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# Determination of Pattern of Multiple Antibacterial Resistance In Clinical Bacterial Isolates of Wound Infections

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author ANU designed the study and wrote the protocol. Author SSS carried out the laboratory analysis, generated all data and wrote the first draft of the manuscript. Author SAA did the literature search and carried out part of the laboratory analysis. Author EJM did literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

The effectiveness of drugs and chemotherapeutic agents used against bacteria has recently declined due to various mechanisms used by the bacteria to prevent their actions. This study aims at detecting the mechanism of multidrug resistance in bacteria isolated from wound infections in patients attending University of Uyo Teaching hospital. Swabs from infected wounds were collected using aseptic methods. Culture and examination was done using standard microbiological techniques. Sensitivity test was done using disk diffusion technique. Curing was done using acridine orange. *Staphylococcus aureus* was the predominant species with 43.3% followed by

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*Pseudomonas aeruginosa* with 31.67%, *Escherichia coli* 11.7%, *Proteus sp.* 8.3% and *Klebsiella pneumoniae* 5.0%. The overall multidrug resistance was 68.3%. The results of the study further reveal that 61.5% of *Staph aureus* were resistant to more than eight antibiotics with multiple antibiotic resistance (MAR) index ranging from 0.22-0.89. Other organisms also exhibited various levels of multiple antibiotic resistance indexes. This study shows that the prevalence of multidrug resistant organisms was high and majority of the organism isolated exhibited plasmid mediated resistance. This identification enhances the development of new approaches to overcome the problem of antibiotic resistance as this causes a huge challenge in the treatment of infections within the community where more people get infected each day.

*Keywords: Acridine orange; antibiotics; multidrug resistance; wound infections.*

## 1. BACKGROUND

Bacteria have been a major cause of infectious disease throughout the history of human race. With the introduction of antibiotics, it was thought that the problem should disappear, however bacteria have been able to evolve the point where they are resistant to antibiotics [1]. The term multi drug resistance therefore applies to a bacterium that is simultaneously resistant to a number of antimicrobial drugs belonging to different classes (chemically) or subclasses through various mechanisms [2]. The effectiveness of currently available microbial drugs is decreasing due to the increasing number of resistant strain causing infections so that available therapeutic options for such organism are severely limited [3].

Antimicrobial drug resistance can be acquired as a result of mutation or acquisition of resistance genes via horizontal gene transfer or can be an innate feature of an organism that is encoded chromosomally [2].

In a research done by Zeleke [4], it was indicated that over the past few years, several studies in African countries had reported the presence of multidrug resistant strains of bacteria isolates from clinical wound infections. This was consecutively ascertained by some researchers [5,6,7]. A study conducted in one of the tertiary hospitals in Ethiopia by Anguzu et al., [8] also revealed that about 51% of the bacteria isolated from open wounds were identified as being multidrug resistant. Multidrug resistances in both the hospital and community environment are important concern to the clinicians, patients and the pharmaceutical industries. The widespread uses of antibiotics together with the length of time over which the drugs have been available in the market have led to major problems of emergence of resistant organisms. Antimicrobial drugs overuse, overdosing, drug prescription with improper susceptibility test, self-medication and long duration of hospitalization was suggested to augment the problem of multidrug resistance in developing nations [9]. The aim of this present study is therefore to determine the pattern of multiple antibiotic resistances in clinical bacterial isolates obtained from wound infections in order to develop new approaches to overcome the problem as it causes a huge challenge in the treatment of infections within the community where more people get infected each day.

## 2. MATERIALS AND METHODS

This study which was carried out between October 2013 and January 2014 comprised of 120 wound samples obtained from both inpatients and outpatients attending the University of Uyo Teaching Hospital (UUTH), Uyo, Nigeria.

Sterile swab sticks were used for the collection of the wound samples. The patients that qualified for the collection of sample for this research were patients with infected wounds. All collected samples were immediately transferred to the Medical Microbiology Laboratory for processing. Aseptic precautions were taken into consideration during the sample collection.

