Prevalence, Sensitivity Profile and Resistance of Gram-Positive Bacteria in Wounds to Conventional Antibiotics

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MA designed the experiment, conducted the study and corrected the manuscript. Author MB corrected the manuscript and did the proof read. Authors IA and JA wrote the first draft of the manuscript. Author OA analyzed the data obtained. All authors read and approved the final manuscript.

ABSTRACT

Aim: The prevalence, sensitivity profile and resistance of Gram-positive bacteria in wounds to commercial antibiotics were ascertained in this study.

Place and duration of study: University of Medical Sciences Teaching Hospital, Akure, Nigeria, between January and June 2019.

Methodology: Wound swabs sample collection, isolation of bacteria, identification of Gram-positive bacteria isolates and antibiotics sensitivity testing of isolated bacteria were determined employing standard protocols.

Result: Three Gram-positive bacteria were isolated and presumptively identified to be S. aureus, S. epidermidis and S. pyogenes. S. aureus had the highest prevalence of 53% followed by S. epidermidis with 42% and S. pyogenes accounting for the least occurrence of 5%. Ninety percent
(90%) of ten *S. aureus* strains were resistant to ciprofloxacin while only 10% had intermediate activity. The least resistance of *S. aureus* strains was against pefloxacin (40%), while to streptomycin, 87.5% of eight *S. epidermidis* strains were resistant and 12.5% had intermediate sensitivity. Susceptibility was observed in *S. epidermidis* against pefloxacin (12.5%) while 50% had intermediate sensitivity and 37.5% were resistant. The highest zone of inhibition of *S. epidermidis* was observed in strain 7 against pefloxacin (16.00±1.00 mm) and in *S. aureus* by strain 5 against pefloxacin (16.50±2.50 mm).

**Conclusion:** Pefloxacin-sensitive *Staphylococcus* and *Streptococcus* species from wound swabs could become resistant overtime and this calls for incessant vigilance on Gram-positive wound bacteria antibiotic-susceptibility appraisal particularly in an antibiotics-abuse setting.

**Keywords:** Antibiotic; antibiotics sensitivity; Gram-positive bacteria; wound swab; prevalence.

1. INTRODUCTION

Every living organism evolves with survival instincts overtime. They do this by adapting to hostile environmental conditions resulting in resistance to harmful substances they are exposed to, majorly antibiotics [1]. Antibiotic resistance of bacteria refers to the genetic capability of bacteria to reduce the efficacy of a particular antibiotic through its physiological or structural traits [2]. Antibiotic resistance has accumulated over the last few decades, to become a major public health challenge globally. This resistance has been attributed to the consistent use and abuse of antibiotics and the reluctance of pharmaceutical industries to produce more efficacious antibiotics due to financial burden [3].

A wound is a type of injury on the skin either from accidents, surgery or puncture resulting in damage of the underlying tissue [4]. Wound contamination refers to the presence of microorganisms within a wound. Wound infection results due to the colonization of wound surface by pathogenic bacteria. Bacteriological studies have shown that wound infection is common and the bacteria types that occupy the wound vary geographically [5]. Wound infection by bacteria could result from direct contact with the pathogen through contaminated surgical equipment during surgery or airborne dispersal from a contaminated environment. Wound infection may also be self-inflicted by physical migration of patient’s endogenous microflora present on the skin, mucous membrane or gastrointestinal tract to the surgical site [6].

Gram positive bacteria include the group of bacteria with thick cell walls which enables them to yield a positive result to the Gram stain test [7]. Gram positive bacteria have played a major role in prolonging wound infections majorly due to their resistance to antibiotics and the continuous upsurge in the number of severely ill patients [8]. The appropriate knowledge of the pathogens and their resistant characteristics would play an important role in wound treatment process as well as infection control and prevention measures. Therefore, this study was intended to determine the antibiotic susceptibility profile of Gram-positive wound bacteria responsible for wound contamination and infection.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Bacteria

A total of 57 wound swabs were collected from patients in medical and surgical wards of University of Medical Sciences Teaching Hospital, Akure, Ondo State, Nigeria. The samples were carefully collected by medical personnel using sterile cotton swabs and immediately transported to the Department of Microbiology laboratory, Federal University of Technology, Akure, Nigeria. The samples were inoculated on nutrient agar and blood agar and incubated at 37 °C for 24 hours [9]. Each colony was sub-cultured to obtain pure cultures and Gram-stained to identify the Gram-positive isolates which were stored at 4°C.

2.2 Identification of Gram-Positive Bacterial Isolates

Bacterial isolates were presumptively identified using colonial characteristics including opacity, colour, elevation, surface, edge and shape and biochemical characteristics including Gram’s reaction, catalase, citrate, urease, oxidase and sugar fermentation tests [10].

