



EVALUATION OF PLASMID MEDIATED DRUGS RESISTANCE AMONG WOUND BACTERIAL INFECTION OBTAINED FROM PATIENTS ATTENDING SOME HOSPITALS IN KANO STATE, NIGERIA

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ABSTRACT: *This study was carried out to evaluate the plasmid mediated drugs resistance among wound bacteria isolates obtained from (352 swabs specimens) patients attending some hospitals in Kano State (National Orthopaedic Hospital Dala, Aminu Kano Teaching Hospital, Infectious Diseases Hospital, Bichi General Hospital and Wudil General Hospital). Swab specimens were obtained aseptically using the randomized sampling method and cultured on Blood and MacConkey agar media and incubated aerobically and anaerobically for 24 hours. Result showed that two hundred and twelve (212, 60.23%) out of 352 harbors some bacteria, which consist of six different species (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogenes*) as isolated from wound swabs specimens. *S. aureus* (39%) and *S. pyogenes* (7%) showed the highest and the lowest prevalences respectively. Antibiotic susceptibility testing was done using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar, where 140 (66%) isolates were susceptible while 72 (34%) isolates were resistant. Bacteria isolates exhibited the highest resistance to Cefixime, Azithromycin and Erythromycin and least resistance to Ciprofloxacin (most effective/active) among the antibiotics used. The resistant isolates were subjected to curing experiment. Plasmid detection and isolation showed that out of 72 resistant isolates, 49 contain plasmids which accounts for 68%. After plasmid curing and second sensitivity test, 38 (78%) isolates that were initially resistant becomes susceptible to the same antibiotics used. Hence, post plasmid-curing sensitivity test revealed increased susceptibility pattern of isolates to the previously used antibiotics. This indicates that plasmid-borne multidrug resistant gene had been denatured by the sodium dodecyl sulfate used as the curing agent. Therefore, this study justifies the need to encourage infection control, and proper treatment to prevent the spread and re-emergence of plasmid-mediated drugs resistant bacteria.*

KEYWORDS: Plasmid Mediated Drugs, Wound, Bacterial, Infection, Nigeria



INTRODUCTION

Hospitals worldwide are facing unprecedented crisis due to increasingly rapid emergence and dissemination of antimicrobial resistant bacterial species in wounds and its environments (Yah *et al.*, 2004a). Resistant bacterial species isolated from burns and surgical wounds are one of the leading causes of nosocomial infections, including pneumonia, urinary tract infections, and bacteremia (Pournaras *et al.*, 2003; Laura *et al.*, 2004). These infections can be particularly severe in cases of an impaired specific or nonspecific defense, such as that in neutropenic or cancer patients (Pollack, 2000). Therefore, acquired resistance to these agents constitutes a major challenge for anti-bacteria chemotherapy, especially when it is associated with resistance to other classes of antibiotics, such as beta lactams, aminoglycosides and fluoroquinolones (Laura *et al.*, 2004; Livermore *et al.*, 2002). Patients with wounds suffer from a significant prolonged stay, disability, deformation, cost of the in-patient treatment and death (Enabulele *et al.*, 1993; Steer *et al.*, 1996; Yah *et al.*, 2004b). A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue (Leaper & Harding, 1998). On the other hand, the term multidrug-resistant (MDR) applies to a bacterium that is simultaneously resistant to a number of antimicrobial drugs belonging to different chemical classes or subclasses through various mechanisms (Magiorakos *et al.*, 2012).

The increasing morbidity and mortality due to wound resulting from antibiotic resistance is on the increase therefore having a high bearing on the socio-economic status on the population (Neghesha *et al.*, 1996). The precise global burden of wounds is not known due to poor data from developing countries. However, in the developed world, the economic burden of chronic wounds is well documented (Macdonald *et al.*, 2010). In the United States, chronic wounds affect 6.5 million patients and the care costs over 25 billion dollars (Roy *et al.*, 2007; Sen *et al.*, 2009). Wound prevalence in India was 15.03/1000 of the population (acute wound was 10.55/1000 and chronic wound 4.48/1000) (Gupta *et al.*, 2004). Over the past few years, several studies in African countries had reported the presence of MDR strains of bacteria identified from clinical and environmental specimens (Zelege, 2002). The point prevalence of chronic wounds at a tertiary hospital in Nigeria was found to be 11% (Iyun *et al.*, 2016). This was consecutively ascertained by the findings of Olayinka *et al.* (2004), Chikere *et al.* (2008), and Nkang *et al.* (2009) all in Nigeria, Zelege (2002) in Ethiopia and Anguzu and Olila (2003) in Uganda.