The isolation of microorganism was carried out by plating out the wound swab on blood and macConkey agar plates respectively. The plates were incubated at 37℃ for 18-24 hours after which they were examined for growth colonies.

From the growth pattern of the organisms obtained from the culture plates, representative colonies were subjected to macroscopic, microscopic and biochemical characterization using standard identification schemes [10,11].

### 2.1 Antibiotic Susceptibility Testing

The susceptibility profile of each of the isolates to nine commonly used antibiotics was determined using the agar disk diffusion method. The

antibiotics used were Ciprofloxacin (2 µg), Gentamicin (10 µg), Ofloxacin (5 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Ceftriaxone (30 µg), Levofloxacin (5 µg), Cefepime (10 µg) and Azithromycin (15 µg). The bacterial inoculum was prepared by suspending freshly grown bacteria in 5ml of sterile peptone water. The peptone water was then inoculated by pouring it onto the Mueller Hinton (MH) agar plates and the excess drained out. The plates were allowed to dry and appropriate antibiotic disks aseptically placed on the agar plate surface using sterile forceps. The plates were then incubated at 37℃ for 24 hours.

Determination of the inhibition zone diameter was done using the Kirby Bauer test method as described by Willey et al. [12]. The degree of susceptibility of the test bacteria to each antibiotic were recorded as either sensitive (S) or resistant (R) respectively. After the susceptibility testing the resistant isolates were inoculated in already prepared Nutrient agar slants and stored for curing.

### 2.2 Curing of Resistant Markers

Curing of the drug resistant isolates was done using 0.10 mg/ml of acridine orange as described by Akortha and Filgona [13]. The stored resistant isolates were cultured at 37℃ for 24 hours in nutrient broth containing 0.10 mg/ml of acridine orange, after which they were agitated to homogenize the content before sub-culturing onto Mueller Hinton agar plates for another susceptibility testing. The cured resistant markers were determined by comparing the pre and post curing antibiogram of isolates i.e, the result of the first susceptibility testing was compared to the one done after the introduction of the acridine orange.

### 2.3 Determination of Multiple Antibiotic Index

Multiple antibiotic resistance index (MAR) was determined using the formula MAR=x/y, where 'x' was the number of antibiotics to which the test isolate displayed resistance and 'y' is the total number of antibiotics tested.

## 3. RESULTS

One hundred and twenty wound samples were analyzed out of which 5 organisms were isolated. The isolated organisms were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus spp* and *Klebsiella pneumoniae*. *Staphylococcus aureus* were 52 representing 43.3%, *Pseudomonas aeruginosa* were 38 and *E. coli* were 14 both representing 31.7% and 11.7% respectively. Others were *Proteus spp*, 10 (8.3%) and *Klebsiella pneumoniae* 6 (5.0%). It could be seen that *Staphylococcus aureus* was the highest while *Klebsiella pneumoniae* was the least isolated organism. This is shown in Table 1.

**Table 1. Organisms isolated from wound infections at the University of Uyo Teaching hospital**

| Organism | No.(%) Isolated |
|---|---|
| *Staph aureus* | 52 (43.3) |
| *Ps aeruginosa* | 38 (31.7) |
| *E. coli* | 14 (11.7) |
| *Proteus spp* | 10 (8.3) |
| *Kleb pneumonia*e | 6 (5.0) |
| **Total** | **120 (100)** |

### 3.1 Number of Isolates Resistant to Antibiotics

Table 2 shows the number of isolates showing resistance to a given number of antimicrobial drugs. None of the isolates were resistant to as low as one antimicrobial agent. Only two out of all the bacterial isolates showed resistance to two antibiotics.

### 3.2 Curing of Resistant Markers

When the isolates were subjected to curing in the presence of 0.10 mg/ml of acridine orange, 16(13.3%) isolates of the total isolates were cured of at least one resistant marker. The highest loss was observed in 5% of the isolates while there was no loss of marker in 14 of the isolates. The highest percentage (16.7%) of isolates seems to have lost 3 markers and the lowest percentage (5%) seems to have lost up to 8 markers. This is presented in Fig. 1.

