PREVALENCE, SENSITIVITY PROFILE AND RESISTANCE OF GRAM-POSITIVE BACTERIA IN WOUNDS TO CONVENTIONAL ANTIBIOTICS

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 bacterial 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.~~taphylococcus~~ aureus*, *S.~~taphylococcus~~ epidermidis* and *S.~~treptococcus~~ 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%. n~~N~~inety 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%)~~. To~~ ,while streptomycin, 87.5% of eight *S. epidermidis* strains were resistant and ~~while~~ 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~~these 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 evolve 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 bacteria responsible for wound contamination and infection.

2. Material and methods

2.1 Sample c~~C~~ollection and ~~Isolation~~ isolation of ~~Bacteria~~bacteria

A total of 57 wound swabs were collected from patients in medical and surgical wards of ~~University~~ university of ~~Medical~~ medical ~~Sciences~~ sciences ~~Teaching~~ teaching ~~Hospital~~hospital, ~~Akure~~akure, ~~Ondo~~ ondo ~~State~~state, Nigeria. The samples were carefully collected by medical personnel using sterile cotton swabs and immediately transported to the ~~Department~~ department of ~~Microbiology~~ microbiology laboratory, ~~Federal~~ federal ~~University~~ university of ~~Technology~~technology, Akure, Nigeria. The samples were inoculated on nutrient agar and blood agar and incubated at 37 ^{o}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 ^{o}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 ^{o}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), ~~amplicl0x~~ (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 ^{o}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].

Comment [S1]: Composition

Comment [S2]: composition

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 Figure 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], Ekwati *et al*. [15] and Baba *et al*. [5] which confirms 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.

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	ND	A	-	A	A	-	A	*Streptococcus pyogenes*

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

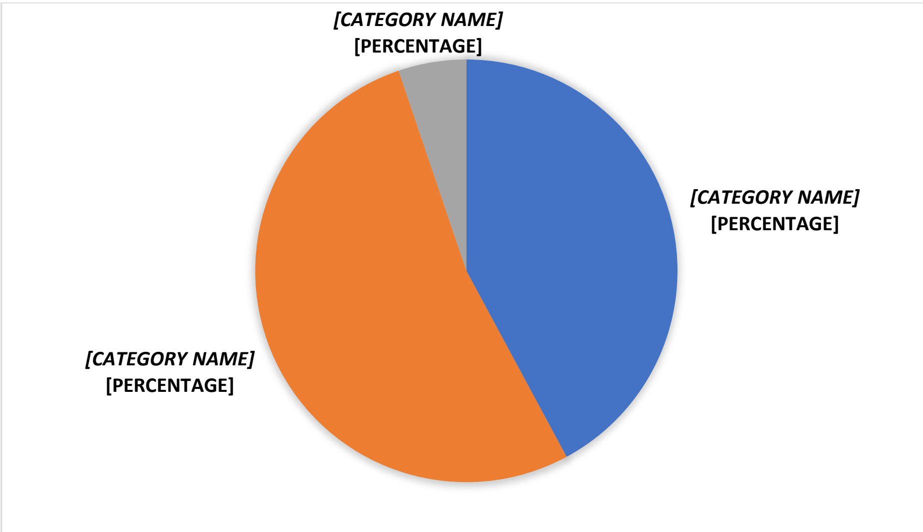

Figure 1- Prevalence of Gram-positive Bacteria in Wound Swab Samples

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.