2.3 Antibiotics Sensitivity Testing

The sensitivity of bacterial isolates to a panel of conventional antibiotics was performed using the
Kirby-Bauer method. Isolates were cultured at 37 °C for 24 hours and standardized to 0.5 McFarland standard as described by Alabi et al. [11]. A panel of ten antibiotics inclusive of pefloxacin (10 µg), gentamicin (10 µg), ampiclox (30 µg), zinacef (20 µg), amoxicillin (30 µg), rocephin (25 µg), ciprofloxacin (10 µg), streptomycin (30 µg), septrin (30 µg) and erythromycin (10 µg) was used to determine the sensitivity of each Gram-positive bacterium. The antibiotics discs were aseptically placed on the inoculated agar plates using sterile forceps and seeded plates were then incubated at 37 °C for 18 hours [12]. After incubation, diameters of zones of inhibition were measured to the nearest millimetre (mm) using a transparent meter rule and interpreted as stated by Clinical laboratory standard institute [13].

2.4 Statistical Analysis

Analysis of data was done using Statistical Package for Social Sciences (SPSS) version 26. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and means separated by Duncan’s New Multiple Range Test. Results are presented as mean± standard error.

3. RESULTS AND DISCUSSION

Three Gram-positive bacteria were isolated from wound swab samples and presumptively identified to be Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus pyogenes (Table 1). The frequency of occurrence of the bacterial isolates is presented in Fig. 1 with S. aureus having the highest prevalence of 53% followed by S. epidermidis with 42% and S. pyogenes accounting for the least occurrence of 5%. This finding is cohesive with the observations from Almeida et al. [14], Ekwiti et al. [15] and Baba et al. [5] which confirm the prevalence of the same isolates in wounds of hospitalised patients. Ohabughiro et al. [16] also reported the dominance of S. aureus and S. pyogenes in their study on orthopaedic wound infection in medical centres in South East Nigeria.

Tables 2-4 show the zones of inhibition of S. epidermidis, S. aureus and S. pyogenes respectively to a panel of ten antibiotics each. The zones of inhibition for S. epidermidis ranged from 6.00 to 16.00 mm, the recorded zones of inhibition for S. aureus ranged from 6.00 to 16.50 mm and the zones of inhibition ranged from 7.00 to 14.50 mm for S. pyogenes. The values of the zones of inhibition showed that S. epidermidis, S. aureus and S. pyogenes isolated from wounds vary in sensitivity to different commercially available antibiotics. However, the overall result showed extensive resistance of bacteria to the antibiotics which agrees with the result of Ohabughiro et al. [16] and Mahat et al. [17]. This elevated level of resistance of the isolates may be as a result of the promiscuous use of antibiotics without laboratory tests and doctor’s prescription.

The antibiotic susceptibility test carried out on the isolated Gram-positive bacteria showed that all ten (10) strains of S. aureus isolates were resistant to erythromycin, gentamicin, ampiclox, zinacef, amoxicillin and rocephin. Ninety percent (90%) of S. aureus strains were resistant to ciprofloxacin while only 10% had intermediate activity. The least resistance of S. aureus strains was against pefloxacin (40%) (Fig. 2). All eight (8) strains of S. epidermidis showed resistance to ciprofloxacin, septrin, erythromycin, gentamicin, ampiclox, zinacef, amoxicillin and rocephin. To streptomycin, 87.5% of the strains were resistant while 12.5% had intermediate sensitivity. Susceptibility (12.5%) was observed against pefloxacin while 50% had intermediate sensitivity and 37.5% were resistant (Fig. 3). A single strain of S. pyogenes was isolated in the experiment. S. pyogenes strain was resistant to eight of the ten antibiotics tested (ciprofloxacin, septrin, erythromycin, gentamicin, ampiclox, zinacef, amoxicillin and rocephin) while intermediate sensitivity was observed against streptomycin and pefloxacin (Fig. 4).

The high level of resistance of bacteria to antibiotics reported in this study agrees with the findings of Nagaraju and Divakar [18] who reported high resistance of bacteria to amoxicillin, streptomycin, ceftriaxone, gentamicin and erythromycin. Another experiment on the susceptibility pattern of Gram-positive bacteria from blood culture by Abebaw et al. [19] also reported the resistance of bacteria to ampiclox, amoxicillin and erythromycin which supports the findings of this study. However, the high resistance of bacteria in this study to ciprofloxacin negates the study of Mohammed et al. [20] who reported that ciprofloxacin had great inhibitory effect on S. aureus. This can be as a result of the level of exposure of the bacterial isolates to antimicrobials which in turn increases its resistance.
### Table 1. Biochemical Tests for the Bacteria Isolates

| Isolate number | Gram’s Reaction | Catalase | Citrate | Oxidase | Urease | Glucose | Fructose | Lactose | Sucrose | Mannitol | Maltose | Probable identity           |
|----------------|-----------------|----------|---------|---------|-------|---------|---------|---------|---------|----------|--------|--------------------------------|
| 1              | +               | +        | -       | +       | A     | A       | A       | A       | -       | A        |        | Staphylococcus epidermidis    |
| 2              | +               | +        | +       | +       | AG    | AG      | AG      | AG      | AG      | AG       |        | Staphylococcus aureus         |
| 3              | +               | -        | ND      | ND      | -     | A       | A       | A       | -       | A        |        | Streptococcus pyogenes        |