### 3.3 Multiple Antibiotic Indexes of Organisms Isolated from Wound Infection

Table 3 shows the multiple antibiotic resistance indexes of the organisms. *Staph. aureus* shows MAR index of 0.22 - 0.89 while *Kleb. pneumoniae* shows MAR index of 0.67 - 0.78. Other organisms also exhibited various levels of multiple antibiotic indices.

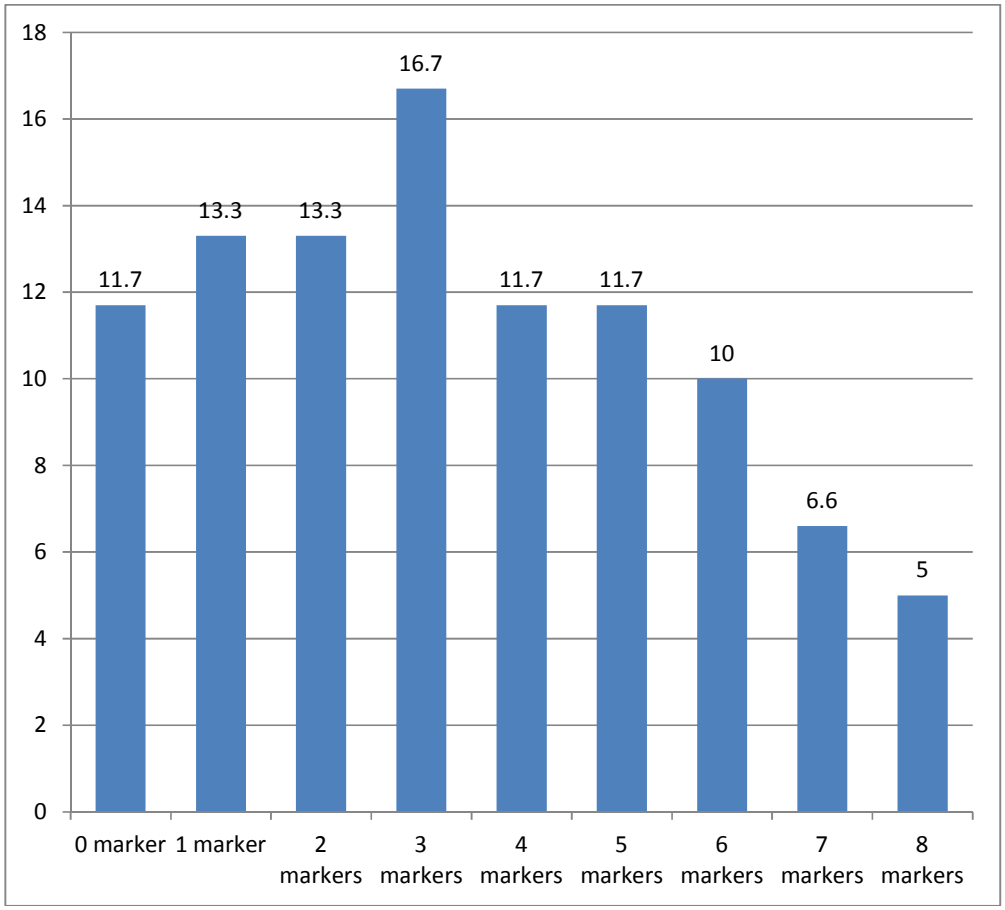


**Fig. 1. Organisms showing loss of resistant markers**

**Table 2. Number of isolates resistant to specified number of antibiotics**

| Organism | No of Isolates | No of Isolates showing resistance to antibiotics | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| *Staph. aureus* | 52 | - | 2 | 4 | 6 | 16 | 14 | 4 | 8 |
| *Ps. aeruginosa* | 38 | - | - | - | 2 | - | 10 | 14 | 12 |
| *E. coli* | 14 | - | - | - | 2 | - | 6 | 6 | - |
| *Proteus spp* | 10 | - | - | - | - | 4 | 6 | - | - |
| *Kleb. pneumoniae* | 6 | - | - | - | - | - | 4 | 2 | - |
| **Total** | **120** | **-** | **2** | **4** | **10** | **20** | **40** | **26** | **18** |