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

Strains	**CPX** **S = ≥21** **I =16-20** **R = ≤15**	**S** **S = ≥16** **I =12-14** **R = ≤11**	**SXT** **S = ≥16** **I =11-15** **R = ≤10**	**E** **S = ≥23** **I =14-22** **R = ≤13**	**PEF** **S = ≥16** **I =13-15** **R = ≤12**	**CN** **S = ≥15** **I =13-14** **R = ≤12**	**APX** **S = ≥17** **I =14-16** **R = ≤13**	**Z** **S = ≥23** **I =15-22** **R = ≤14**	**AM** **S = ≥17** **I =14-16** **R = ≤13**	**R** **S = ≥23** **I =20-22** **R = ≤19**
1	12.00 ± 1.00^{bc}	8.50 ± 0.50^{a}	10.50 ± 0.50^{abc}	9.00 ± 1.00^{ab}	13.00 ± 1.00^{c}	7.50 ± 0.50^{a}	8.00 ± 1.00^{a}	9.00 ± 1.00^{ab}	10.00 ± 1.00^{abc}	10.50 ± 1.50^{abc}
2	14.00 ± 1.00^{d}	8.50 ± 0.50^{abc}	8.50 ± 0.50^{abc}	9.00 ± 0.00^{abc}	13.00 ± 1.00^{d}	6.00 ± 0.00^{a}	8.00 ± 0.00^{ab}	9.00 ± 0.00^{abc}	12.00 ± 2.00^{cd}	11.00 ± 2.00^{bcd}
3	13.50 ± 1.50^{b}	7.50 ± 0.50^{a}	8.50 ± 0.50^{a}	7.50 ± 0.50^{a}	13.50 ± 0.50^{b}	7.50 ± 0.50^{a}	12.00 ± 1.00^{b}	13.00 ± 1.00^{b}	8.50 ± 0.50^{a}	8.50 ± 0.50^{a}
4	15.00 ± 1.00^{d}	9.00 ± 0.00^{ab}	9.00 ± 1.00^{ab}	7.00 ± 1.00^{a}	14.00 ± 0.00^{cd}	9.00 ± 1.00^{ab}	10.00 ± 2.00^{abc}	11.00 ± 1.00^{abc}	10.00 ± 1.00^{abc}	12.00 ± 2.00^{bcd}
5	14.00 ± 1.00^{d}	9.00 ± 1.00^{abc}	7.00 ± 1.00^{ab}	8.00 ± 1.00^{abc}	11.50 ± 1.50^{cd}	6.50 ± 0.50^{a}	6.50 ± 0.50^{a}	10.50 ± 0.50^{bc}	9.00 ± 2.00^{abc}	8.50 ± 0.50^{abc}
6	14.00 ± 1.00^{c}	7.50 ± 0.50^{a}	6.50 ± 0.50^{a}	6.50 ± 0.50^{a}	9.00 ± 1.00^{ab}	6.50 ± 0.50^{a}	6.50 ± 0.50^{a}	11.00 ± 1.00^{b}	7.00 ± 1.00^{a}	7.00 ± 1.00^{a}
7	15.00 ± 1.00^{e}	10.00 ± 1.00^{cd}	7.00 ± 1.00^{ab}	8.00 ± 1.00^{abc}	16.00 ± 1.00^{e}	6.50 ± 0.50^{a}	7.00 ± 1.00^{ab}	11.50 ± 0.50^{d}	7.50 ± 0.50^{abc}	9.50 ± 0.50^{bcd}
8	13.50 ± 0.50^{a}	13.50 ± 2.50^{a}	9.00 ± 1.00^{a}	12.50 ± 0.50^{a}	12.50 ± 0.50^{a}	12.50 ± 1.50^{a}	13.50 ± 2.50^{a}	13.00 ± 1.00^{a}	10.00 ± 0.00^{a}	11.50 ± 0.50^{a}

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= Perfloxacin; CN= Gentamicin; APX= Ampliclox; Z= Zinacef; AM= Amoxicillin; R= Rocephin

Table 3: Zones of inhibition (mm) of *Staphylococcus aureus* isolated from wound swabs against antibiotics