Getting to know the drug resistance patterns possessed by etiologic agents of wound infections will greatly improve chemotherapeutic approaches in the treatment of wound infections. In this study, the knowledge of types of bacterial pathogens and antimicrobial resistance pattern can optimize treatment and decrease disease morbidity and mortality rates of surgical wound infections. The information obtained, will be used to update the present knowledge on multi antibiotics resistance in our community and will definitely help in developing proper measures aimed at controlling antibiotics resistant bacteria, and improving the quality of antibiotics prescription and usage among wound patients. This will also help to trace the resistance pattern among wound isolates in our environments where such trials have not been documented.



MATERIALS AND METHODS

This research was partly conducted in the below named hospital and partly in Microbiology laboratory, KUST Wudil. 352 samples were collected from some hospitals in Kano State, namely Aminu Kano Teaching Hospital; National Orthopedic Hospital, Dala-Kano; Infectious Diseases Hospital, Kano, Wudil; and Bichi General Hospital.

Ethical approval was obtained from Kano State Ministry of Health and Ethics Committee of the above named hospitals before the study moved on. Similarly, the patients or guardians (in cases of children) were issued with a consent form.

Sample Collection

A wound swab was obtained from each patient by the Levine's technique after cleaning the wound surface with saline. Samples were collected from pus of wounds, abscesses and other skin lesions using sterile cotton swabs. Sample collection was conducted by medical officers in the out-patient clinic and in the wards using commercially available sterile cotton swabs and following existing departmental guidelines. Only one swab per patient was collected after carefully cleaning the wound with normal saline in order to prevent surface contamination (Levine *et al.*, 1976). The samples were transported in an ice box to the microbiology laboratory at KUST, Wudil. (Chesbrough, 2006).

Bacteriological Culture and Examination

Swabs were inoculated on Nutrient agar, MacConkey agar and Chocolate agar and incubated at 37°C aerobically (N.A and MacConkey agar), and anaerobically (Chocolate agar) for 24 hours. This was done by following standard microbiological techniques (Chesbrough, 2006). Pure culture of the isolates was made by picking a single colony from the overnight colonies of bacteria and then sub-cultured on fresh plates. Bacterial colonies were picked and then sub-cultured into nutrient agar (Oxoid) slant and incubated at 37°C for 24-48 hours, and later stored in the refrigerator for further use (Cowan *et al.*, 2003; Chesbrough, 2006; CLSI, 2010). The isolates were subjected to Gram staining to differentiate Gram-positive from Gram-negative organisms.

Biochemical Test

Isolates were phenotypically identified using biochemical test (Biomérieux, Marcy l'Etoile, France) as previously described by APHA (2018), Chesbrough (2012), Burkhard *et al.* (2004), and Baker *et al.* (2001).

Sensitivity Test/Multidrug-resistance Testing

Susceptibility to antibiotic chemotherapy was done by the Kirby-Bauer disc diffusion method, and interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCL, 2001; CLSI, 2010). The sensitivity pattern was assessed using commercially prepared antibiotic discs, produced by CelTech Diagnostic, for Gram-positive and Gram-negative bacteria which contained different classes of antimicrobials usually prescribed in those hospitals. For Gram-positive, the antibiotic discs used include (code: BDR002) Imipenem/Cilastatin (IMP 10ug), Cefuroxime (CXM 30ug), Ofloxacin (OFX 5ug), Erythromycin (ERY 15ug), Gentamycin (GN 10ug), Azithromycin (AZN 15ug),