**Table 3. Multiple antibiotic resistance (MAR) indices of bacteria isolated from wound infection**

| Mar | *S. aureus* | *P. aeruginosa* | *E. coli* | *Proteus* | *Kleb pneumoniae* |
|---|---|---|---|---|---|
| 0.22 | 1 | - | - | - | - |
| 0.33 | 2 | - | - | - | - |
| 0.44 | 3 | 1 | 1 | - | - |
| 0.56 | 8 | - | - | 2 | - |
| 0.67 | 7 | 5 | 3 | 3 | 2 |
| 0.78 | 2 | 7 | 3 | - | 1 |
| 0.89 | 3 | 6 | - | - | - |

## 4. DISCUSSION

Wound infection has always been a major complication in the hospital setting [14], and it has been regarded as the most common nosocomial infection especially in patients undergoing surgery [15]. The pathogens isolated from wound infections differ and depend on the site of infection.

In this study, the isolated bacteria were *Staphylococcus aureus* 52(43.3%), *Ps. aeruginosa* 38(31.7%), and *E. coli* 14(11.7%). Others were *Proteus species* 10(8.3%) and *Klebsiella pneumoniae* 6(5.0). The possible suggested reason for the high frequency of isolation of these bacteria may be related to the fact that they are normal flora in healthy person. When they overcome the natural resistance of the skins and soft tissue in the cases of burns, they can easily disseminate [16,17]. Moreover, these bacteria are commonly found in the hospital environment which might increase wound infection rate and cross contamination among admitted patients. This study looked at the resistance of bacterial isolates of wound infections against more than one antibiotic at one of the tertiary hospitals in Nigeria, and found that the level of multi-resistance to antibiotics in the hospital has increased but is however relatively low compared to reports from other countries [5]. Widespread use of antibiotics has undoubtedly caused the epidemics of antimicrobial resistance worldwide. Unfortunately, resistance in some species has developed to the level that no clinically available treatment is effective. Prevention and control strategies will require the application of epidemiological and behavioral approaches, as well as the research technologies aimed at the basic mechanisms of drug resistance. Bacteria have elaborated mechanisms to achieve antibiotic resistance one of which is by the fine-tuning of expression of genetic information. The majority of the organisms isolated in this study exhibited plasmid mediated resistance as they were cured by the acridine orange methodology.

Antibiotic resistant profiles of the isolated bacteria within the study period showed that the organisms were multiple antibiotics resistant as evidenced by the exhibition of multiple resistances to the majority of antibiotics tested. Multiple antibiotic resistances have been reported to occur through different mechanisms like, modification of drug target site or reduction of cell permeability to the drug which has been established with development of resistance of *Pseudomonas spp.* and *Staphylococci*, with Gentamicin [18], and production of β-lactamase enzymes which destroy β-lactam ring of β-lactam antibiotics, which renders the drug useless as observed with *Staphylococci and Pseudomonas spp*. [19]. Efflux pump to reduce antibiotics concentration in the cell is another mechanism used by *Staphylococci and Pseudomonas spp.* to develop resistance to levofloxacin and ofloxacin [19,20].

Prevalence of these multidrug resistant isolates in the wounds is a cause of concern because of its attendant effect. It implies that most of the commonly used antibiotics will not be useful in the management of wound infection with such pathogens, infected wounds would take longer to heal, and cost of treatment would increase. It also means patients will stay longer in the hospitals with higher likelihood of transmitting such pathogens to other patients. Resistance to multiple classes of antimicrobial agents in gram-negative and Gram-positive bacteria has been described to be more often due to efflux of the drugs out of the bacterial cell [21,22]. Therefore, it is important that management of hospitals adopt rational use of antibiotics to avoid epidemic outbreak of drug resistant bacterial infections.

