



## ***In-vitro* Antibiotic Susceptibility Tests of Bacterial Isolates from Abdominal Wound Infection in a Nigerian Teaching Hospital**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author AAA was involved in the conception, design and literature search. He also contributed significantly to the writing of the discussion section. Author SKO was involved in the study design and literature search. Author KIO was involved in statistical analysis, study design and protocol and literature review. All the authors have read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The trend of increasing antibiotic resistance has been reported from various centres. The aim of this study was to look at the pattern of resistance of bacterial isolates from abdominal wound infections and determine its magnitude in a bid to establish appropriate antibiotic stewardship program in the centre.

**Study Design:** A prospective cross sectional study that looked at pattern of antibiotic susceptibilities in isolated organisms from infected laparotomy wounds.

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**Place and Duration of Study:** Department of Surgery (General Surgery Unit) and Department of Medical microbiology, Federal Teaching Hospital, Gombe; between January 2012 and December 2012.

**Methods:** All adult patients (eighteen years and above) who had either emergency or elective laparotomy for one indication or the other were recruited into this study. Wound inspection was done on days 3, 5, 7; swabs were taken in infected cases under aseptic condition and processed according to microbiological standards.

**Results:** Eighty five (38.1%) patients developed wound infection out of the 223 that met the inclusion criteria. This consists of 157 (70.4%) males and 66 (29.6%) females. Their ages ranged between 18 and 80 years. Males developed wound infection more than females. Dirty wounds had the highest infection rate. The most common isolates were *Klebsiella spp* (34%), *Staphylococcus aureus* (30.4%) and *Proteus spp* (19.6%). Multidrug resistance (>50%) to commonly used antibiotics such as amoxicillin-clavulanate, cotrimoxazole and gentamicin were seen in many isolates.

**Conclusion:** The emergence of multidrug resistant organisms calls for collaborative efforts and judicious use of antimicrobial agents among clinicians.

*Keywords: Wound infection; microorganisms; multi-drug resistance.*

## 1. INTRODUCTION

Surgical site infection (SSI) as recently defined by the Centres for Disease Control and Prevention (CDC) is one occurring after surgery in the part of the body where the surgery took place [1]. The extent of this might range from involvement of layers of the anterior abdominal wall (incision site) to involvement of the deep space/peritoneal cavity or specific organs within the abdominal cavity (organ/space) [1]. Surgical wounds are generally classified based on their degree on microbial contamination into clean, clean contaminated, contaminated and dirty wounds [2]. The susceptibility of a wound to infection is therefore directly related to each class of the wound. For clean wounds, gram positive organisms from the skin flora are usually the cause of infection while in other classes of wounds, polymicrobial aerobic and anaerobic organisms closely resembling the normal endogenous microflora of the affected organ are the usual isolates [3-5].

Some of these organisms have developed resistance to the commonly used antimicrobial agents over time. This is as a result of injudicious use of such drugs on the part of the patients and indiscriminate prescription on the part of clinicians [6,7]. This prospective study therefore aimed to look at the pattern of antibiotic resistance by organisms isolated from abdominal wound infection following laparotomies in a Nigerian teaching hospital.

## 2. METHODS

### 2.1 Study Centre and Design

This is a prospective cross sectional study carried out at the Federal Teaching Hospital, a tertiary health care centre which also serves as a referral centre located in Gombe, North –Eastern Nigeria from January 2012 to December 2012.

### 2.2 Recruitment of Study Participants and Sample Collection

The inclusion criteria were all adult patients (eighteen years and above) who had either emergency or elective laparotomy in the General Surgery unit at Federal Medical Centre, Gombe, North eastern, Nigeria and who subsequently developed surgical site infection within thirty days of surgery. [Eighteen years was the cut-off age for pediatric patients in the study centre as at the time of this study]. Informed consent was obtained from all patients recruited into the study and relevant clinical information entered into a proforma designed for the study. Ethical approval was obtained from the Institutional Ethics Review Board.