Amoxicillin/Clavulanate (AUG 30ug), Cefotaxime (CTX 25ug), Ceftriaxone Sulbactam (CRO 45ug), Cefixime (ZEM 5ug), Levofloxacin (LBC 5ug), and Ciprofloxacin (CIP 5ug). For Gram-negative isolates, the antibiotic discs used include (code: BDR003) Nitrofurantoin (NF 300ug), Cefuroxime (CXM 30ug), Ceftriaxone Sulbactam (CRO 45ug), Ampiclox (ACX 10ug), Cefixime (ZEM 5ug), Levofloxacin (LBC 5ug), Amoxicillin/Clavulanate (AUG 30ug), Cefotaxime (CTX 25ug), Imipenem/Cilastatin (IMP 10ug), Ofloxacin (OFX 5ug), Gentamycin (GN 10ug), and Nalidixic Acid (NA 30ug). These drugs were selected based on prescription frequencies and availability in the market.

Plasmid DNA Isolation and Detection

Pure isolates in broth were taken to the center for biotechnology research at Bayero University Kano, where plasmid isolation was carried out using a commercial plasmid isolation kit (ZR Plasmid Miniprep™- Classic) as previously described by Sambrook and Russel (2001) and Ranjbar *et al.* (2007). 0.5ml of the overnight culture was centrifuged. The supernatant was discarded. 200µl of P1 Buffer was added to the pelleted cells. 200 µl of P2 Buffer was added and mixed. It was incubated at 25⁰C for 2 minutes. 400 µl of P3 Buffer was added and mixed. It was centrifuged at 16.000 × g for 2 minutes. The supernatant was loaded inside the Zymo-spin™ IIN column and was centrifuged for 30 seconds. The flow through was discarded. 200 µl of Endo-Wash Buffer was added to the column in a collection tube and centrifuged for 30 seconds. 400 µl of plasmid Wash Buffer was added and centrifuged for 1 minute. The spin column was placed in a new microcentrifuge tube and 30 µl of DNA Elution Buffer was added and centrifuged for 30 seconds.

Plasmid Curing

Plasmid curing of the isolates was carried out using sodium dodecyl sulphate (SDS) as described by Ehiaghe *et al.* (2013). 9ml of freshly prepared nutrient broth was inoculated with 1ml overnight culture of resistant isolates; the resultant mixture was grown for 4 hours to allow for minimal growth of the microorganisms. 1µl of 10% sodium dodecyl sulphate curing agent was added to 9ml nutrient broth culture, and then incubated at 37⁰C for 24 hours. 1ml of the cured culture was inoculated into 9ml freshly prepared nutrient broth and incubated at 37⁰C for 24 hours. The overnight broth culture was then used to carry out post susceptibility test on Muller Hinton agar plate with the necessary antibiotic disc placed and incubated for 24 hours at 37⁰C for analysis.

Sensitivity Test of the Cured Isolates

Susceptibility of the cured isolates to the same antibiotic discs used in the first sensitivity was done by using Kirby-Bauer disc diffusion method, and interpreted according to Clinical Laboratory Standards Institute (CLSI, 2010). The overnight growth culture (obtained after plasmid curing) was inoculated on Mueller Hinton agar using a swab stick and the plates were incubated at 37⁰C for 24 hours. The plates were then read and the result was recorded as either susceptible or resistant by measuring the zone of inhibition. In other words, resistance markers expressed after curing was regarded as being chromosome-mediated while those that were not expressed were regarded as plasmid-mediated.



Data Analysis

Data generated in this study was statistically analyzed using appropriate statistical methods (SPSS version 16 and Microsoft Excel) and presented in forms of tables. Descriptive statistics including average, proportion, frequencies, and percentages were used to summarize the data.

RESULTS

A total of three hundred and fifty-two (352) wound swab specimens from patients at AKTH and NOH Dala, IDH Kano, General Hospital Wudil and General Hospital Bichi were analyzed. A total of 212 clinical bacterial isolates were isolated from all locations, giving overall prevalence of 60.23%. However, prevalence of wound infection from NOHD, AKTH, IDH, BGH and WGH is 69%, 47%, 46%, 73% and 71% respectively. On the basis of gender, generally, the result shows that males are more infected (69%) than females with 31%.

