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Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* among wound patients in Alex Ekwueme Federal University Teaching Hospital Abakaliki.

Chibuike Kingsley U[1], Iroha Ifeanyi R[2], Okeh Emmanuel N[1]
[1] Department of Medical Laboratory Science, Gregory University, Uturu, Abia State, Nigeria
[2] Department of Applied Microbiology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Abstract
Wound infection occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. The present study determined the Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Among Wound Patients in Alex Ekwueme Federal University Teaching Hospital Abakaliki. A total number of 229 wound samples were collected from wound patients and analyzed using standard microbiology techniques to determine the presence and distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates. Antibiotic susceptibility testing and multidrug resistance of these isolates were performed using a modified Kirby-Bauer disk diffusion method. Out of the 229 wound samples analyzed, Gram positive and negative bacteria were isolated in 132 (57.6 %) samples, out of which, *Pseudomonas aeruginosa* was 39.4 % while 60.6 % was *S. aureus*. The possible reason for the high frequency of *S. aureus* and *P. aeruginosa* may be that these bacteria are commonly found in the hospital environment. In addition, *S. aureus* is a normal flora of healthy person (especially on skin), so whenever there will be breaks and cuts on skins and soft tissue, it can easily disseminate.

Keywords: Wound, infections, distribution

Introduction
A wound refers to an injury to the skin or underlying tissues or organs. Dermal wounds are often caused by surgery, trauma, and chemicals or as a result of diseases (Agyare, 2013) [1]. Intentionally created dermal wounds can be incisional, whereby the wound is brought about by surgical cutting into the skin with a scalpel or excision wound created when a part of the skin is cut off. The process of tissue repair after an insult to the tissue (wound) is called 'wound healing'.

Wound healing is an intricate process in which usually the skin repairs itself. The process involves four overlapping phases: haemostasis (ceasation of bleeding), inflammation, proliferation, and remodeling (Pandith, 2013) [10].

Wound infection occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulitis around a wound (Pandith, 2013) [10].

The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors, including the type, site, size, and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load, and the combined level of virulence expressed by the types of microorganisms involved.

Gram-positive bacteria (such as *Staphylococcus aureus*) possess an inner cytoplasmic membrane and thick peptidoglycan layer functionalised with teichoic acids. These anionic glycopolymers play roles in regulating cell morphology and division, cell adhesion and defence against temperature, osmotic and toxic stresses (Romaniuk and Cegelski, 2018) [11].

Gram-negative bacteria (such as *Pseudomonas aeruginosa*) have a thin peptidoglycan layer sandwiched between an inner cytoplasmic membrane and outer lipopolysaccharide membrane. The latter component offers osmo-protection, regulating the permeability of the cell and the influx and efflux of nutrients and toxins, whilst the presence of outer membrane proteins, or porins, creates channels that permit the passage of molecules in and out of the cell (Delcour, 2009) [4]. The study aimed at to determine the Isolation and Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from Wound Patients in Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Methodology
Study Area
This study was carried out at Alex Ekwueme Federal University Teaching Hospital Abakaliki, and Ebonyi State University Abakaliki. Both are in Abakaliki town, the capital city of Ebonyi State. Alex Ekwueme Federal University Teaching Hospital Abakaliki is located at 6.32ºN latitude and 8.12ºE longitude and is situated at an elevation of 117 meters above sea level, while Ebonyi State University Abakaliki is located at 6.20ºN latitude and 8.6ºE longitude. Abakaliki is populated and inhabited by indigenes and people from other parts of Nigeria (Ezegwui *et al.*, 2013) [6].

Specimen collection
Wound swabs were collected from 229 wound patients from March 2023 to May 2024. The Swabs were collected following aseptic methods and adhering to the Standard Operating procedure of the General outpatient department.

Laboratory Analysis

The media used were: (Nutrient Agar (NA), MacConkey Agar (MA), Cysteine Lactose Electrolyte Deficiency (CLED), Peptone Water (PW), Mueller-Hinton Agar (MHA), Preparation of Simmon's Citrate Agar, Preparation of Peptone Water). All media were prepared following strict adherence to the manufacturer's instructions.

Identification of Isolates

The organisms were identified based on gram staining and their reactions to standard microbiology biochemical tests which include: (Motility test, Indole test, Oxidase test, Catalase test, Methyl red test, Voges Proskauer test, Simmon's Citrate Agar, Coagulase test, Deoxyribonuclease (DNA-ase test) test, Nitrate reduction test (NR), Triple Sugar – Iron test).

Results

Morphology and biochemical characteristics of *Pseudomonas aeruginosa and Staphylococcus aureus* from wound samples of patient attending Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA).