Strains	CPX S = ≥21 I =16-20 R = ≤15	S S = ≥16 I =12-14 R = ≤11	SXT S = ≥16 I =11-15 R = ≤10	E S = ≥23 I =14-22 R = ≤13	PEF S = ≥16 I =13-15 R = ≤12	CN S = ≥15 I =13-14 R = ≤12	APX S = ≥17 I =14-16 R = ≤13	Z S = ≥23 I =15-22 R = ≤14	AM S = ≥17 I =14-16 R = ≤13	R S = ≥23 I =20-22 R = ≤19
1	13.00 ± 1.00^{c}	7.00 ± 1.00^{a}	9.00 ± 1.00^{abc}	6.00 ± 0.00^{a}	12.00 ± 1.00^{bc}	8.00 ± 2.00^{ab}	10.00 ± 2.00^{abc}	9.00 ± 1.00^{abc}	7.50 ± 0.50^{a}	9.50 ± 0.50^{abc}
2	12.00 ± 1.00^{de}	7.00 ± 1.00^{a}	8.50 ± 0.50^{abc}	7.50 ± 1.50^{ab}	13.50 ± 0.50^{e}	7.50 ± 0.50^{ab}	9.00 ± 1.00^{abc}	10.50 ± 0.50^{bcd}	7.50 ± 0.50^{ab}	11.00 ± 1.00^{cde}
3	14.00 ± 1.00^{d}	7.00 ± 1.00^{a}	10.50 ± 0.50^{abcd}	11.00 ± 3.00^{abcd}	13.00 ± 1.00^{cd}	7.00 ± 1.00^{a}	9.00 ± 1.00^{abc}	10.50 ± 0.50^{abcd}	8.00 ± 1.00^{ab}	11.50 ± 0.50^{bcd}
4	12.00 ± 1.00^{d}	7.50 ± 0.50^{a}	9.50 ± 0.50^{abcd}	8.00 ± 1.00^{ab}	12.00 ± 1.00^{d}	7.00 ± 1.00^{a}	9.00 ± 1.00^{abc}	10.50 ± 0.50^{bcd}	8.00 ± 1.00^{ab}	11.50 ± 0.50^{cd}
5	13.50 ± 0.50^{cd}	9.00 ± 0.00^{ab}	7.00 ± 1.00^{a}	7.00 ± 1.00^{a}	16.50 ± 2.50^{d}	6.00 ± 0.00^{a}	12.00 ± 2.00^{bc}	8.50 ± 0.50^{ab}	7.00 ± 1.00^{a}	9.50 ± 0.50^{ab}
6	17.00 ± 1.00^{c}	10.00 ± 1.00^{ab}	7.00 ± 1.00^{a}	9.50 ± 0.50^{ab}	12.50 ± 1.50^{b}	7.00 ± 1.00^{a}	7.00 ± 1.00^{a}	12.50 ± 2.50^{b}	7.00 ± 1.00^{a}	11.00 ± 1.00^{ab}
7	12.00 ± 1.00^{bc}	7.50 ± 0.50^{a}	12.00 ± 2.00^{bc}	9.50 ± 0.50^{ab}	13.00 ± 1.00^{c}	7.00 ± 1.00^{a}	6.50 ± 0.50^{a}	8.50 ± 0.50^{a}	7.00 ± 1.00^{a}	8.50 ± 0.50^{a}
8	12.50 ± 1.50^{bcd}	12.00 ± 2.00^{bcd}	8.50 ± 0.50^{ab}	9.00 ± 1.00^{abc}	15.00 ± 1.00^{d}	7.50 ± 0.50^{a}	8.50 ± 0.50^{ab}	13.00 ± 1.00^{cd}	13.50 ± 1.50^{d}	14.00 ± 2.00^{d}
9	9.00 ± 1.00^{ab}	8.00 ± 1.00^{ab}	9.00 ± 1.00^{ab}	7.50 ± 1.50^{a}	9.50 ± 0.50^{ab}	10.00 ± 2.00^{ab}	12.00 ± 1.00^{b}	7.00 ± 1.00^{a}	7.00 ± 1.00^{a}	7.50 ± 1.50^{a}
10	12.50 ± 1.50^{b}	7.50 ± 0.50^{a}	7.00 ± 1.00^{a}	8.50 ± 0.50^{a}	13.00 ± 1.00^{b}	7.00 ± 1.00^{a}	7.50 ± 1.50^{a}	7.00 ± 1.00^{a}	7.00 ± 1.00^{a}	10.50 ± 1.50^{ab}