The distribution of etiologic agents of wound infections generally (from all named hospitals) showed that *S. aureus* (39%) was found to be more predominant, followed by *Ps. aeruginosa* (22%), *Pr. mirabilis* (15%), *K. pneumoniae* (9%), *E. coli* (8%), and *St. pyogenes* (7%) as the least, as shown in Figure 1. However, taking the individual hospitals into consideration, the distribution of etiologic agents of wound infections were summarized in Figure 2.

In terms of susceptibility and resistivity, the results show that out of 212 isolates, 140 (66%) are susceptible while 72 (34%) are resistant. Out of the resistant isolates (72), *S. aureus* ranked highest in resistivity to antibiotics with 32 (44%), followed by *Ps. aeruginosa* 15 (21%), *Pr. mirabilis* 12 (17%), *K. pneumoniae* 7 (10%), *E. coli* 4 (5%), and then *St. pyogenes* with 2 (3%), as shown in Figure 3. However, the susceptibility and resistivity of the isolates in all the hospitals (independently) has been shown in figure 4.

However, the result revealed a high resistance of the bacteria isolates to Cefixime (93%) followed by Azithromycin (87%), Erythromycin (81%), Cefotaxime and Cefuroxime (75% each), Imipenem/Cilastatin and Gentamicin each with 69%, and also, Levofloxacin and Nalidixic Acid with 63% both, Ofloxacin, (56%), Nitrofurantoin (50%), Ceftriaxone (37%), Amoxicillin (34%), Ampiclox (31%) and finally Ciprofloxacin (19%) with as the lowest. Similarly, the susceptibility of the bacteria to the antibiotics oppose the previous arrangement of the antibiotics (high susceptibility to Ciprofloxacin and low susceptibility to Cefixime).

After plasmid detection and isolation, the result shows that out of 72 resistant isolates, 49 contained plasmids, accounting for 68% of the total resistant isolates, while plasmids were not detected in 23 (32%). Resistant isolates with plasmids (plasmid-borne multidrug resistant bacteria) were subjected to plasmid curing (using sodium dodecyl sulfate) followed by second sensitivity test, and the result shows that 38 (78%) isolates that were initially resistant became susceptible to the same antibiotics used prior to plasmid curing, while 11 (22%) were still resistant. This means that the initially-resistant isolates were now susceptible to the drugs to which they were once resistant as they had now lost their resistance markers.

