Original Research Article

ISOLATION AND IDENTIFICATION OF BACTERIA FROM WOUND SEPSIS AMONG PATIENTS AT OBIGWE ORTHOPEDIC CLINIC, AMORJI UBAHA, OKIGWE

ABSTRACT

Infection in wound delays healing and may cause wound breakdown, herniation, and complete wound dehiscence. This study investigated the isolation and identification of bacteria associated with wound sepsis. A total number of ten (10) swab samples were collected at random from the wound surface of patients with infected wounds. The swabs with the samples were analyzed using standard microbiological procedures. Bacterial isolates were characterized using their colonial, microscopic, and biochemical properties. Identification was with reference to Bergey's Manual. It was observed that *Escherichia coli* is the most frequently occurring isolates with a percentage occurrence of (25%) followed by *Staphylococcus aureus* with a percentage of (20%), then *Pseudomonas aeruginosa* (20%), *Klebsiella pneumonia* (20%), and *Streptococcus pyogenes* (15%). The overall distribution of bacterial isolates from the wound samples indicated that the wound samples from the male patients have a higher percentage distribution of (80%) than the samples obtained from the female patients with (20%). This study revealed that various bacteria, including opportunistic bacteria, are found in different wounds. Some of the wounds were infected with more than one bacteria species at a time. Although complete eradication of wound infections is not possible, however, by adopting prompt, clean surgical procedures, proper care of wounds, and antibiotics, the incidence of wound infection may be limited to a minimum.

***Keywords**: Antibiotics; Escherichia coli; Klebsiella pneumonia; Pseudomonas aeruginosa; Staphylococcus aureus; Wound sepsis;*

1. INTRODUCTION

Wounds refer to injuries that break the skin, either breaking the cell membranes or a laceration, usually resulting in damage to underlying tissues [1]. They are usually a result of violence, accidents, and surgery leading to the loss of the integrity of the first line of defense (skin) and exposure of the subcutaneous layer. Wound provides a moist, warm, and nutritive environment conducive to microbial and colonization, which results in infection [1]. Wounds make sepsis more expected following the entry of microorganisms into the wound if not treated well. Sepsis is a toxic condition resulting from the spread of bacteria or their toxic products from a focus of infection [2]. Wound sepsis occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host's natural immune system. The subsequent invasion and dissemination of microorganisms in viable tissue provoke 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 [3].

Wound infection poses a global health challenge leading to delay in wound healing and surgical complications like dehiscence or wound breakdown [4]. Despite the latest advances in technology, wound infection has been identified as the most common nosocomial infection, especially in patients undergoing surgery [4], resulting in a prolonged hospital stay, increased trauma care, treatment costs, and more demanding general wound management practices [1].

Evidence from bacteriological studies has shown that many bacteria species are universally involved in wound infection. This variation is due to geographical locations, bacteria resident on the skin, clothing at the wound site, the time between the wound, and examination [3]. Several bacterial genera cause wound infections [5]. Isolates found in cases of wound infections include *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella aerogenes, Escherichia coli, Staphylococcus epidermidis, Streptococcus pyogenes,* and *Streptococcus faecalis*, *Candida albicans* and *Candida tropicalis* have also been implicated as etiological agents [6]. These microorganisms are transmitted into the wound through various ways, including direct contact with the organisms, which are either transferred from surgical equipment airborne dispersal from the environment contaminated with microorganisms deposited onto the wounds. Contamination could also occur through one-self by physical migration of the patient's endogenous flora on the skin, mucous membrane, or gastrointestinal tract to the surgical site [7].

In treating wound infections, several antibiotics are used. Ideally, proper antibiotics are given after the culture and sensitivity of isolates from the wound swab, pus, or infected tissue. Unfortunately, this practice is uncommon among physicians, especially in developing countries. Consequently, improper use of broad and narrow-spectrum antibiotics leads to drug resistance [8]. The control of wound infections has become more challenging due to pervasive bacterial resistance to antibiotics and increased infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL) producing gram-negative bacteria, and polymicrobial flora [3]. Therefore, the effects of antimicrobial resistance cannot be over-emphasized, and it can increase complications and costs associated with procedures and treatment [9].