Table 1 showed the result of morphological and biochemical characteristics of the bacteria isolated from wound swab samples. The test result revealed Gram-negative rod bacteria, motile, catalase negative, oxidase positive, indole negative, methyl red and Voges-Proskauer negative and the isolate was identified as *Pseudomonas aeruginosa*, on the other hand, the characteristics of Gram-positive organisms in cocci shape bacteria, non-motile, coagulase positive, catalase positive, oxidase negative, indole negative, methyl red and Voges-Proskauer positive were observed and identified as *Staphylococcus aureus*.

Percentage distribution of Bacterial Isolates from Wound Swabs Infected Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Table 2 showed that out of the 229 wound swabs analyzed, Gram positive and Gram-negative bacteria were isolated in 132 (57.6 %) samples, indicating distribution of 39.4 % (52/132) *Pseudomonas aeruginosa* and 60.6 % (80/132) *S. aureus*. This indicates that *S. aureus* is most prevalent and may pose a serious threat to the patients and the hospital.

Table 1: Morphology and biochemical characteristics of *Pseudomonas aeruginosa and Staphylococcus aureus* from wound samples of patient attending Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA)

Gram	Shape	Coagulase	Catalase	TSI	Oxidase	Motility	VP	Methyl red	Indole	Citrate	Glucose	Lactose	Galactose	Arabinose	Sorbitol	Xylose	Fructose	Bacterial isolates
-	Rod	-	-	A	+	+	-	-	-	+	+	-	+	-	-	+	+	*P. aeruginosa*
+	Cocci	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	+	*S. aureus*

KEYS: A/A- Acid/Alkaline, A-Acid, A/G-Acid/Gas, VP-Voges-Proskauer, TSI-Triple Sugar Iron, (+)- positive, (-)- negative

Table 2: Percentage Distribution of Bacterial Isolates from Wound Swabs of Infected Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA)

Bacterial isolates	Frequency	Percentage (%)
Staphylococcus aureus	80	60.6
Pseudomonas aeruginosa	52	39.4
Total	132	100

Discussion of Results

In this study, the most common isolate was *S. aureus* 88 (60.6 %) as shown in Table 2, which was also reported in many other studies to be the predominant bacteria (40–60 % of the total bacteria) isolated from different types of wounds (Davies *et al.*, 2004; Gjødsbøl *et al.*, 2006; Körber *et al.*, 2010; Urbancic-Rovan and Gubina, 2000) [3, 7, 9, 14]. *P. aeruginosa* 52 (39.4 %) was the Gram-negative bacterium detected out of 132 isolates, which is also in agreement with other reports of Burmølle *et al.*, 2010 [2]; Davies *et al.*, 2004 [3]; Serra *et al.*, 2015 [13]; Gjødsbøl *et al.*, 2006 [7], who reported that *S. aureus* and *P. aeruginosa* play a central role, colonizing about 93.5 % and 52.2 % of patients with chronic leg ulcers, respectively. This finding is also in line with the work of Guan *et al.*, 2021 [8], who reported that the most common bacteria isolates among patients with chronic wounds were *S. aureus* (29.2 %), *E. coli* (11.4 %), *P. aeruginosa* (11.0 %), *Proteus mirabilis* (8.0 %) and *Klebsiella pneumoniae* (5.8 %).

The possible reason for the high frequency of *S. aureus* and *P. aeruginosa* may be that these bacteria are commonly found in the hospital environment. In addition, *S. aureus* is a normal flora of healthy person (especially on skin), so whenever there will be breaks and cuts on skins and soft tissue, it can easily disseminate.

It is well documented that bacteria such as *S. aureus* and *P. aeruginosa* produce very destructive virulence factors, responsible for maintaining infection and delay healing in chronic wounds. *S. aureus* causes clinically relevant infections mostly because of its virulence factors such as coagulase, catalase, clumping-factor A and leucocidines (Dissemond, 2009) [5]. Similarly, the production of an elastase by *P. aeruginosa* has been associated to its pathogenicity in the wound environment (Schmidtchen *et al.*, 2003) [12].

Conclusion

Wound infections are underestimated problems that result into a chronic disease and the detection of microbial species and pathogens distribution patterns are important aspects, often underestimated, in order to limit the spread of bacteria isolates. It is well documented that bacteria such as *S. aureus* and *P. aeruginosa* produce very destructive virulence factors, responsible for maintaining infection and delay healing in chronic wounds. *S. aureus* causes clinically relevant infections mostly because of its virulence factors such as coagulase, catalase, clumping-factor A and

leucocidines (Dissemond, 2009) [5]. Similarly, the production of an elastase by *P. aeruginosa* has been associated to its pathogenicity in the wound environment (Schmidtchen *et al.,* 2003) [12].

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