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Microbial population of wound isolates and socio demographic characteristics in patients attending clinic in national orthopaedic Hospital, Enugu, Nigeria

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Abstract

Background: The microbial population of wound isolates remains a public health concern because of its role in spread of infections. The microorganisms present in wound swab of patients have been reviewed in order to ascertain the presence of emerging and re -emerging pathogens in hospital environment. These microorganisms are generated from nosocomial and opportunistic origin as well as immune compromised individuals. The purpose of this study is to determine the occurrence of microorganisms in wounds of patient's attending clinic in National Orthopedic Hospital Enugu, Nigeria.

Methodology: A cross sectional study of 230 wound patients was selected using convenient sampling technique. Conventional methods and 16SrRNA sequencing was used for characterization of some organisms that could not be identified in the conventional methods. Questionnaire structured from literature review was also deployed for demographic studies obtained from consented participants.

Results The frequency distribution of wound isolates showed an increase in *Pseudomonas aeruginosa* 7(10%) and *Klebsiella pneumonia* 6(8.6%) respectively. Mixed growths had a notable increase of 42(18.3%), while culture with no bacteria growth had 37(16.1%). *Staphylococcus aureus* was prevalent with 32(13.9%), *Staphylococcus epidermis* 15(6.5%) and the least isolates had 1(1.4%), Male gender had the highest wound isolates of one hundred and fourty one (141) with mean \pm SD 21.11 \pm 13.608 and median of 19.00 while the female had 88 with mean \pm SD 20.31 \pm 13.457, median of 16.50. The socio demographic data findings for participant's age were higher in the range of 21-40yrs (94) and 41-60yr (74) while the least was >80 years (5). The mean age of the 230 participantswas 43.15 \pm 17.284yrs.Wound type had a notable increase in accident cases (63)27.4%, surgical cases (44)19.1% and lowest was laceration cases (2) 0.9%, The most prevalent population of wound cases according to occupational status was observed in artisans (148) 64.3%, students (33)14.3%, civil servants (29)12.6%, least population was the retired (20)8.7%. The portion of wound site was highest in the leg region (174), trunk (22) and the hand (12).

Conclusion: it is advocated that proper measures such as adequate sanitation, personal hygiene, sterilization of hospital equipment and environment should be beefed up to prevent the spread of these microbes to the public.

Keywords: Microbial population; Wound; Occurrence; Socio demographic; Enugu; Nigeria

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1 Introduction

Microbial population of wound isolates consists of gram negative and gram positive organisms detected in wound infections as a result of nosocomial or opportunistic pathogens and novel emerging pathogens generated in hospital settings (1, 13, 14). Most of these isolates display remarkable role in antibiotic resistance by means of their virulent structures and enzymatic inactivation inform of defense mechanism for survival, therefore, contributing to poor wound healing (4, 5, 7). Previous studies have shown that gram negative organisms are more prevalent in wound culture than gram positive organisms (2,1). However, gram positive bacteria such as *Staphylococcus aureus* have been reported to be dominant in wound in the initial phase of infection, while in the second week, gram negative bacteria such as Pseudomonas aeruginosa, Actinetobacter baumanni, Klebsiella species and others are seen to be prevalent in wound causing sepsis if they enter the blood system (15,3). Microorganisms during unfavorable condition can share mutual relationship with each other in order to survive and these may appear in wound culture as mixed growths (6, 16). Wound is an injury on biological tissue, including the skin and organ tissues (8, 10). Wound infection can be categorized into acute and chronic infection depending on the time of healing or wound repair (8, 11,15). Acute wound heals within 6-12 weeks while chronic wound takes a longer time and healing processes are hindered (15,8). Wounds that have highest risk of infection are bites, open punctures, contaminated wounds, wounds sutured in an unsterile environment, scrapped or removed dead wound tissues(8). Higher risk have been reported in children, elderly people, alcoholics, those with addiction to narcotics, or people with mental illness or disability (7.8). Higher percentage of male gender distribution of wounds was prevalent than in female gender (2,6). Socio demographic data on age wise distribution of wound cases were frequent in children and adolescent in range 0-40yrs and declined at 41-60yrs (9,11). Higher percentage of male gender distribution of wounds was prevalent than in female gender(2,6). In terms of wound types, accident cases was rated high(52%),burns(32%),surgical cases(16%).Wound types had notable increase at 20-30yrs(25%), 80yrs above (1.79%) (7, 10). The site portion affected by wound was commonly in these areas; foot, leg, forearm and knee (17).

Objective of the study is to determine the occurrence of microbial population of wound isolates and its socio demographic characteristics.

2 Material and method

2.1 Study setting and design

Enugu state is densely populated and rated at 460 /km² and Orthopaedic Hospital Enugu is located at Abakpa junction Abakaliki road, Enugu. It is one of the three National Orthopaedic Hospitals in Nigeria. It is a tertiary hospital that serves about a third of Nigerians population in the Eastern and Southern path of Nigeria. A cross sectional study was used for this research. Convenient sampling method was employed for collection of samples. Wound swabs were collected from patients from March 2023 to September 2023.

