



Isolation and Identification of Some Bacteria Associated with Wound Sepsis Among the Patients Attending General Hospital Minna, Nigeria

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ABSTRACT

The study is aimed at assessing the antibiotic susceptibility of some bacteria isolates from wound sepsis of patients attending General Hospital Minna, Niger State, Nigeria. One hundred (100) wound swabs were obtained and cultured on Blood agar and MacConkey agar, 55 samples were positive by culture. The isolates were subjected to biochemical tests for possible identification. Antibiotic susceptibility testing of the isolates was done using disc diffusion method in conformity with McFarland standards. The bacteria isolated from wound swabs were *Staphylococcus aureus* 26 (47.27%), *Escherichia coli*, 10(18.18%), *Pseudomonas aeruginosa* 8 (14.54%), *Streptococcus pyogenes* 4(7.27%), *Klebsiella pneumoniae*, 3 (5.45%), *Proteus sp* ,3 (5.45%) and, *Klebsiella ozanae*, 1 (1.81%). The percentage susceptibility results showed that *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%). *Escherichia coli* showed high susceptibility to Septrin (90%), Augmentin (90%), Tarivid (80%) and Nalidixic acid (70%). *Klebsiella ozanae* exhibited 100% susceptibility to seven (7) of the antibiotics. *Pseudomonas aeruginosa* and *Proteus sp* isolates were susceptible to seven (7) antibiotics, but resisted Ciprofloxacin, Gentamycin and Septrin. *Klebsiella pneumoniae* showed resistance to Ciprofloxacin (75%), Augmentin (75%) and Streptomycin (63%) only among the antibiotics used in the study. Statistical analysis using chi-square test of association indicated that the variables, age, sex, site of wound, area of domicile and occupation of the patients showed statistical significance ($P < 0.05$), type of wound was however statistically insignificant ($P > 0.05$). The frequency of single and multiple drug resistance is highly alarming and is a growing threat to the control of infectious diseases globally.

Keywords: Identification, Bacteria, Wound, Sepsis

INTRODUCTION

A Wound is any injury to the body, either from accidents, violence and surgery, which could involve the breaking of membrane or laceration and usually damage the underlying tissues. Sepsis on the other hand is a toxic condition resulting from the spread of bacteria or their toxic products from a focus of an infection (Russell, 2011). Infections are very important causes of illnesses and deaths among surgical patients (Church *et al.*, 2006). Nosocomial infections are much pronounce in patients with wounds, and this is due to the nature of the wound itself, immune-compromise, age, time and depth of the wound in combination with type and dose of virulence of the infecting organism. Host resistance on the use of antibiotics has been reported to be important in

the establishment of a wound infection, as well as the systemic dissemination of the colonizing organism (Bowler *et al.*, 2001; Nichols *et al.*, 2001)..

The contamination as a result of presence of pathogenic microorganisms in a wound with no systemic-local tissue involvement might result in infection. Apart from causing discomfort to the patients, the effect may vary from being an ordinary nuisance to a delay in wound healing, and other major problems such as wound dehiscence, gas gangrene and tetanus. A

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prolonged hospital admission could result in adding to the cost of the treatment and management of the patient. Bacteriological studies have shown that wound infection is a universal infection, and that the bacteria types present in the wounds vary from one geographical location to the other.

The control of wound infections has become more challenging due to wide spread of bacteria resistance to antibiotics and to a greater incidence of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and polymicrobial flora (Bouchillon *et al.*, 2004). Infections shows a manifestation of organisms successfully colonized by entering the body, which reproduces and initiate an inflammatory response which are characterized by the classic signs of redness, rise in temperature of the body, pains, and fever (George, 2005). Most humans who are sick, weak and malnourished, have cancer or are diabetic have increased susceptibility to chronic or persistent infection. Individuals with a suppressed immune system are particularly susceptible to infections (George, 2005).

Wound infections are caused by the multiplication of microorganisms in the surgical site of an immune-compromised host. Microorganisms are transmitted into the wound through several ways, these could be through direct contact with the organisms, which are either transfer from surgical equipment or the hands of surgeons or nurses, air borne dispersal from the environment contaminated with microorganisms that are deposited onto the wounds, contamination could also occur through one-self, by physical migration of the patients own endogenous flora which are present on the skin, mucous membrane or gastrointestinal tract to the surgical site (Murray *et al.*, 2008).

