

Antibacterial Susceptibility Pattern of Bacteria Associated With Wound Infections in Benue State University Teaching Hospital Makurdi, Nigeria

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Abstract: Data on the isolated bacteria causing wound infections is currently needed in Nigeria to determine the best management practice and antibiotics to be adopted in wound infection treatment to reduce the cost, pains and improve recovery of affected patients. This work is aimed at identifying bacteria isolated from wound infections in Benue State Teaching Hospital Makurdi Nigeria. Samples were collected from ward patients. Swabs were collected from these patients using standard medical procedures and analyzed using cultured nutrient agar medium and cystine lactose electrolyte deficient agar (CLED) medium. *Klebsiella* spp, *Streptococcus* spp, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were bacteria isolated from wound infections. The abundance of these bacteria causing wound infection increases from *Streptococcus* spp (10%), *Klebsiella* spp (20%), and *Pseudomonas aeruginosa* (30%) to *Staphylococcus aureus* (40%). Both culture methods showed the same abundance pattern. Antimicrobial sensitivity pattern and inhibition of bacteria identified with wound infections were different across sampled antibiotics. However, this study revealed the bacteria responsible for wound infections and their antibiotic sensitivity pattern. The outcome could be useful in modelling antibiotics for the management of bacterial wound infections.

Key word: Antibacterial, *Klebsiella* spp, sensitivity, teaching hospital, wound

INTRODUCTION

Bacteria play crucial roles in the Earth's ecosystem, exerting a significant impact on both terrestrial and aquatic environments (Brown, 2015). Their constant efforts are vital for the cycling of essential nutrients like carbon, nitrogen, and sulfur (Bayer *et al.*, 2000). Additionally, bacteria play an important function in the nitrogen cycling process, as emphasized by James (2011). Bacteria serve various advantageous purposes, including their involvement in the creation of conventional foods like yoghurt, cheese, and vinegar. They also play crucial roles in biotechnology and genetic engineering, contributing to the production of substances such as drugs and vitamins. Additionally, Microbes like bacteria are essential tools in agriculture, human and animal digestion, and the biological control of pests (Frankel *et al.*, 2004). Despite this significant importance, bacteria have been identified as one of the causing factors for wound deterioration and decay (Lieberman and Neal, 2001). Although wound healing is a gradual process, that requires proper hygiene. Care should be taken to constantly clean wounds and management of the

patients affected wounds to reduce the possible invasion of bacteria that might prolong the healing process (Xin *et al.*, 2002).

Wounds are infected when microbes colonize a cut or opened injury, this can be responsible for either a worsening of the wound condition or a delay in the process of healing (Ainsworth and Plunkett, 2007). While most wounds contain some bacteria, infections occur when the body's immune defenses are unable to effectively manage the growth of bacteria (Mattes, 1998). The skin typically harbours bacteria, commonly referred to as flora, which is generally harmless when the skin is undamaged. However, if a wound disrupts the protective barrier created by the skin, the normal flora can proliferate in the injured area (Tonneson *et al.*, 2000). The primary bacteria that cause infections of wounds are often various *Staphylococcus* spp groups specifically *Staphylococcus aureus* (Halimi, 2003). Additionally, contamination from other body parts can contribute to wound infections. Increased risk of wound infection may result from inadequate wound dressing techniques and unhygienic conditions (Mikkelsen *et al.*,

2007). However, there are common causes of infected wounds like animal bites as well as trauma caused by physical injuries, post-surgical and burn wounds (Ismail, 2005).

Indicators of wound infection encompass an unpleasant odour characterized by fever or generalized chills, along with inflammation or escalating redness surrounding the wounded area. Additional signs involve heightened pain, swollen lymph nodes especially in the groin, neck or armpit, red lines on the skin extending away from the wound site, the presence of pus or drainage, on the wound and so on (Contardi *et al.*, 2017).