Patients' wounds were inspected on post-operative days 3, 5 and 7 for local evidence of wound infection. The diagnostic criteria for clinically infected wound were based on the definition provided by the Centres for Disease Control and Prevention (CDC) and the National Healthcare Safety Network (NHSN) [2].

Wound swabs were collected from patients who had suspected or clinically infected wounds. This was done under aseptic procedure, cleansing the wound site with sterile gauze soaked in normal saline; parting the wound edges and dipping the sterile cotton-tipped specimen collection stick to the base of the wound and firmly rotating it while avoiding contact with the wound edges. The specimen were capped and labeled appropriately and thereafter sent to the Medical Microbiology laboratory. Microscopy of the specimen was done using gram staining technique. Each smear was examined at high magnification using an oil immersion (x100) objective lens. Gram positive organisms appeared blue/purple, while Gram negative organisms appeared pinkish red [8].

### 2.3 Culture

The samples collected were inoculated on blood agar, chocolate agar (Oxoid, Basingstoke, UK) and MacConkey agar (Fluka medica) plates using a sterile platinum wire loop. MacConkey and blood agar plates were incubated aerobically at a temperature of 35-37°C for 18-24 hours, while chocolate agar plates were incubated in a candle jar to facilitate the growth of fastidious organisms. Growth on the culture plates were examined macroscopically for colonial morphology. The colonies were subjected to appropriate biochemical tests for identification and classification [9].

### 2.4 Biochemical Confirmation

Biochemical test such as carbohydrate fermentation, oxidase production, catalase utilization, coagulase production, indole production, citrate utilization and ability to produce urease were employed in addition to microscopic findings to identify the organisms [10]. *Klebsiella spp* was identified as gram negative bacilli, non-motile, lactose fermenting, indole negative with a positive citrate utilization reaction. *Staphylococcus aureus* was identified as gram positive cocci with positive catalase and coagulase reactions. *Escherichia coli* was identified as gram negative bacilli, motile, lactose fermenting, positive indole and negative citrate reaction. *Proteus spp* were identified as gram negative bacilli, non-lactose fermenting with positive urease and negative oxidase reactions, swarming and motile.

### 2.5 Antibiotics Susceptibility Testing

Antibiotic susceptibilities were determined on Mueller-Hinton agar (Oxoid, Basingstoke, UK) by

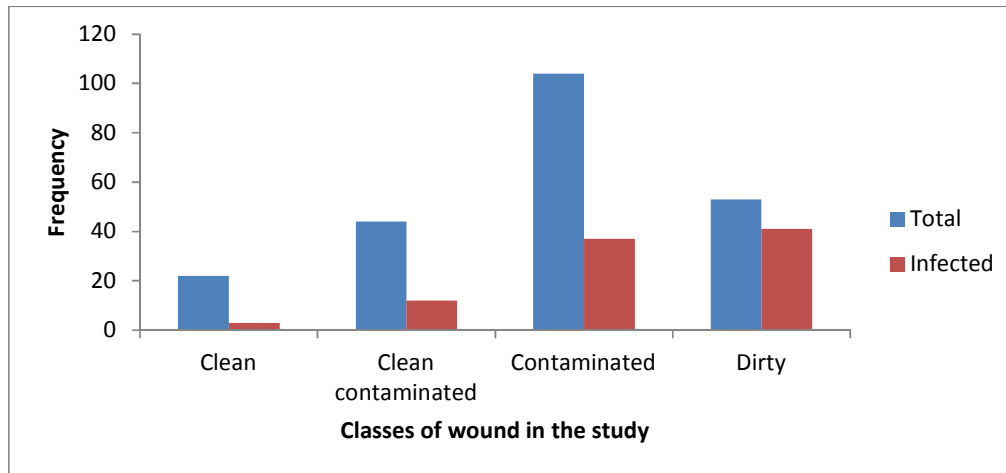
standard disk diffusion procedures. The inoculum in each peptone water broth was standardized by McFarland's standard. The antibiotic discs were applied on two different 90 mm petri dishes, allowed to pre-diffuse for about 20 minutes and incubated at 37°C overnight: penicillin (10 units), ciprofloxacin (10 µg), gentamicin (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), sparfloxacin (30 µg), amoxicillin-clavulanic acid (30 µg). The control strains were run simultaneously with the test organisms. Positive antibiotic response was interpreted by the presence of zone of inhibition around the test organism based on Clinical Laboratory Standards Institute (CLSI) criteria [11].

