, and Levofloxacin and strongly resisted Amoxil. *E. coli* showed high susceptibility to Septrin,–Augmentin, Tarivid and Nalidixic acid.

Pseudomonas aeruginosa showed susceptibility to most of the antibiotics, but resisted Ciprofloxacin, Augmentin and Streptomycin. This might be as a result of inappropriate use of antimicrobial agents (antibiotics) and the intrinsic ability of the isolates to survive in their environment. This work disagrees with the study done in Ethiopia with average susceptibility of *Staphylococcus aureus* and *Streptococcus spp* isolates (0%) and *E. coli* isolates (4.5%) (Mulu *et al.*, 2012) but is relative to the research carried out in Minna General Hospital Nigeria as reported by Baba *et al.* (2016). The results of antibiotic susceptibility showed that *Streptococcus pyogenes* strongly resisted Amoxil.

The biochemical characterization and differentiation into both Gram Positive and Gram Negative isolate further revealed and confirmed the isolate nutritional and metabolic capabilities so as to know the kind of drug that will be used to targets it as it affects patient outcome. There is an association between the demographic factor; sex, age, occupation, area of domicile of patients and occurrence of bacteria from the wound sepsis.

CONCLUSION

The study identified that most of the bacterial isolates from the wound are

susceptible to most of the antibiotics with few of them being resistant.

This research addresses the microbiological aspect that are critical to the successful management of bacteria in wound and resistant rate of these bacteria to single and multiple antibiotics which is really alarming and a threat to the control of infectious diseases. The demographic factor; sex, age, occupation, area of domicile of patients was associated with the occurrence of bacteria from the wound sepsis. The frequency of single and multiple drug resistance is highly alarming and is a growing threat to the control of infectious diseases globally.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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