The bacteria agents often associated with wound infections include *Staphylococcus* species, *Pseudomonas* species, *Klebsiella* species, *Proteus* species, *Streptococcus* species, and *Escherichia coli* as well as anaerobes such as *Clostridium* and *Bacteriodes* species (Vanessa, 2006). Bacteria found in the wound can be categorized into four different conditions namely: contamination, colonization, critical colonization and infection. Contamination simply means existence of bacteria within a wound. All chronic wounds are contaminated.

Bacteria are low in number and non-replicating. Colonization is the next step in the evolving relationship of microbes with the host. Bacteria are now replicating and a larger population is now established in the wound.. Critical colonization is a stage where the confrontation between bacteria and host creates the first fissure to develop within the superficial compartment of the wound. Infection is defined as the point where bacteria begin to invade deep compartments and break through the layers of host defense to damage tissues. A critical density or burden of bacteria has been reached and wound healing become stalled and reversed (Safdar *et al.*, 2006).

METHODOLOGY

Sample Collection

Questionnaires were administered to determine the age, gender, occupation, educational qualification and area of domicile. The swab sticks were used to collect different wound type: Trauma, Postoperative wound, Abscess, Ulcer, Burn wounds and Diabetic foot ulcer and location Leg, Abdomen, Hand, Foot, Ankle, Back, Head and neck, Breast, Chest from patients with infected wounds.

Isolation of bacteria from wound swabs: The total number 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°C, the plates were examined for growth. The plates having growth were sub-cultured into MacConkey agar, and pure cultures were obtained and transferred into Nutrient agar slants for further identifications.

Identification of Bacterial isolates

Identification was carried out by sub-culturing from the Nutrient agar slants into freshly prepared MacConkey and Blood agar in sterile petri dishes using the streak method. The pure culture of the isolates were subjected to biochemical tests , namely : Catalase, Coagulase, Indole, Citrate utilization, Urase, Methyl red- Voges Proskauer, Motility and Sugar fermentation for identification after Gram staining (Cheesbrough, 2002).

Antibiotic susceptibility test

This was carried out by preparing sterile Mueller – Hinton agar and standardized as instructed by the manufacturer. The media were dispensed into petri dishes and allowed to solidify. The inocula were also prepared in test tubes in respect to 0.5 McFarland's standard. The test isolates were inoculated on the Mueller- Hinton agar using a sterile swab stick, after which an antibiotic disc was placed onto the inoculated plates and were incubated at 37° C for 24hours. The plates were read and the zones of inhibition were measured to the nearest mm (Cheesbrough, 2002).

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: Percentage occurrence of the bacteria isolates from wound sepsis of patients attending General Hospital Minna.

Bacteria	Total number	Percentage
<i>S. aureus</i>	26	47.3
<i>E.coli</i>	10	18.2
<i>P. aeruginosa</i>	8	14.5
<i>S. pyogenes</i>	4	7.3
<i>K. pneumoniae</i>	3	5.5
<i>Proteus sp</i>	3	5.5
<i>K. ozanae</i>	1	1.8
Total	55	100.0

The percentage occurrence of the different bacteria isolated as presented in Table 1 indicated that *Staphylococcus aureus* was the most predominant with 26 (47.3%) isolates. This is followed by *Escherichia coli* with 10 (18.2%) isolates. *Klebsiella ozanae* occurred least with 1 (1.8%) isolate.

Table 2: Percentage susceptibility of Gram-negative isolates to some antibiotics.

Antibiotics	<i>E. coli</i> (n =10)	<i>Klebsiella pneumoniae</i> (n =8)	<i>P. aeruginosa</i> (n =3)	<i>Proteus sp</i> (n =3)	<i>K. ozanae</i> (n =1)
Tarivid	80	88.	66	66	100
Reflacine	70	75	100	100	100
Ciprofloxacin	40	25	0	0	0
Augmentin	90	25	66	100	100
Gentamycin	30	50	0	33	0
Streptomycin	60	38.	100	66	100
Ceporex	50	88.	66	100	100
Nalidixic acid	70	75	100	66	100
Seprin	90	50	33	33	0
Amplicin	50	75	66	100	100