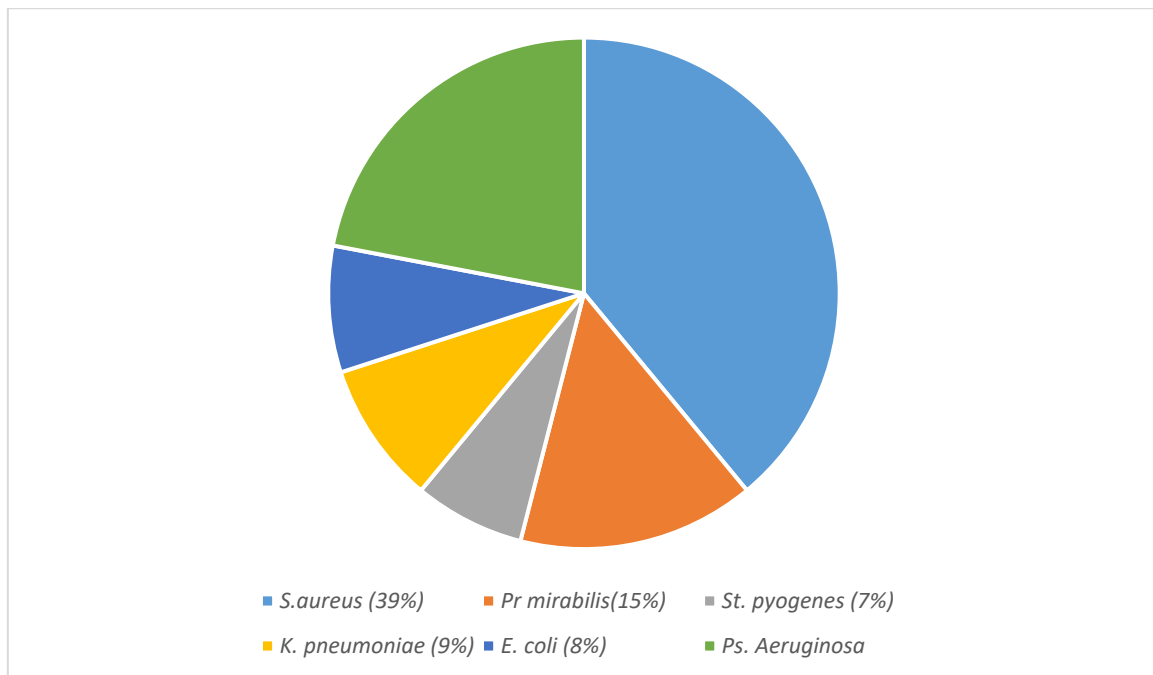


Figure 1: Distribution of the Isolates in Percentage (%)