The overall multidrug resistance in this study was 68.3%. This is in agreement with Godebo et al., [2] who recorded 68.15 in Ethiopia but in disagreement with Biadglegne et al., [23] who recorded 98.6% also in Ethiopia. This difference in resistance could be attributed to the difference in population demographics and other environmental factors. The overall multi-drug resistance (MDR) of *Staphylococcus aureus* is 61.5% out of which 3.8% was resistant to only two antibiotics and 11.5% resistant to as high as 8 antibiotics. This result is so far off when compared to Godebo's 86.2% *Staphylococcus aureus* resistance.

This finding agrees with previous studies carried out in Ethiopia where average resistance of 52% in *Staphylococcus aureus* moved up to 75%.

All the isolates showed 98.3% resistance to cefepime. This is higher than that recorded by Oladipo et al. [24] which recorded 56.06%. Pseudomonas showed 100% resistance to cefepime, while Klebsiella showed a 66.67% resistance to cefepime. In a study carried out by Oladipo et al. [24] in Ogbomoso, Oyo State in Nigeria, Pseudomonas showed a 59.3% resistance and Klebsiella showed a 52.94% against cefepime. This difference may be as a

result of difference in clinical practices, and difference in the demographics of the population examined. The isolates also showed high resistance to Azithromycin. This corroborates the work done by Chayani et al. [25] which showed that azithromycin exhibited a high resistance to most enterobacteria and was only sensitive when used on Salmonella-Shigella species. The multiple antibiotic index recorded in this study reveals that *Staph. aureus* exhibited multiple resistance index in the range of 0.22-0.89 while *Kleb. pneumoniae* exhibited MAR in the range of 0.67-0.78. Proteus also had the MAR index of 0.56- 0.67.

Knowledge of the molecular mechanisms of antibiotic resistance is essential for developing new approaches to overcome this problem one of which may be the development of inhibitors of resistance enzymes. These inhibitors can be administered as co-drugs with the antibiotics, thereby blocking resistance and rescuing the antimicrobial activity of the drugs. Another strategy to overcome resistance is to improve the delivery or otherwise enhance the accessibility of antibiotics to their sites of action. All the alternative strategies to overcome resistance require expanded knowledge of the molecular mechanisms of antibiotic resistance, their origins and evolution, and their distribution throughout bacterial populations and genomes.

## 5. CONCLUSION

In conclusion therefore, there is an urgent need for more prudent use of antibiotics and review of marketing policies, bearing in mind that the drug manufacturing industries will need to remain profit-making in the event of better controlled use of antibiotics and the development of agents with a more discriminate, narrower spectrum of activity. For these problems to be put under control requires international collaboration and strengthened alliances among the research, medical, and pharmaceutical communities.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Dzidic Senka, Jagoda Suskovic, Blazenka Kos. Antibiotic resistance mechanisms in bacteria: Biochemical and genetic Aspects. *Ruder Boskovic Institute,* Department of Molecular Genetics, Zagreb, Croatia; 2007.
2. Godebo, Girma, Gebre kibru. Multidrug resistance isolates in infected wounds at Jimma hospital, Ethiopia. Afr J Microbiol Res. 2013;2:288-291.
3. Centre for Disease Control. (C.D.C). Estimated increase in the Etiology of antimicrobial resistance bacteria for 1990-2010.
4. Zeleke WT. Multiple antimicrobial resistance in bacterial isolates from clinical and environmental sources of Jimma Hospital. Ethiop J Scien. 2002;25:295-302.
5. Olayinka AT, Onile BA, Olayinka BO. Prevalence of multi-drug resistant (MDR) Pseudomonas aeruginosa isolates in surgical units of Ahmadu Bello University teaching hospital, Zaria, Nigeria: an indication for effective control measures. Ann Afr Med. 2004;3:13-16.
6. Chikere CB, Chikere BO, Omoni VT. Antibiogram of clinical isolates from a hospital in Nigeria. Afr J Biotech. 2008;7: 4359-4363.
7. Nkang A.O, Okonko I.O, Mejeha OK Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria. J Microbiol Antimicrobial. 2009;1:19-26.
8. Anguzu JR, Olila D. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Int J Med Sci. 2003;4:18-31.
9. Magiorakus AP, Srinivasan A, Carey RB. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268-281. PubMed Abstract.
10. Cowan ST, Steel KJ. Manual for the Identification of Medical Bacteria ($4^{th}$ Edition). Cambridge University Press. London. 1985;217.
11. Holt JG, Krieg NR, Sneath PHA, Stately JT, Williams ST. Bergey's Manual of Determinative Bacteriology, ($9^{th}$ edn), Baltimore, Williams and Wilkins. 1994; 787.