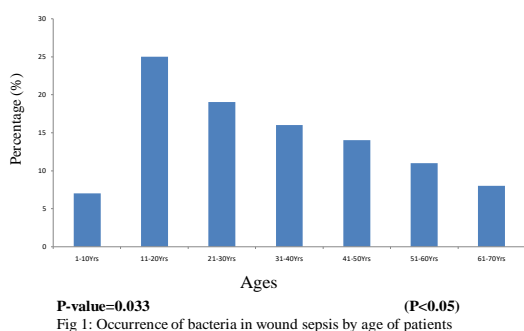
The percentage susceptibility results revealed that *E. coli* showed high susceptibility to Septrin (90%), Augmentin (90%), Tarivid (80%) and Nalidixic acid (70%). *Klebsiella ozanae* exhibited 100% susceptibility to all the antibiotics, but showed 0% susceptibility to Septrin, Gentamycin, and Ciprofloxacin. *Klebsiella pneumoniae* showed susceptibility to most of the antibiotics, but

resisted Ciprofloxacin, Augmentin and Streptomycin.. *Pseudomonas aeruginosa* and *Proteus sp* isolates showed susceptibility to all the antibiotics, but resisted Ciprofloxacin, Gentamycin and Septrin (Table 2).

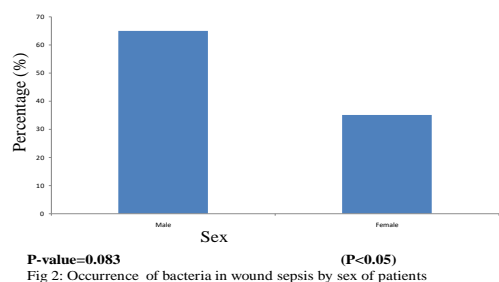
Table 3: Percentage susceptibility of Gram-positive isolates to some antibiotics

	<i>S. aureus</i> n=26	<i>S. pyogenes</i> n=4
Ciprofloxacin	65	100
Norfloxacin	69	50
Gentamycin	19	75
Amoxil	38	25
Streptomycin	92	50
Rifampicin	96	75
Erythromycin	18	75
Chloramphenicol	57	100
Ampiclox	23	75
Levofloxacin	88	100

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 3).

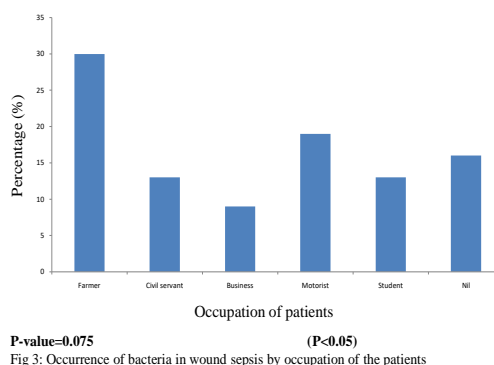


The graph clearly showed statistical significance (P<0.05) in the distribution of ages, of patients (Fig.1).

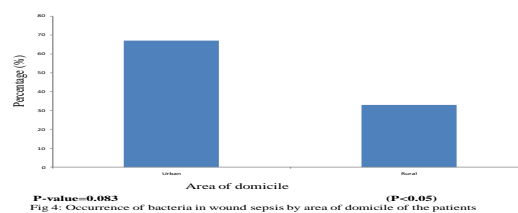


There is an association between the sex of patients and occurrence of bacteria from wound sepsis, because mainly male patients are

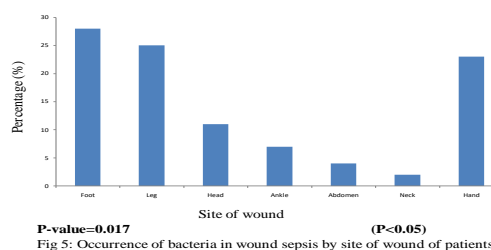
involved with occupations such as farming, motorcycling etc. The distribution on the graph indicated statistical significance (P<0.05) (Fig. 2).



The statistical analysis using chi-square(X²) test of association showed statistical significance between the occupation of the patients and the occurrence of the bacteria isolates from wound sepsis. This is evident in the trend of distribution of the occupation of the patients as shown in the curve (Fig.3).



The statistical analysis using chi-square(X²) test of association showed statistical significance between the area of domicile of the patients and the occurrence of the bacteria isolates in wound sepsis. This is evident in the trend of distribution of the patients as shown in the curve (Fig.4).