Studies have shown that microorganisms are responsible for wound infections (Daniel, 1999), but debates persist about the precise mechanisms through which these microorganisms cause infections. Additionally, wound colonization commonly involves a multitude of microorganisms, often with pathogenic potential (Cline *et al.*, 2002). Indeed, any wound is susceptible to infection. In cases of infection, failure of the wound to heal can lead to heightened patient trauma, elevated treatment expenses, and increased demands on general wound management practices. Despite advancements in medical practices and efforts to address septic conditions, septic wounds remain a frequent cause of morbidity (Eriksen *et al.*, 2005). As wound colonization commonly involves multiple potentially pathogenic microorganisms, every wound carries some risk of infection. Infection remains a significant complication of wounds, leading to increased morbidity and potential mortality. Wound infection poses a substantial challenge in wound management and is a significant contributor to healthcare costs worldwide. Research indicates that the average duration of hospital stays doubles, and corresponding hospitalization costs increase significantly when postoperative surgical wound infections develop. Gosling *et al.* (2003); hence, it was crucial to identify the causative

organism and assess antimicrobial sensitivity patterns to mitigate infections and promote judicious antimicrobial use. This research intends to identify the source and evaluation of antimicrobial susceptibility patterns of bacteria accountable for inducing wound infections in patients attending the State University Teaching Hospital in Benue State (BSUTH), Makurdi, Nigeria. The investigation was prompted by concerns about antibiotic misuse and overuse, which can contribute to the development of resistance, highlighting the necessity of this study.

MATERIALS AND METHODS

Study location: Makurdi is tropically dominated by Guinea savannah type of vegetation with an annual rainfall of about 1000 mm. The temperature ranges between 2.7°C - 24.7°C and the maximum of 29.7°C - 33.7°C (Metrological Service Department, Makurdi). There are two seasons; the wet (April – October) and dry (October – April) seasons each year.

Ethical clearance: Sample collection approval was granted by the ethical instituted committee panel of BSUTH, Makurdi, Nigeria, with an issuance of a letter of ethical clearance approval. The written informed consent questionnaire was filled out by all patients who participated in the study.

Sample collection: Swab samples were obtained from patients attending the BSUTH, Makurdi, Nigeria. The collected samples were carefully enveloped in sterilized aluminium foil paper to safeguard against contaminants and promptly conveyed to the lab within the Department of Microbiology at Joseph Sarwuan Tarka University, previously known as Federal University of Agriculture, Makurdi, Nigeria.

Sterilization method: All glassware used in the course of this work, such as measuring cylinders, pipettes, beakers, test tubes, and Petri-dish, were sterilized in the oven at 160°C and wire loop and inoculating needle were sterilized by flaming in a Bunsen

burner using blue flame to redness and cool before use.

Microbiological analysis: Pretreatment of samples serial dilution up to five folds (10^{-5}) in test tubes was carried out on samples, using sterile pipettes in each case. It was carried out with 1ml of the various stock solutions from the different samples in nine (9) ml in a tube of distilled water or dilution blank into 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} respectively.

Preparation of media: All media used were (nutrient agar, cystine lactose electrolyte deficient (CLED) agar and Mueller-Hinton agar) for inoculation and isolation of organisms were prepared aseptically. In most cases, the culture medium to be used was weighed in the required grams and poured into properly labelled conical flasks. Distilled water up to 500 ml was added into each of the conical flasks and was corked using cotton wool and aluminum foil. Media was sterilized in an autoclave at a temperature of 250°F i.e. 121°C for 15-30 minutes and was allowed to cool down to a temperature of 45°C before this was poured into Petri-dishes (Chesbrough, 2000).

Inoculation of samples: The serial dilution of 0.5 ml of each diluent with a dilution of 10^3 and 10^5 was inoculated into duplicate sterile Petri dishes using a sterile pipette. Thereafter, the prepared media (nutrient agar, CLED) were poured into the Petri dishes already inoculated, swirled to spread, and then allowed to solidify. Plates for bacteria growth were incubated at 37°C for a whole day i.e. 24hrs to ensure the growth of discrete colonies. Isolation of pure culture for further identification was carried out in which one discrete colony/ isolated, was taken from each culture plate and further sub-cultured onto a nutrient agar plate. Incubation for 24 hours at 37°C to obtain pure cultures for stock on agar slants was carried out.