Knowledge of the causative agents of wound infection has proven helpful in selecting empiric antimicrobial therapy and on infection control measures taken in health institutions. Therefore, this study aims to identify and characterize isolates from wound sepsis to select the proper antimicrobial agent needed for their eradication.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 10 wound swab samples were collected from patients at Obigwe Orthopaedic Clinic, Amorji Ubaha, Okigwe, using labeled sterile swab sticks. The wound was first cleansed with normal saline (irrigation) to remove superficial debris and a swab of purulent exudates; wound secretion was aseptically obtained using sterile cotton swab sticks for each patient. The wound specimen was collected on moistened cotton swab sticks to swab across the wound surface in a zigzag motion, without contaminating the skin commensals. The swabs taken were immersed in a small tube and labeled. Soon after collection, each sample was taken to the laboratory of microbiology, Abia State University, Uturu.

2.2 Preparation of Samples

On getting to the laboratory, the samples were stored immediately in a refrigerator at a temperature of $4^{o}C$ to prevent the microbes' proliferation.

2.3 Culture of Samples

The collected swab specimens were streaked on freshly prepared nutrient agar and placed in the incubator. The swab specimens were incubated for 24 hours at $37^{0}C$; the plates were examined for growth. Isolates from the labeled nutrient agar plates were further sub-cultured into MacConkey agar (selective for only gram-negative enteric organisms) and blood agar using a sterile wire loop, and pure cultures were obtained. According to manufacturers ' instructions, all growth media used in his process were prepared and autoclaved appropriately.

2.4 Morphological characterization, gram reaction, and microscopic examination of isolates.

Before isolating the pathogens involved in wound sepsis, macroscopic examinations (for cultural characteristics such as colony size, pigmentation, elevation, and margin) were studied after incubation. Different morphological features of the yielded colonies, including color, size, shape, margin elevation, and texture, were recorded.

Following the macroscopic examination, the Gram staining technique was used in this experiment to help identify pathogens by their Gram reaction. According to Cheesbrough [10], the procedure was carried out as follows; the smear was prepared from an overnight culture on a clean, grease-free glass slide. The smear was left to air dry. After that, the slide was rapidly passed three times through the flame of a Bunsen burner and then allowed to cool before staining. The fixed smear was covered with Crystal violet stain for 60 seconds and then washed in slow-running clean tap water. The smear was tipped off all the water before being covered with Lugol's iodine (mordant) for 60 seconds. The stain was washed off in slow-running clean tap water. Acetone was used to decolorize the smear rapidly and immediately washed in slow-running clean tap water. Finally, the smear was covered with Safranin stain for 2 minutes and washed in slow-running clean tap water. The back of the slide was wiped clean and placed in a draining rack for the smear to air dry. A drop of oil was added to the dried smear got examined under the microscope with x100 oil immersion objective.

2.5 Biochemical tests

After microscopic examination, the isolates were characterized using the following biochemical tests: catalase test, coagulase test, citrate test, oxidase test, and indole test using the standard operating procedure in Cheesbrough [10]. After biochemical tests, the ninth edition of Bergey's Manual of Determinative Bacteriology was used to identify the pathogens from wound sepsis as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia, and Streptococcus pyogenes*.

2.6 Percentage occurrence of isolates

The percentage occurrence of isolates was determined by dividing the number of times a particular isolate appeared by the total number of isolates and then multiplied by hundred (%).

3. RESULTS

3.1 Morphological identification, biochemical identification, gram reaction of bacterial isolates from the wound sepsis.

Table 1 shows the Morphological identification, Biochemical Identification, and Gram Reaction of bacterial isolates from the wound sepsis. The bacterial isolates obtained from this study include; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia, and Streptococcus pyogenes*.

3.2 Percentage occurrence of the isolates from the wound sepsis.

Table 2 shows the percentage occurrence of the isolates from the wound sepsis. It was observed that *E. coli* is the most frequently occurring isolate with a percentage occurrence of 5(25%), followed by *Staphylococcus aureus* 4(20%), *Pseudomonas aeruginosa* 4(20%), *Klebsiella pneumoniae* 4(20%), and *Streptococcus pyogenes* 3(15%).

3.3 Distribution of bacteria isolates from the wound swab samples.

Table 3 shows the distribution of bacteria isolates from the wound swab samples. The overall distribution of bacterial isolates from the wound samples indicated that the wound samples from the male patients have a higher percentage distribution of (80%) than the samples obtained from the female patients with (20%).