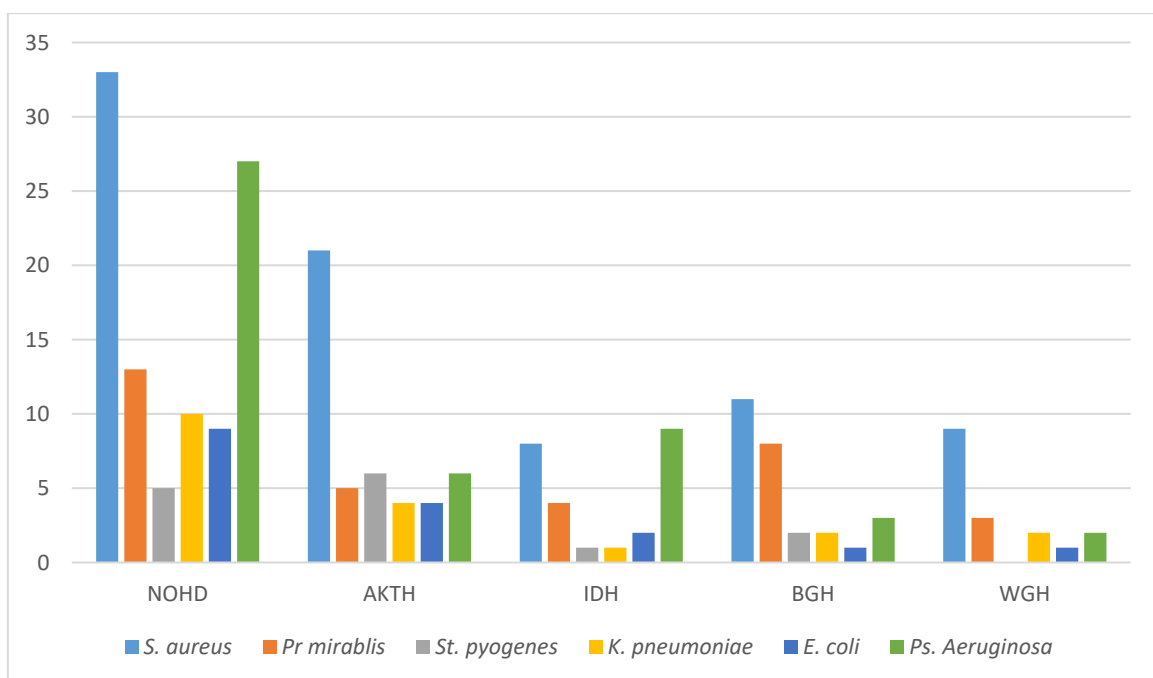


Figure 2: Distribution/Occurrence of Organisms Isolated in Each of the Hospitals

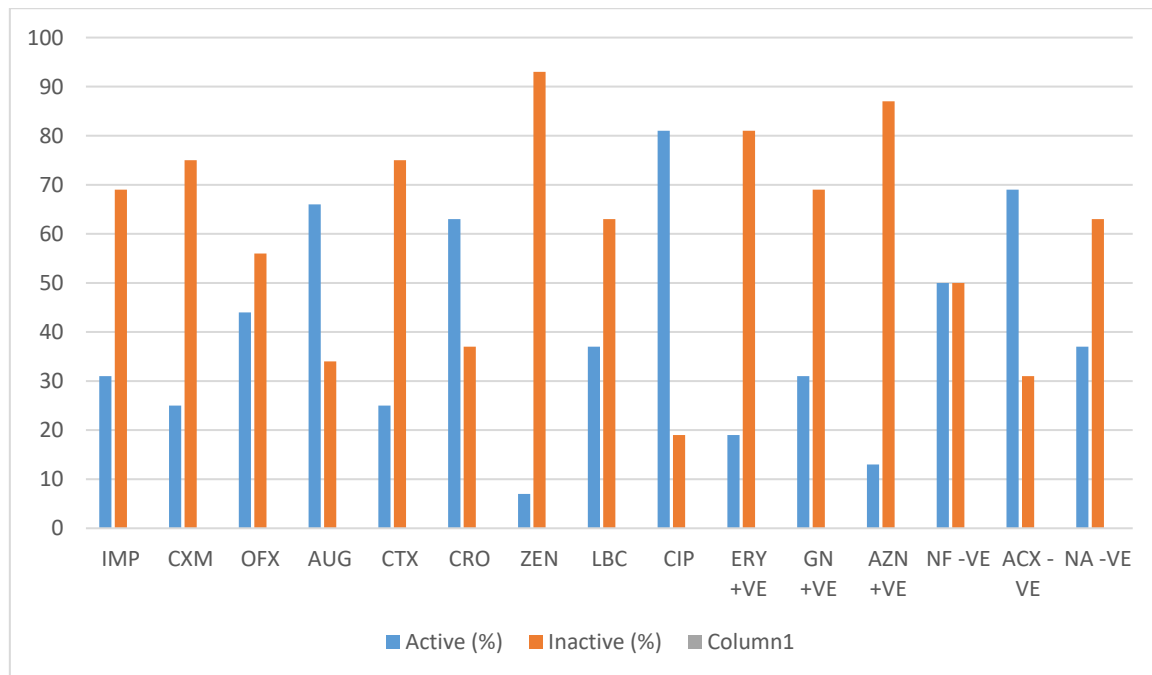


Figure 3: Antibiotics Activity Profile to the Isolates in Percentages (%)