Identification of isolates: Based on cultural, morphological, and biochemical characterization of standard microbiological

procedures described by Chesbrough (2006) isolates were identified as follows:

Gram reaction: The objective of this procedure distinguish Gram-positive organisms from Gram-negative ones. A sterilized wire loop in a red-hot Bunsen burner flame was used and brought to cool. Subsequently, a loopful of bacterial growth was collected from the agar plate and, application onto a grease-free clean microscopic slide was ensured. After adding one drop of normal saline, the mixture was emulsified or homogenized and heat-fixed by passing it over a flame three times. The sample on glass was then submerged with crystal violet for 30–60 seconds, washed off, and decolourized with acetone until no colour ran off the slide, followed by an immediate rinse in running tap water. To complete the staining process, the sterilized glass slide was stained with the counterstain (safranin) for one minute, washed off using distilled water (H_2O), and placed in a rack with cotton wool.

Biochemical tests: The test was carried out on isolated organisms based on their ability to produce enzymes or gases.

Catalase test: In a sterile test tube, three millilitres (3 ml) of hydrogen peroxide (H_2O_2) were introduced. Subsequently, a sterilized glass rod was employed to gather the organism and inoculate it onto the H_2O_2 . The solution was then monitored for the immediate formation of active bubbles, indicating a catalase-positive culture. Conversely, the absence of bubbles indicated a catalase-negative culture.

Coagulase test: To create two thick suspensions, two drops of saline were applied to a clean, grease-free microscope slide approximately 2 cm apart. Subsequently, a colony of the test bacteria was meticulously added to each spot. Following this, a loopful of blood plasma was introduced into the suspension and gently mixed. The glass slide was held and tilted for one minute. The presence of cell clumping in the bacterial suspension mixed with plasma is indicative of a positive coagulase test. The absence of clumping

indicates a negative test. The second suspension which no plasma was added served as control.

Indole test: Each bacteria isolate was grown (sub-cultured) in 5 ml of peptone water for 24 hours at 35°C and the addition of two drops of Kovacs reagent was ensured. The colour change from pale yellow to pink or cerise red indicates a positive result and no colour change with the indole reagent remaining pale yellow indicates a negative result.

Citrate test: The conical flask was sterilized, and 2.4 grams of Simon citrate was added to 100 ml of distilled water and further sterilized in an autoclave for 15 minutes. The solution was allowed to cool, after which the Simon citrate (media) was dispensed into a slant bottle and allowed to gel. A sterile wire loop was used to take a loopful of the bacteria inoculated into the Simon citrate medium and left for 24 hours. Colour change from the initial green to blue is a positive result while the absence of color change is a negative result.

Oxidase test: A Petri dish containing a fitted filter paper was utilized in this procedure. The filter paper was saturated with 2 to 3 drops of a newly prepared oxidase reagent. Subsequently, a colony of the test bacteria was smeared onto the filter paper using a sterilized wire loop. The appearance of a blue-purple colour within a few seconds was considered an indication of a positive oxidase test, while a no colour change or colour change longer than two minutes indicates a negative oxidase test result.

Colony count:

This was performed manually by counting the colonies formed on culture plates by illuminated transmitted light. Discrete colours appearing on the plate after appropriate incubation were counted and recorded. The total bacterial counts were obtained by counting discrete colonies on nutrient agar with the assumption that each colony was raised from one single

bacterium. Microorganisms present in the original sample were calculated using the formula:

$$\text{Count (cfu/ml)} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of sample plated}}$$

Where the volume of the sample plated is = 1 ml (Cheesbrough, 2000).

Antimicrobial sensitivity test: Each bacterial isolate underwent sub-culturing in 5 ml of peptone water for 24 hours at 35°C. A mixture of 16 grams of Mueller Hinton agar and 200 ml of distilled water was sterilized in an autoclave for fifteen minutes and brought to cool. Using a sterile pipette, 1 ml of the isolate was turned over into a Petri dish, and the media was poured into the dish, and left to gel. A sensitivity disc was placed on the agar, and the setup was allowed to incubate for 18-20 hours to assess the zone of inhibition.

To measure the zone of inhibition, a metric ruler was employed. The widest diameter of the circular zone was manually measured by placing the ruler across the zone. A larger zone of inhibition indicated greater susceptibility to the antibiotic. If the observed zone equaled or exceeded the standard zone specified by the Clinical and Laboratory Standards Institute (CLSI, 2006), the microorganisms studied are considered sensitive to the antibiotic. Conversely, a smaller zone than the standard observed size of microorganisms was categorized as resistant (Tenover, 2006).