Key: + is Positive; - is Negative; A is Acid producing; AG - Acid and gas producing; ND – Not determined

### Table 2. Zones of inhibition (mm) of *Staphylococcus epidermidis* isolated from wound swabs against antibiotics

| Strains | CPXs ≥21 | SS ≥16 | SXTS ≥16 | ES ≥23 | PEFS ≥21 | CNS ≥15 | APXS ≥17 | ZS ≥23 | AMS ≥17 | RS ≥23 |
|---------|---------|--------|----------|--------|----------|---------|----------|--------|---------|--------|
| I =16-20 | R = ≤15 | I =12-14 | I =11-15 | I =14-22 | I =13-15 | I =14-16 | I =15-22 | I =14-16 | I =20-22 |        |

Results are presented as mean ± SE. Values carrying the same alphabet in similar row are not significantly dissimilar (P=0.05); Key: CPX= Ciprofloxacin; S= Streptomycin; SXT= Septrin; E= Erythromycin; PEF= Perfoxacin; CN= Gentamicin; APX= Ampicilox; Z= Zinacef; AM= Amoxicillin; R= Rocephin
### Table 3. Zones of inhibition (mm) of *Staphylococcus aureus* isolated from wound swabs against antibiotics

| Strains | CPXS = ≥21 | SS = ≥16 | SXTS = ≥16 | ES = ≥23 | PEFS = ≥16 | CANS = ≥15 | APXS = ≥17 | ZS = ≥23 | AMS = ≥17 | RS = ≥23 |
|---------|------------|----------|-------------|-----------|------------|-------------|------------|----------|-----------|----------|
|         | I =16-20   | I =12-14  | I =11-15    | I =14-22  | I =13-15   | I =13-14    | I =14-16   | I =15-22  | I =14-16   | I =20-22 |
|         | R = ≤15    | R = ≤11   | R = ≤10     | R = ≤13   | R = ≤12    | R = ≤13     | R = ≤14    | R = ≤13   | R = ≤14    | R = ≤19   |
| 1       | 13.00±1.00* | 7.00±1.00** | 9.00±1.00*** | 6.00±0.00* | 12.00±1.00** | 8.00±2.00** | 10.00±2.00** | 9.00±1.00*** | 7.50±0.50** | 9.50±0.50*** |
| 2       | 12.00±1.00** | 7.00±1.00** | 8.50±0.50** | 7.50±1.50** | 13.50±0.50** | 7.50±1.00** | 9.00±1.00** | 10.50±0.50** | 7.50±0.50** | 11.00±1.00** |
| 3       | 14.00±1.00** | 7.00±1.00** | 10.50±0.50** | 11.00±3.00** | 13.00±1.00** | 7.00±1.00** | 9.00±1.00** | 10.50±0.50** | 8.00±1.00** | 11.50±0.50** |
| 4       | 12.00±1.00** | 7.50±0.50** | 9.50±0.50** | 8.00±1.00** | 12.00±1.00** | 7.00±1.00** | 9.00±1.00** | 10.50±0.50** | 8.00±1.00** | 11.50±0.50** |
| 5       | 13.50±0.50** | 9.00±0.00** | 7.00±1.00** | 7.00±1.00** | 16.50±2.50** | 6.00±0.00** | 12.00±2.00** | 8.50±0.50** | 7.00±1.00** | 9.50±0.50** |
| 6       | 17.00±1.00** | 10.00±1.00** | 7.00±1.00** | 9.50±0.50** | 12.50±1.50** | 7.00±1.00** | 7.00±1.00** | 12.50±2.50** | 7.00±1.00** | 11.00±1.00** |
| 7       | 12.00±1.00** | 7.50±0.50** | 12.00±2.00** | 9.50±0.50** | 13.00±1.00** | 7.00±1.00** | 6.50±0.50** | 8.50±0.50** | 7.00±1.00** | 8.50±0.50** |
| 8       | 12.50±1.50** | 12.00±2.00** | 8.50±0.50** | 9.00±1.00** | 15.00±1.00** | 7.50±1.00** | 8.50±0.50** | 13.00±1.00** | 13.50±1.50** | 14.00±2.00** |
| 9       | 9.00±1.00** | 8.00±1.00** | 9.00±1.00** | 7.50±1.50** | 9.50±0.50** | 10.00±2.00** | 12.00±1.00** | 7.00±1.00** | 7.00±1.00** | 7.50±1.50** |
| 10      | 12.50±1.50** | 7.50±0.50** | 7.00±1.00** | 8.50±0.50** | 13.00±1.00** | 7.00±1.00** | 7.50±1.50** | 7.00±1.00** | 7.00±1.00** | 10.50±1.50** |