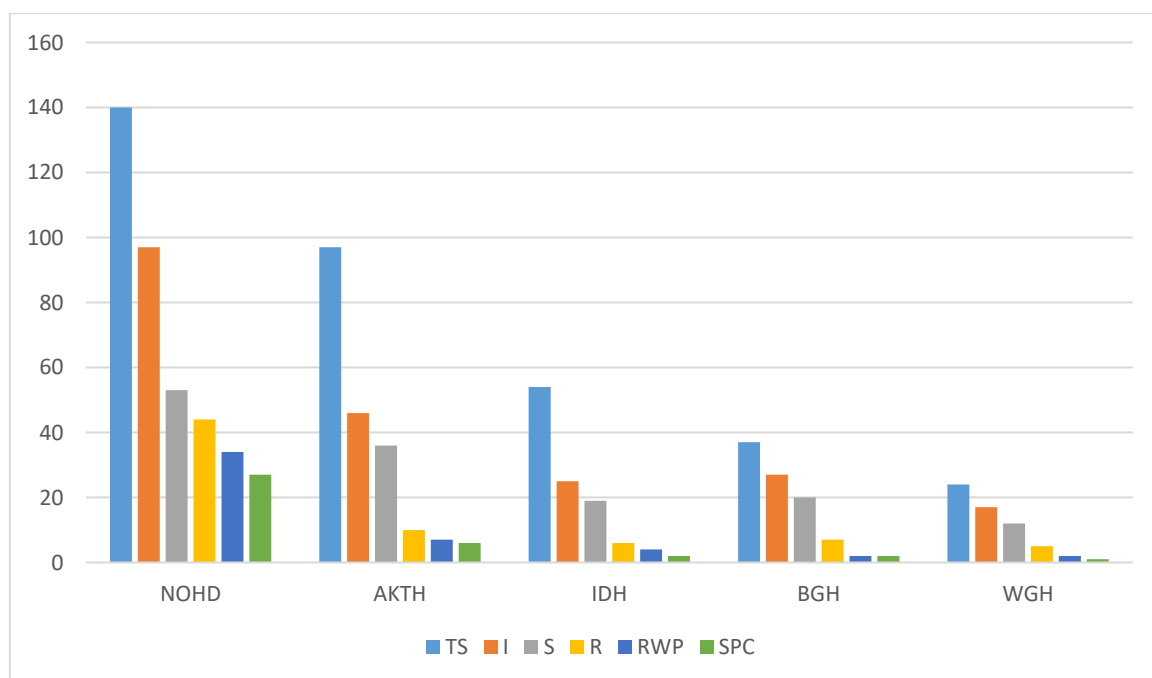


Figure 4: General Summary of the Result

KEY: TS - total number of samples, I - number of infected samples, S - number of susceptible isolates, R - number of resistant isolates, RWP - number of resistant isolates with plasmids, and SPC - number of susceptible isolates after plasmid curing.



Finally, the result of this finding was summarized in Figure 4. This figure shows the following: total number of samples, number of infected samples, number of susceptible isolates, number of resistant isolates, number of resistant isolates with plasmids and number of susceptible isolates after plasmid curing.

DISCUSSION

This finding revealed a high prevalence of wound infection with 212 (60.23%) out of 352 wound swabs. Although this is higher than an earlier report of 33.7% (Ethiaghe *et al.*, 2016) and 39.9% (Mohammed *et al.*, 2013; Oni *et al.*, 2006), it is still higher when compared to the result of Dellinger *et al.* (2005) who reported a prevalence rate of 9.6% and the result of Sule *et al.* (2002) who reported a prevalence rate of 11%. The World Health Organization (WHO, 2011) gave a prevalence of 5-34% of SSI and this is also lower than the result of this study. However, statistically lower prevalence (39.8%) of wound infection was recorded among patients in NAUTH while the prevalence of wound infection among study participants in UBTH was 27.6% (Ethiaghe *et al.*, 2016). The findings in this research are lower than that of Reiyee *et al.* (2014) who reported a prevalence rate of 75% in surgical wounds and 86.13% in another report (Shitu *et al.*, 2002).

The distribution of etiologic agents of wound infections generally (from all named hospitals) is in agreement with some works, where *S. aureus* predominated the other bacterial isolates (Aisha *et al.*, 2013; Christopher *et al.*, 2011; Ojo *et al.*, 2014). The finding is contrary to the report of 85% Gram-negative (*Ps. aeruginosa* as the most prevalent followed by *E. coli*) and 15% Gram-positive with only *S. aureus* as the Gram-positive isolate (Shitu *et al.*, 2002). There were some little variations of distribution of the isolates from the locations. However, the finding contradicts that of some previous researchers; some reports showed *E. coli* (52%) as the dominant followed by *Ps. aeruginosa* (36.1%), *S. aureus* (8.3%) and *Pr. mirabilis* (2.8%) (Ethiaghe *et al.*, 2016), with *E. coli* and *Ps. aeruginosa* ranking highest in distribution (Ezebialu *et al.*, 2016; Sulochana *et al.*, 2014). In another work, *E. coli* (34.4%) was mostly isolated from surgical wounds infections (Nejad *et al.*, 2011). However, in other studies, *Ps. aeruginosa* have been mostly recovered from post-operative surgical wounds despite the site of infection and location of specimens due to its high survival characteristics in hospital environment (Christopher *et al.*, 2011; Dalhatu *et al.*, 2014). It is ranked as the second among nosocomial pathogens isolated from hospitals, often contaminating hospital equipment such as wound dressing sinks and other surgical apparatus, and even antibiotic resistant strains can survive in supposedly sterile equipment used in the hospitals, making it a dangerous nosocomial pathogen widely distributed in the hospital environments where they are particularly difficult to eradicate (Masa'deh & Jaran, 2009). The high prevalence of isolates is probably due to poor hygienic practice in the area which may have been the cause of the high rate of multidrug bacterial surgical wound infections among the patients. Bacterial infections of wounds in hospital wards may be attributed to the overcrowding of hospital wards and lack of basic facilities for standard hygiene conditions, which is common in sub-Saharan African countries including Nigeria (Fontana *et al.*, 2000).