2.2 Study Population, sample size calculation and subject selection and criteria

The study population comprised male and female of different age groups (outpatients and inpatients) were recruited in National Orthopaedic Specialist Hospital Enugu, Nigeria.

The sample size for this research was obtained by using the Naing's sample size determination formula; Naing *et al.*,(10) Calculation formula: $N=z^2 \times p$ (1-p)/d² N=minimum sample size

P=prevalence of pathogenic bacteria isolates in wound (82%), Aisha *et al.*, (18). D=desired level of significance=0.05 (5%), Z= the level of statistical significance of the expected result, in this case 1.96 for 95% confidence interval using the prevalence rate of a previous study Aisha *et al.*, (10),the sample size was calculated as 227 which was adjusted to 230 participants. The participants eligible were those with wounds (both male and female) of different age groups. Only those without wound were excluded. Written informed consent was obtained from each participant.

2.3 Ethical Considerations

Ethical approval was sought for and obtained from the Ethical Committee National Orthopaedic Specialist Hospital, Enugu, Nigeria.

2.4 Sampling Technique

Convenient sampling technique was employed in the collection of wound samples from patients who met the inclusion criteria during clinical consultation hours. The subjects' participation in the study was voluntary. The subjects were properly informed on the nature, merits and aim of the study before sample collection.

2.5 Sample collection and demographic

Wound swabs were collected from the participants attending National Orthopaedic Specialist Hospital, Enugu, Nigeria. Questionnaires were structured from the literature review and administered to all the participants before collection of their samples in order to highlight and educate them on the study. The demographic data taken into consideration were age, gender, wound types and occupation and wound sites. Participants on and without antibiotics were all included.

2.6 Laboratory analysis of samples

Wound swabs: Sterile cotton swabs (Bio rapid, USA) were labeled with participant's identity numbers and samples were obtained after cleaning the affected area with normal saline before gently inserting the wound swab on the affected site using the tipped area of the cotton swabs. The wound samples was inserted back into the swab container after each collection prior to analysis, asceptically cultured and incubated aerobically and anaerobically overnight at 35°C for isolation of bacteria respectively in MacConkey agar (Bio Pro, IVD), Chromatic detection agar media (Liofilchem, Italy), Nutrient agar (Oxoid,UK) was used for blood agar and chocolate agar).Identification: Single colonies of the bacteria isolates was observed in the different bacteria culture media. Some of the cultures that had mixed growth were separated and sub cultured to get a pure colony. Gram staining test was done to differentiate the gram negative bacteria from the positive ones. Analytic Profile Index (API-20E), (Biomerieux, France), Enterosystem 18 R, (Liofilchem, Italy).Triple sugar iron agar (TSIA) (L-S Biotech, USA) was used in identification of the wound isolates. The isolates that were not identified using conventional methods were characterized using 16SrDNA sequencing (12).

Data analysis: Data obtained was analyzed using Statistical package for Social Science (SPSS) version 23 software, M-S Excel. Descriptive statistical analysis was employed such as Frequency, Percentage, Mean \pm SD, pie charts and bar chart. Social demographic of participants were also included in the study using the structured questionnaires.