RESULTS

Table 1 shows the frequency and percentage of bacteria isolated from wound infections on nutrient agar. The frequency and percentage of *Staphylococcus aureus* was 11 (55%), followed by *Pseudomonas aeruginosa* with a frequency of 7 and a percentage of 35%, while *Streptococcus* spp had the lowest frequency of 2 and a percentage of 10%.

Table 1: Frequency and percentages (%) occurrence of bacteria isolated from wound infections on nutrient agar

Isolates	Frequency	Percentage (%)
<i>S. aureus</i>	11	55
<i>P. aeruginosa</i>	7	35
<i>Streptococcus</i> spp.	2	10

Also, Table 2 shows the number of bacteria isolated from wound infections on CLED. *Staphylococcus aureus* has the highest frequency of (8) 40%, followed by *Pseudomonas aeruginosa* with a frequency

of (6) 30%, *Klebsiella* spp has a frequency of (4) 20%, while *Streptococcus* spp had the least frequency and percentage of 4 and 10% respectively.

Table 2: Frequency and percentage (%) of occurrence of bacteria isolated from wound infections on CLED

Isolates	Frequency	Percentage (%)
<i>S. aureus</i>	8	40
<i>Streptococcus</i> spp.	2	10
<i>P. aeruginosa</i>	6	30
<i>Klebsiella</i> spp	4	20

The antimicrobial sensitivity profile of all the bacterial isolates from wound infections and their zones of inhibition are shown in Table 3. *Staphylococcus aureus* shows a high sensitivity to amoxicillin, ciprofloxacin, ampiclox, gentamycin, and streptomycin but is resistant to erythromycin, rocephin, septrin augmentin, tarivid and sparfloxacin. The *Streptococcus* spp are highly sensitive to ciprofloxacin, amoxicillin, rocephin, septrin, erythromycin and resistant to chlorophenicol, sparfloxacin, augmentin, gentamycin, and pefloxacin.

Also, *Klebsiella* spp is highly sensitive to augmentin, gentamycin, pefloxacin and resistant to streptomycin, septrin, amoxicillin, sparfloxacin, and chlorophenicol. In addition, the antimicrobial sensitivity profile of *Pseudomonas aeruginosa* and its zone of inhibition in Table 3 indicates that *Pseudomonas aeruginosa* was highly sensitive to amoxalin, ciprofloxacin, streptomycin, pefloxacin, amoxicillin and gentamycin and resistant to septrin, augmentin and chlorophenicol.

Table 3: Antimicrobial sensitivity profile of bacterial isolates and their zones of inhibition

Isolates	GEN	ERY	REP	AMP	ZNF	SEP	STR	AUG	AMX	CPX	TVD	SFC	PEF	CHL	AXN
<i>Staphylococcus</i>	S (15)	R (-)	R (-)	S (20)	S (15)	R (-)	S (17)	R (-)	S (20)	S (15)	R (-)	R (-)	-	-	-
<i>Streptococcus</i>	R (-)	S (15)	S (10)	-	S (15)	S (20)	R (-)	R (-)	S (15)	S (17)	-	R (-)	R (-)	R (-)	-
<i>Klebsiella</i>	S (15)	-	-	-	-	R (-)	R (-)	S (18)	R (-)	-	-	R (-)	S (15)	R (-)	-
<i>P. aeruginosa</i>	S (13)	-	-	-	-	R (-)	S (16)	R (-)	S (15)	S (18)	-	-	S (17)	R (-)	S(20)

Key: R = Resistance, S = Sensitive, GEN = gentamicin, ERY = erythromycin, REP = rocephin, AMP = ampiclox, ZNF = zinacef, SEP = septrin, STR = streptomycin, AUG = augmentin, AMX = amoxicillin, CPX = ciprofloxacin, TVD = tarivid, SFC = sparfloxacin, PEF = pefloxacin, CHL = chlorophenicol, AXN = amoxallin - = not used.