Table 1: Characterization and identification of isolates from Wound Sepsis

Specimen	Shape	Size	Margin	Color	Elevation	Texture	Gram reaction	CA	CI	CO	IN	OX	Probable isolates
Male 1A	Circular	Small	Entire	Dark pink	Flat	Moist	Negative bacilli (pairs)	+	-	-	+	-	*E. coli*
Male 1B	Circular	Small	Entire	Golden-yellow	Convex	Smooth	Positive cocci (cluster)	+	+	+	-	-	*Staphylococcus aureus*
Male 2A	Circular	medium	Entire	off-white	Flat	Smooth	Negative bacilli (chains)	+	+	-	-	+	*Pseudomonas aeruginosa*
Male 2B	Ovoid	Small	Entire	Grayish white	Raised	Smooth	Positive cocci (chains)	-	-	-	-	-	*Streptococcus pyogenes*
Male 3A	Circular	medium	Entire	Pink	Convex	Mucoid	Negative bacilli (pairs)	+	+	-	-	-	*Klebsiella pneumoniae*
Male 3B	Circular	Small	Entire	Golden-yellow	Convex	Smooth	Positive cocci (clusters)	+	+	+	-	-	*Staphylococcus aureus*

Male 4A	Circular	Small	Entire	Dark pink	Flat	Moist	Negative bacilli (pairs)	+	-	-	+	-	*E. coli*
Male 4B	Circular	Large	Entire	Creamy	Convex	Mucoid	Negative bacilli (chains)	+	+	-	-	-	*Klebsiella pneumoniae*
Male 5A	Circular	Small	Entire	Off white	Flat	Smooth	Negative bacilli (pairs)	+	+	-	-	+	*Pseudomonas aeruginosa*
Male 5B	Ovoid	Small	Entire	Grayish white	Raised	Smooth	Positive cocci (chains)	-	-	-	-	-	*Streptococcus pyogenes*
Male 6A	Circular	Medium	Entire	Pink	Convex	Mucoid	Negative bacilli (short chains)	+	+	-	-	-	*Klebsiella pneumoniae*
Male 6B	Ovoid	Small	Entire	Grayish white	Raised	Smooth	Positive cocci (chains)	-	-	-	-	-	*Streptococcus pyogenes*
Male 7A	Circular	Small	Entire	Dark pink	Flat	Moist	Negative bacilli (clusters)	+	-	-	+	-	*E. coli*
Male 7B	Circular	Large	Entire	Green	Flat	Mucoid	Negative bacilli (pairs)	+	+	-	-	+	*Pseudomonas aeruginosa*
Male 8A	Circular	Small	Entire	Dark pink	Flat	Moist	Negative bacilli (pairs)	+	-	-	+	-	*E. coli*

Male 8B	Circular	Small	Entire	Golden yellow	Convex	Smooth	Positive cocci (clusters)	+	+	+	-	-	*Staphylococcus aureus*
Female 1A	Circular	Medium	Entire	Pink	Convex	Mucoid	Negative bacilli (clusters)	+	+	-	-	-	*Klebsiella pneumoniae*
Female 1B	Circular	Small	Entire	Golden yellow	Convex	Smooth	Positive cocci (clusters)	+	+	+	-	-	*Staphylococcus aureus*
Female 2A	Circular	Small	Entire	Dark pink	Flat	Moist	Negative bacilli (pairs)	+	-	-	-	+	*E. coli*
Female 2B	Circular	Large	Entire	Green	Flat	Mucoid	Negative bacilli (pairs)	+	+	-	-	+	*Pseudomonas aeruginosa*

KEY: a = MacConkey agar, b = Blood agar, + = Positive, - = Negative, CA. = Catalase, CI. = Citrate, CO. = Coagulase, IN. = Indole, OX=Oxidase,MR.=MethylRed.

Table 2: Percentage occurrence of bacterial isolates from wound sepsis

ISOLATES	NO OF ISOLATES	PERCENTAGE OCCURRENCE (%)
Escherichia coli	5	25
Staphylococcus aureus	4	20
Pseudomonas aeruginosa	4	20
Klebsiella pneumoniae	4	20
Streptococcus pyogenes	3	15
Total	20	100

Table 3: Distribution of Bacteria Isolates from the wound swab samples.