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= Pefloxacin; CN= Gentamicin; APX= Ampliclox; Z= Zinacef; AM= Amoxicillin; R= Rocephin

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

Strains	CPX S = ≥21 I =16-20 R = ≤15	S S = ≥16 I =12-14 R = ≤11	SXT S = ≥16 I =11-15 R = ≤10	E S = ≥23 I =14-22 R = ≤13	PEF S = ≥16 I =13-15 R = ≤12	CN S = ≥15 I =13-14 R = ≤12	APX S = ≥17 I =14-16 R = ≤13	Z S = ≥23 I =15-22 R = ≤14	AM S = ≥17 I =14-16 R = ≤13	R S = ≥23 I =20-22 R = ≤19
1	12.50 ± 1.50^{bcd}	13.00 ± 2.00^{cd}	7.00 ± 1.00^{a}	7.50 ± 1.50^{ab}	14.50 ± 0.50^{d}	7.00 ± 1.00^{a}	9.00 ± 1.00^{abc}	8.00 ± 2.00^{abc}	8.00 ± 2.00^{abc}	12.50 ± 1.50^{bcd}

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= Pefloxacin; CN= Gentamicin; APX= Ampliclox; Z= Zinacef; AM= Amoxicillin; R= Rocephin

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, ampliclox, 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%) (Figure 2). All eight (8) strains of *S. epidermidis* showed resistance to ciprofloxacin, septrin, erythromycin, gentamicin, ampliclox, 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 (Figure 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, ampliclox, zinacef, amoxicillin and rocephin). Intermediate sensitivity was observed against streptomycin and pefloxacin (Figure 4).

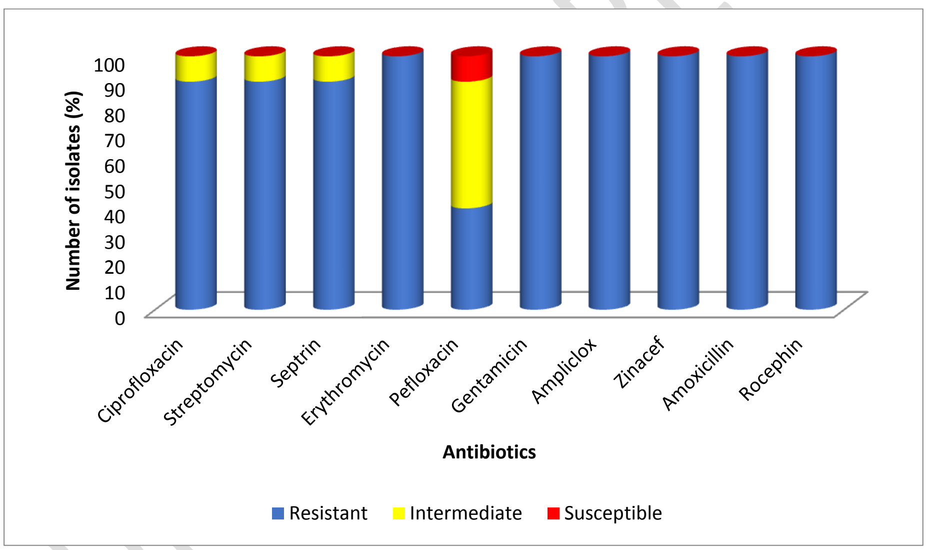

Figure 2: Sensitivity pattern of *Staphylococcus aureus* strains isolated from wounds