12. Willey M, Sherwood M, Woolverton J. Mechanisms of multidrug resistance in bacteria. Prescott, Harley and Klein's Microbiology. 8th edn. 2011;849-853.
13. Akortha EE, Filgona J. Transfer of gentamicin resistance genes among enterobacteriaceae isolated from the outpatients with urinary tract infections attending 3 hospitals in Mubi, Adamawa State. Scientific Research and Essay. 2009;4(8):745-752.
14. Balley, Love. Wound infection in short practice of surgery. 24th edition. Mc graw hill companies New York; 2004;118-119.
15. Dionigi R, Rovera F, Dionigi G, Imperator A, Ferrari A, Dionigi R. Risk factors in surgery. J Chemother. 2001;13:6-11.
16. Khanal LK, Jha BK. Prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) among skin infection cases at a hospital in Chitwan, Nepal. Nepal Med Coll J. 2010;12:224-228. PubMed Abstract.
17. Flamm RK, Weaver MK, Thornsberry C Factors associated with relative rates of antibiotic resistance in *Pseudomonas aeruginosa* isolates tested in clinical laboratories in the United States from 1999 to 2002. Antimicrobial Agents Chemotherapy. 2004;7:2431-2436.
18. Shaw KJ, Rather PN, Hare RS Molecular genetics of aminoglycoside resistance gens and familial relationship of aminoglycoside-modifying enzymes. Journal Microbiol Rev. 1993;57:138-163.
19. Neu HC. Synergy of fluroquinolones with other antimicrobial agents. Rev. Infect. Dis. 1989;11(Suppl. 5):S1025-S1035.
20. Spear BS, Shoemaker NB, Salyers Bacterial resistant to tetracycline: mechanisms, transfer and clinical significance. Clinical Microbiology Rev. 1992;5:387-399.
21. Nikaido H. Antibiotic resistance caused by Gram negative multidrug efflux pumps. Clin. Infect. Dis. 1998;27(Suppl. 1)S32-41.
22. Levy SB. Antibiotic Resistance Infection Control. Ann Intern Med. 1983;4:195.
23. Biadglegne F, Abera B, Alem A. Bacterial isolates from wound infection and their antimicrobial susceptibility pattern in Felege Hiwot Referral Hospital, North West Ethiopia. Ethiop J health Sci. 2009;19:173-177.
24. Oladipo EK, Ayandele AA, Amao JA, Lateef A. Cefepime-resistance in clinical isolates of *Pseudomonas aeruginosa* and *Klebsiella* spp in Ogbomoso. B Jn Med & Medical Res. 2013;4(20):2-5.
25. Chayani N, Tiwari S, Sarangi G, Mallick B, Mahapatra. A-Role of azithromycin against clinical isolates of family Enterobacteriaceae, A comparison of its MIC by three different methods. Ind J Med Microbiol. 2009;27(2):107-110.



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