DISCUSSION

The study identified four (4) species of bacteria associated with infected wounds. They included *Pseudomonas* spp, *Staphylococcus* spp, *Streptococcus* spp, and *Klebsiella* spp. Two of these species were observed to be more dominant among the isolates as reported in previous studies carried out by Mehedi *et al.* (2013). The authors reported that *Pseudomonas aeruginosa* was the most frequent isolate (46.1%) next was *Staphylococcus* spp. However, in this study, the reverse was the case as *Staphylococcus* spp had 55% and *Pseudomonas* spp had 35%. Therefore, their dominance in the sampled wounds was an indication of the fact the duo bacteria are the major inhabitants of wounds. According to the work reported by (Ananth *et al.*, 2014), *Staphylococcus* spp was the predominant bacterial strain in all 100% of the samples collected followed by *Pseudomonas aeruginosa*. Similarly, various bacterial isolates from various wound infections were reported by Valarmathi *et al.* (2013) to include *Staphylococcus aureus* (54.1%), *Klebsiella pneumonia* (20.8%), *Pseudomonas aeruginosa* (16.6%) and *Escherichia coli* (8.3%). They opined that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were commonly found in wound infections.

This study establishes *S. aureus* (40%) as the commonest bacterial infection followed by *Pseudomonas* spp. (30%), then *Streptococcus* spp. (10%) and *Klebsiella* spp. (20%). This result agrees with studies carried out within various parts of the country including Maidugri (Gadzama, *et al.*, 2007), Ibadan (Okesola and Kehinde, 2008), Ekpoma (Isibor *et al.*, 2008) and Benin-city (Egbe, *et al.*, 2011), some other foreign countries (Anbumani *et al.*, 2006). The elevated prevalence of *Staphylococcus* spp infection is attributed to its potential endogenous origin. Additionally, this may arise from environmental contamination, such as surgical instruments. *Staphylococcus aureus*, the most common bacterium on skin

surfaces, enters wounds easily as it can disrupt the natural skin barrier of humans (Nwankwo *et al.*, 2017). *Pseudomonas* spp. (30%) emerged as the next most frequent bacterial isolated. These bacterial species are commonly reported as the predominant pathogens in burns, and their high frequency may be linked to the anaerobic conditions of wounds, as suggested by Nwankwo *et al.* (2017). *Streptococcus* spp. and *Klebsiella* spp. (20%) were the third occurring bacteria found in this work. The bacteria are not spread through the air. Patients in hospitals face potential exposure to bacteria, particularly when using ventilators, with *Streptococcus* spp being a concern since it can be isolated from the mouths and nostrils of humans. Additionally, patients with intravenous catheters or surgical wounds are susceptible. Wounds may become infected if a patient touches their nose and then uses the same hand on the wound. Overcrowding in hospital wards has been identified as a vital factor responsible for the high rate of nosocomial infections (Amadi *et al.*, 2009).

The antimicrobial sensitivity profile of *Staphylococcus aureus*, as assessed against selected commercial antibiotics, revealed its tendency as resistant to a broad antibiotic spectrum. Specifically, *Staphylococcus aureus* in our study demonstrated resistance to erythromycin at a rate of 86.4%. This finding aligns with the work of Gelaw (2011) and Shamsuzzaman *et al.* (2003). The notable resistance of *Staphylococcus aureus* to these antibiotics is due to the presence of a Staphylococcal cassette chromosome, *mec* (SCC*mec*) a large genetic mobile element, which carries *mecA* genes, conferring it with a diminished ability to bind with antibiotics (Kurlenda, *et al.*, 2009). Notwithstanding, *Staphylococcus* spp was observed to be sensitive to gentamycin, ampiclox, zinacef, amoxicillin, ciprofloxacin, and septrin. *Streptococcus aureus* was found to be sensitive to zinacef, amoxicillin, ciprofloxacin, septrin, erythromycin, and rocephin. Also, *Klebsiella* was observed to be sensitive to gentamycin, augmentin, and

pefloxacin. According to Mohammed *et al.* (2013), vancomycin emerged as the most effective antibiotic against Gram-positive bacteria. In a separate study conducted in Kenya, gentamicin was found to be effective against isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* (Andhoga *et al.*, 2002). *S. aureus* and *P. aeruginosa* remain the predominant isolates from this study further agreeing with the publication of (Ochei and Kolhathar, 2000) which listed the duo bacteria as the most common types associated with wounds.

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