The statistical analysis using chi-square(X²) test of association showed statistical significance between the site of wound of the patients and the occurrence of the bacteria

isolates in wound sepsis. This is evident in the trend of distribution of the patients as shown in the curve (Fig.5).

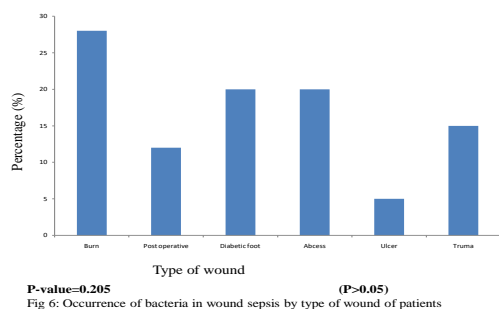


Fig 6: Occurrence of bacteria in wound sepsis by type of wound of patients

The statistical analysis using chi-square(X^2) test of association showed that there is no significant difference between the type of wound of the patients and the occurrence of the bacteria isolates in wound sepsis. This is evident in the trend of distribution of the patients as shown in the curve (Fig.6).

DISCUSSION

The predominant bacteria isolated were *Staphylococcus aureus*, 26 (47.27%), *Escherichia coli* 10 (18.18%), *Pseudomonas aeruginosa* 8 (14.54%), *Streptococcus pyogenes*, 4 (7.27%), *Proteus* sp, 3 (5.54%), *Klebsiella pneumoniae* 3 (5.54%) and *Klebsiella ozanae* 1 (1.81%). The high prevalence of *S. aureus* in the infections may be because it is an endogenous microbial flora. Infections with this 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 India (86-100%) and Pakistan (98%) (Basu *et al.*, 2009). A number of researches carried out previously on wound infection from Africa and different parts of the world showed that *S. aureus* and *E. coli* were the most frequent isolates (Bhatt *et al.*, 2006).

Bacteria isolated from only 55 burn wound swabs from the total 100 swab indicated that 55% of examined burn patients had invasive burn wound infections, this finding is supported by investigation of Moonery *et al.*(2002), who

explained that the burn wound infection is one of the most important and potentially serious complications that occur in the acute period, following injury. Raja *et al.*(2007) demonstrated that the infectious complications are considered a major cause of morbidity and mortality and the type and amount of bacteria in the injured tissues influence wound healing. 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 burn wounds in our hospitals. In the present study, the most commonly isolated bacteria from burned patients were *S. aureus* and *P. aeruginosa*. 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 30 (54.54%) was more than that of gram negative bacteria 25 (45.45%). This finding negates the report of Obritsch *et al.*(2004), who reported that the rate of gram negative bacteria isolation from burn wound was more than twice that of gram-positive, and it was noticed that *Klebsiella* sp. was the pathogen less isolated constituting 3.33% followed by *P.aeruginosa* (23.33 %) and *S. aureus* (15.33%).

Resistance to some antibiotics was very low. Percentage susceptibility results 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 (75%). *E.coli* showed high susceptibility to Seprin (90%), Augmentin (90%), Tarivid (80%) and Nalidixic acid (70%). *Pseudomonas aeruginosa* showed susceptibility to most of the antibiotics, but resisted Ciprofloxacin (75%), Augmentin (75%) and Streptomycin (63%). 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 *S. aureus* and *Streptococcus* sp isolates (0%) and *E.coli* isolates (4.5%) (Mulu *et al.*, 2012).

The results of antibiotic susceptibility showed that *Streptococcus pyogenes* strongly resisted Amoxil (75%). *P. aeruginosa*, and *Klebsiella ozanae* 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 burn wound were resistant to Gentamicin, a commonly used antibiotic against Gram-positive infections.

Chi-square test of association to assess the association between different variables revealed that the variables, ages, sex, occupation, area of domicile, site of wound, indicated statistical significance ($P < 0.05$), except type of wound that is statistically insignificant ($P > 0.05$). It equally includes that there is an association in the occurrence of bacteria in wound of males (65%) than in females (35%). The reasons being that, in African countries, mainly males are involved in occupations such as farming, construction works, transportation and industrial works, where the likely exposure to trauma is common. This is in agreement with studies reported by Gautam *et al.* (2013) in different parts of Africa and other countries (Amaran *et al.*, 2013).

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