In terms of susceptibility and resistivity, the results show that out of 212 isolates, 140 (66%) are susceptible while 72 (34%) are resistant. This is largely due to the fact that bacterial isolates develop resistance by different mechanisms (mechanisms of drug resistance). Prevalence of



multidrug resistant isolates were found to be relatively higher than the one reported by Shitu *et al.* (2002) with 28.7% of the isolates. This high resistance pattern is an indication of the multidrug resistance strategies possessed by the clinical bacteria isolates. Many studies have been carried out on multidrug resistant bacteria and such resistance may be promoted due to numerous reasons, particularly, poor adherence to hospital antibiotic policy and excessive and indiscriminate use of broad spectrum antibiotics (Garba *et al.*, 2012; Akoachere *et al.*, 2014).

The result, which revealed a high resistance of the bacterial isolates to some of the used antibiotics, is not in agreement with some researches, with high resistance of the bacteria isolates to antibiotics found to be Cefixime (94%) followed by Cefuroxime (68%), Augmentin (68%), Ofloxacin (54%), Ciprofloxacin (52%), Ceftazidime (48%), Gentamicin (42%), Imipenem (14%), Erythromycin (0%) and Oxacillin (0%), whereas in NAUTH, the isolates displayed the same highest percentage resistance to Augmentin and Cefixime (94%) followed by Cefuroxime (79%), Ceftazidime (75%), Ciprofloxacin (69%), Ofloxacin (58%), Gentamicin (53%), Imipenem (17%), Erythromycin (17%) and Oxacillin (17%) (Ethiaghe *et al.*, 2016). The high resistance the isolates showed to the various antimicrobial agents used in this study may in part be due to various factors such as inappropriate usage of antibiotics and drug resistance mechanisms possessed by the bacterial isolates. Cephalosporins and Penicillins have been found to be highly resisted by wound pathogens. In another work, Ceftazidime and Augmentin were mostly resisted by wound etiologic bacteria (Eduardo *et al.*, 2008). This is mostly likely due to the presence of Cephalosporinase and Penicillinase enzymes which prevent the action of the Beta-lactam ring structure of the antibiotics (Livermore, 1995; Fontana *et al.*, 2000). The resistance shown to the used antibiotics in this study may be due to various factors like misuse or inappropriate use of antibiotics and drug resistance mechanisms possessed by the bacterial isolates.

In terms of plasmid detection and isolation, this finding is higher when compared to 34.4% and 28.2% of resistant isolates from NAUTH and UBTH respectively (Ethiaghe *et al.*, 2016). Subsequent plasmid curing (using sodium dodecyl sulfate) and post-curing sensitivity shows that 38 (78%) isolates that were initially resistant became susceptible to the same antibiotics used prior to plasmid curing while 11 (22%) were still resistant. This is almost in line with a report of Ethiaghe *et al.* (2016) where all 32 plasmid-borne resistant isolates became susceptible after plasmid curing. This means that the initially-resistant isolates were now susceptible to the drugs to which they were once resistant as they had now lost their resistance markers. This suggests that plasmid-borne multidrug resistant genes had been denatured by the sodium dodecyl sulfate used as the curing agent.

CONCLUSION

The findings demonstrated an increasing incidence of wound infections with multidrug resistance; they also highlighted the emergence of multidrug plasmid-mediated resistance among the isolates. The findings proved the existence of *S. aureus* as the leading cause of wound infection with *E. coli* as the least frequent. Meanwhile, male youth are the most exposed to wound infections compared to other groups. High resistance to Cefixime (93%) followed by Azithromycin (87%) antibiotics was observed also among these isolates. However, 78% of the cured isolates that were initially resistant became susceptible to the same antibiotics used prior to plasmid curing.



The findings in this study justify the need to strengthen infection control to prevent the spread of plasmid-mediated multidrug resistant bacteria. Therefore, efforts to promote the appropriate use of antibiotics are paramount to avoid therapeutic failure; the correct choice of antibiotics should be made only after antibiotic sensitivity testing.

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