Results are presented as mean ± SE. Values carrying the same alphabet in similar row are not significantly dissimilar (P>0.05); Key: CPXS= Ciprofloxacin; SS= Streptomycin; SXTS= Septin; ES= Erythromycin; PEFS= Pefloxacin; CANS= Gentamicin; APXS= Ampliclox; ZS= Zinacef; AMS= Amoxicillin; RS= Rocephin

### Table 4. Zones of inhibition (mm) of *Streptococcus pyogenes* isolated from wound swabs against antibiotics

| Strains | CPXS = ≥21 | SS = ≥16 | SXTS = ≥16 | ES = ≥23 | PEFS = ≥16 | CANS = ≥15 | APXS = ≥17 | ZS = ≥23 | AMS = ≥17 | RS = ≥23 |
|---------|------------|----------|-------------|-----------|------------|-------------|------------|----------|-----------|----------|
|         | I =16-20   | I =12-14  | I =11-15    | I =14-22  | I =13-15   | I =13-14    | I =14-16   | I =15-22  | I =14-16   | I =20-22 |
|         | R = ≤15    | R = ≤11   | R = ≤10     | R = ≤13   | R = ≤12    | R = ≤13     | R = ≤14    | R = ≤13   | R = ≤14    | R = ≤19   |
| 1       | 12.50±1.50** | 13.00±2.00** | 7.00±1.00** | 7.50±1.50** | 14.50±0.50** | 7.00±1.00** | 9.00±1.00** | 8.00±2.00** | 8.00±2.00** | 12.50±1.50** |

Results are presented as mean ± SE. Values carrying the same alphabet in similar row are not significantly dissimilar (P>0.05); Key: CPXS= Ciprofloxacin; SS= Streptomycin; ES= Erythromycin; PEFS= Pefloxacin; CANS= Gentamicin; APXS= Ampliclox; ZS= Zinacef; AMS= Amoxicillin; RS= Rocephin
Strain 7 of *S. epidermidis* was susceptible to pefloxacin with zone of inhibition of 16.00±1.00 mm (Table 2). *S. aureus* strain 5 was also susceptible to pefloxacin with zone of inhibition of 16.50±2.50 mm (Table 3). *S. pyogenes* displayed intermediate sensitivity to pefloxacin at 14.50±0.50 mm (Table 4). Generally, the least resistance of Gram-positive bacteria in this study was against pefloxacin (Figs 2-4). Hence, it can be opined that pefloxacin is an effective antibiotic in treatment of bacterial infections. Sani et al. [21] also reported the high effectiveness of pefloxacin against Gram-positive bacteria from wounds.

Owing to the great resistance of bacteria against antibiotics, combination therapy (use of more than one antibiotic) can be an effective treatment option [22]. However, the indiscriminate use of antibiotics must be reduced to the barest minimum to avoid further increase in the rate of resistance. Another alternative is the use of natural products such as plant extracts as antimicrobials. These natural products have
multiple mechanism of action against bacteria and it is therefore difficult for bacteria to develop resistance against them [23]. However, in the use of these natural products, there is a need for standardisation for appropriate dosage and to prevent other adverse effects that can result from the use of herbal and other natural products [24].

4. CONCLUSION

Gram-positive bacteria isolated from wound swab samples are *S. aureus*, *S. epidermidis* and *S. pyogenes*. The sensitivity of Gram-positive bacteria in wounds to antibiotics varies proving the need for antimicrobial sensitivity testing
before drug prescription. The result of antibiotics sensitivity testing in this study showed high resistance to commonly used antibiotics. The least resistance was to pefloxacin. However, pefloxacin-sensitive *Staphylococcus* and *Streptococcus* species from wound swabs could become resistant over time, hence, the need for caution in the use of antibiotics without prescription. Combination therapy and the use of natural products as antimicrobials are promising solutions to the problem of increasing resistance of bacteria to antibiotics.

**CONSENT**

As per international standard or university standard, patient’s written consent has been collected and preserved by the author(s).

**ETHICAL APPROVAL**

Ethical approval for the collection of wound swabs from patients of Medical Sciences Teaching Hospital, Akure, Nigeria was collected from Ondo State Health Research Ethics Committee, Ministry of Health, Ondo State with number NHREC/18/08/2016.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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