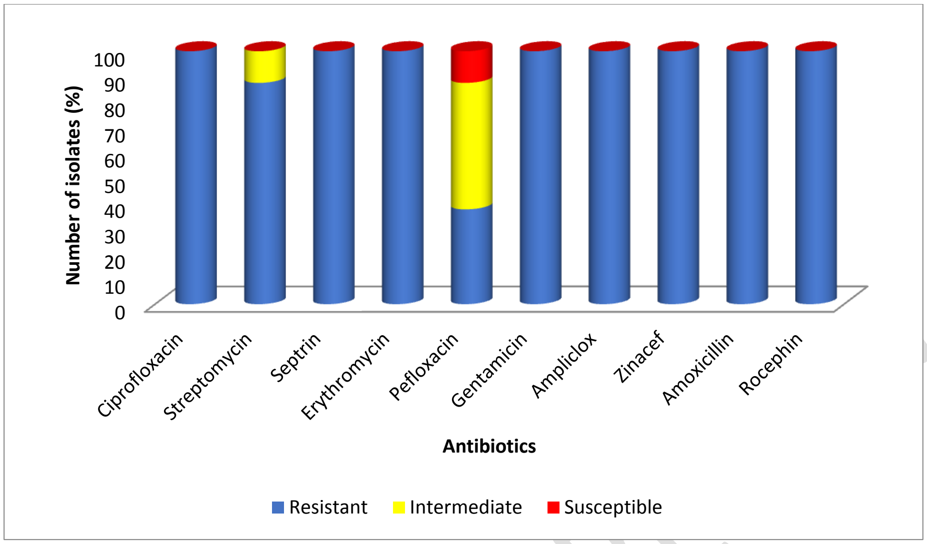

Figure 3: Sensitivity pattern of *Staphylococcus epidermidis* strains isolated from wounds

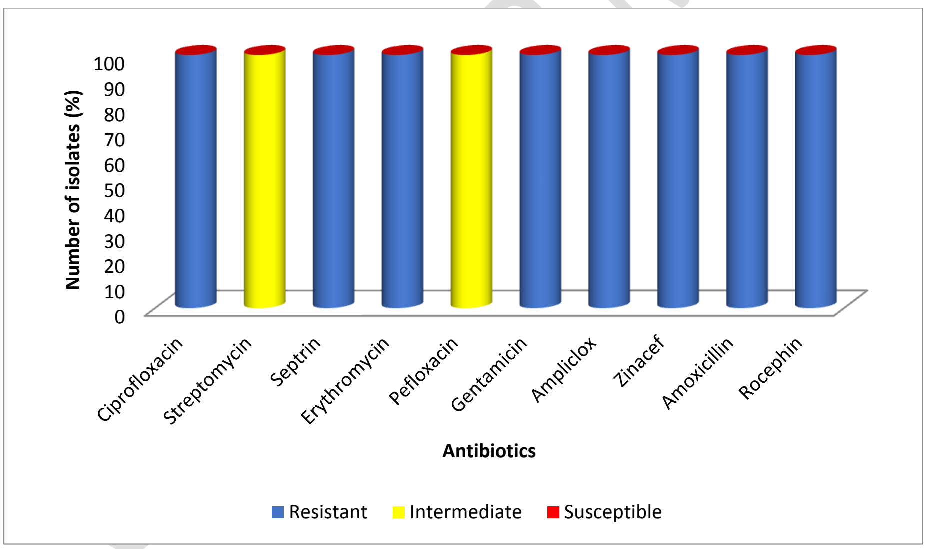

Figure 4: Sensitivity pattern of *Streptococcus pyogenes* strain isolated from wound

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 ampicillin, 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.

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 (Figures 2-4). Hence, it can be opined that pefloxacin is an effective antibiotic in treatment of bacterial infection. 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 overtime, 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.

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.

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