3 Results

The data obtained from the research were arranged accordingly and subjected to statistical analysis such as frequency, percentage, pie chart, bar chart, mean \pm SD, A total of 230 participants were recruited in this study. The relative sizes of microbial population of wound isolates recorded the highest size as mixed bacteria growth. Out of the 230 samples, pure isolates were 32 in number; twenty two (21) gram negative and eleven (11) gram positive organisms as seen in figure 1.0. The wound isolates obtained were Staphylococcus aureus(32.3%), Staphylococcus epidermis (14.1%), Staphylococcus saprophyticus (11.1%), Pseudomona aeruginosa (10%), Pseudomonas luetola (2.9%), pneumoniae flourescens(1.4%),Klebsiella Pseudomonas (8.6%), Klebsiella ozaenae(1.4%),Klebsiella vulgaris(1.4%),Escherichia coli oxytoca(1.4%),Serratia marcescens (7.1%),Proteus mirabilis (5.7%),Proteus (4.3%),Klebsiella michiganensis (2.9%), Providentia stuartii(1.4%),Salmonella enterica(1.4%),Salmonella odonifera planticola (1.4%),*Candida* albicans (2.0%),Raoutella (8.6%), Raoutella ornithinolytica(4.3%),Citrobacter freundii(1.4%), Acinetobacter baumannii, Lactobacillus acidiophilus(2.0%), Enterobacter faecalis(7.1%), Enterococcus faecium(7.1%),Enterobacter cloacae (2.9%),Streptococcus pyogenes(14.1%),Clostridium perfrigens %), Clostridium diphtheria (2.0%), Clostridium ulcerans (1.4%). Streptococcus pneumonia (2.0%), Haemolytic staphylococcus (1.0%). The frequency distribution of gram negative bacteria isolates showed an increase in *Pseudomonas aeruginosa* 7(10%) and Klebsiella pneumonia 6(8.6%) respectively and the least isolate had 1(1.4%). Mixed growth was the highest with 42(18.3%) while culture with no bacteria growth had 37(16.1%). Staphylococcus aureus was prevalent with 32 (13.9%), Staphylococcus epidermis 15(6.5%), and other pathogenic, non -pathogenic emerging bacteria were isolated as observed in figure 1.1 below. Male gender had the highest wound isolates of one hundred and fourty one (141) with mean \pm SD 21.11 ± 13.608 and median of 19.00 while the female had 88 with mean \pm SD 20.31 \pm 13.457, median of 16.50. The socio demographic data findings for participant's age was higher in the range of 21-40yrs (94) and 41-60yr (74) while the least was >80 years (5). Mean age of 230 participants was 43.15 ± 17.28 yrs as shown in figure 1.3 Male gender (63%) were mostly affected than female gender (37%) respectively as seen in figure 1.2 The wound type had a notable increase in accident cases (63)27.4%, followed by surgical cases (44)19.1% and lowest in laceration cases (2) 0.9%, as shown in figure 1.2. The most prevalent population of wound cases according to occupational status was observed in artisans (148) 64.3%, civil servants (29)12.6%, Students (33)14.3% while the least population was the retired (20)8.7% as seen in figure 1.4. The portion of wound site was highest in the leg region (174), trunk (22) and the hand (12) as seen in table 1.0.

4 Discussion

The occurrence of microorganisms in wound isolates constitutes a community of pathogenic, non pathogenic emerging and re-emerging organisms which are similar to those reported in other research work (1,2,15). The relative sizes of microbial population of wound isolates recorded the highest size as mixed bacteria growth, indicating that wounds are more likely to be contaminated or have mix-infection (16, 6). This may be linked to poor environmental sanitation, poor infection control in health care settings, poor hygiene and indiscriminate administration of antibiotics (2, 13). The mean age of 43.15+17.28 yrs of 230 participants, falls within the range with other authors as in Moro *et al* (15). Male gender (63%) was mostly affected than female gender (37%) respectively and had the greatest number of wounds. This study is similar with most researcher's work as reported in (9,15) The wound type had a notable increase in accident cases (63)27.4%, followed by surgical cases (44)19.1% and lowest in laceration cases (2) 0.9%. The most prevalent population of wound cases according to occupational status was observed in artisans (148) 64.3%, civil servants (29)12.6%, students (33)14.3% while the least population was the retired (20)8.7%. This study corresponds with most researchers work as provided by (7, 8, 10). A likely explanation may be that the young and adolescent are prone to wound infection due to their life style, risks, norms, custom and nature of job (4,19,11). The portion of wound site was highest in the leg region (174), trunk (22) and the hand (12). This is an indication that the leg portion is active and in use than other parts of the body. Some researchers have reviewed studies on the wound site with similar results (17). The findings in this study shows significant association between wound infection and age groups of young and adolescent >20-40 yrs and 41-60yrs. which is similar to other researchers (9,11). Wound cases according to occupation may be as a result of occupational hazard encountered in the field of work(17).



Figure 1 The distribution of relative sizes of all the microorganisms isolated in wound swab



Figure 2 Percentage distribution of microorganisms in wound swabs



Figure 3 The frequency distribution of wound types according to sex



Figure 4 Bar chart showing the socio demographic status according to age





Table 1 Socio demographic characteristics according to wound sites

Variables	Frequency
Wound sites n-208	
Leg	174
Hand	12
Trunk	22

Limitations of study

This study is only in a hospital setting and does not include those receiving treatment at various tradtional treatment centers involving wound patients thereby limiting generalization of the findings in this study.

5 Conclusion

This study provides information on the prevalence of microorganisms in wounds as a result of nosocomial infection in hospital environment. The findings in this study shows significant association between wound infection and age groups of young and adolescent. It is an eye opener for the health care practitioner to improve in their surveillance system and improve in sanitation, personal hygiene from the health workers and patients, adequate sterilization of instruments and proper handling of materials for wound dressing as well as adherence to antibiotics administration in order to promote wound healing and reduce the spread of these infectious organisms to the public and hospital environment.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Contributions of authors

LNC and NRA devised and planned the study. LNC wrote the first draft of the manuscript and reviewed literature along with NRA, CVU and EIB. EAD, CLD, ISE and COA collected clinical samples from subjects. LNC performed laboratory analysis and reviewed the manuscript. All authors read and approved the final manuscript.

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Statement of ethical approval

Ethical approval was obtained from institutional review board on date of 24/05/2022.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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