ISOLATES	PERCENTAGE DISTRIBUTION OF ISOLATES (%)		
	No of isolates	**Male**	**Female**
Escherichia coli	5	4(80%)	1(20%)
Staphylococcus aureus	4	3(75%)	1(25%)
Pseudomonas aeruginosa	4	3(75%)	1(25%)
Klebsiella pneumoniae	4	3(75%)	1(25%)
Streptococcus pyogenes	3	3(100%)	0(0%)
Total	20	16(80%)	4(20%)

4. DISCUSSION

Wound sepsis is one of the most common infections in which bacterial flora proliferates and delays wound healing. It is a significant complication of wounds with a significant increase in costs, morbidity, and potential mortality [2]. Wound sepsis occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host's natural immune system. The subsequent invasion and dissemination of microorganisms in viable tissue provoke a series of local and systemic host responses [11]. Wound sepsis is one of the most common hospital-acquired infections, a significant cause of morbidity, and accounts for 70-80% mortality [12]. The development of such infections represents delayed healing, causes anxiety and discomfort for patients, longer stays at hospitals, and significantly adds to the cost of healthcare services [13]. This study evaluated the bacteriology of patients' infected wounds. In this study, the predominant bacteria isolated were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia,* and *Streptococcus pyogenes* (Table 1). This conforms with earlier reports by Mahat et al. [14], which indicated that *S. aureus*, *Streptococcus spp, Enterococcus spp, Pseudomonas spp, E. coli, Acinetobacter Baumannii, E. aerogens, K. pneumonia, Streptococcus pyogenes,* and *C. freundii* were among the most commonly isolated bacteria from the wound. The report of Adeyemi (2012) in the same vein agrees with the findings of this work that the predominant bacteria isolated were *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus species, Bacillus species, Klebsiella aerogenes, Klebsiella oxytoca, Enterobacter species, Proteus mirabilis, Proteus Vulgaris, Pseudomonas aeruginosa,* and *Streptococcus pyogenes*. This is also similar to what was earlier reported by Aizza et al. [15] and Bessa et al. [16], in their studies to explore the common bacterial pathogens responsible for wound infection and determine the antibiotic susceptibility pattern of the bacterial isolates.

From the findings in this study, it was observed that *Escherichia coli* is the most frequently occurring isolates with a percentage occurrence of (25%) followed by *Staphylococcus aureus* with a percentage of (20%) then *Pseudomonas aeruginosa* (20%), *Klebsiella pneumonia* (20%), and *Streptococcus pyogenes* (15%). In all, Gram-negative bacilli are more prevalent than Gram-positive bacteria. The prevalence of 70% and 30%, respectively, is similar to the previously reported prevalence of 67% and 32% by [11]. Our report shows that *Escherichia coli* is the most common isolated Gram-negative bacilli, while *S. aureus* is

Gram-positive bacteria. This is consistent with earlier reports [17]. The trend in the rate of isolation of Gram-negative bacilli over the study period is that of an increase, while that of Gram-positive bacteria is one of decrease. Although these changes are not statistically significant, they are clinically meaningful. The two most critical bacterial pathogens are *S. aureus* and *E. coli.* This report shows an increase in their rates of isolation. This also is of clinical importance. The overall distribution of bacterial isolates from the wound samples indicated that the wound samples from the male patients have a higher percentage distribution of (80%) as compared to the samples obtained from the female patients (20%) (Table 3). This is similar to the findings of Aizza et al. [15], who reported that male patients with wound infection were 20% higher than female patients. The higher growth positive cases in male patients (58.8%) than in females (42.2%) was observed in this study and was supported by the similar studies carried out by Mahat et al. [14]. The slight difference noted is due to our social behavior where males are given superiority to the female and, if they get diseased, are brought immediately to hospitals compared to females for treatment.

5. CONCLUSION

This study concluded that contracting wound infection remains an ongoing problem. The main culprit for the wound infection is trivial organisms like *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella*, etc. Although complete eradication of wound infections is not possible, the incidence of wound infection may be limited to a minimum by taking preventive measures and adopting prompt, clean surgical procedures and proper care of wounds. Otherwise, wound infections may lead to the morbidity and mortality of a high count, especially in children.

6. RECOMMENDATIONS

The result obtained from this study will be helpful for policymakers in evaluating the infection control measures in hospitals. Routine antimicrobial susceptibility testing before antibiotic administration is highly recommended since it can play a pivotal role in limiting wound infections to a minimum. As the emergence of drug-resistant bacteria is less likely when there is empirical drug therapy, it fastens the process of wound healing; hence the appropriate use of drugs decreases the cost of wound infection treatment.

CONSENT

As per international standard or university standard written participant consent has been collected and preserved by the authors.

ETHICALAPPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

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