## 3. RESULTS

Two hundred and twenty three patients met the inclusion criteria. This consists of 157 (70.4%) males and 66 (29.6%) females. Their ages ranged between 18 and 80 years. Eighty five (38.1%) patients had wound infection; 59 (69.4%) were males while 26 (30.6%) were females (M:F=2.3:1). Twenty two patients had clean wounds out of which three (13.6%) patients had SSI; 44 patients had clean contaminated wounds out of which 12 (27.3%) patients had SSI; 104 patients had contaminated wounds out of which 37(35.6%) patients had SSI while 53 patients had dirty wounds out of which 41 (77.4%) patients had SSI. Patients with dirty wound had the highest infection rate while those with clean wound had the lowest (Fig. 1). Single bacterial isolate was seen in 56 (65.9%) patients and the organisms comprised *Klebsiella spp* (34%), *Staphylococcus aureus* (30.4%), *Proteus spp* (19.6%), *Providencia* (12.5%) and *Escherichia coli* (3.6%), while mixed infection was seen in 21(24.7%) patients. The responses of the isolated organisms as single and mixed isolates to the various tested antibiotics are shown in Tables 1 and 2 respectively.

## 4. DISCUSSION

The pattern of isolated microorganisms from the surgical wounds in this study is similar to the profile that has been observed from other related studies within and outside Nigeria [12-16]. The resistance pattern demonstrated to the tested antibiotics could be seen to vary and a similar scenario has been widely reported [17-19]. The development of this resistance pattern could be attributed to injudicious use of antimicrobial agents which is common place in most third world countries as there are no rule prescription of antibiotics.



**Fig. 1. Showing the distribution of patients according to different classes of wound**

In Nigeria for example, it is a free for all as there has not been any regulations that govern who prescribes what. Antibiotics abound everywhere as over the counter drugs and clinicians most times do not wait before they start antibiotics as an empirical treatment. If they had to wait for culture result, patient may have sepsis. This is same for whole world especially after malignancy operations and complications. This is the common cause of antibiotic resistance [20,21]. The continuous exposure of microbial agents to these drugs over time eventually lead to reduced efficacy borne out of genetic modification of the target receptors on the microorganisms. Some of these organisms which are ubiquitous within the hospital environment have developed resistance to the commonly used antibiotics needed to suppress their proliferation.

The resistance to the commonly used antibiotics occurs through various plasmid-mediated mechanisms. These mechanisms include: decreased intracellular concentration of antibiotics (either increased efflux or reduced influx of the drug); neutralization by inactivating enzymes ( $\beta$ -lactamase); alteration of the target receptor on which the drug is to act and complete elimination of the target on which the drug is to act [22,23].

Bacterial isolates in this study were both monomicrobial and polymicrobial. Monomicrobial isolates were predominant and comprised largely (67%) of gram negative, aerobic organisms while *Staphylococcus aureus* was the only gram positive pathogen isolated. The gram negative organisms were largely from the intestinal flora

as these were seen more in clean contaminated, contaminated and dirty wounds.

*Klebsiella spp* demonstrated a very good response to the cephalosporins, gentamicin and quinolones. However, there was a poor response to the use of penicillin and amoxicillin/clavulanic acid. The findings from Benue, North central, Nigeria [24] is comparable to ours but at variance with reports from Lagos [25] and Abuja [26] where these organisms showed a high pattern of resistance (>60%) to cephalosporins and gentamicin. Other workers from Ethiopia [27] and India [28] had reported similar resistance profile by *Klebsiella spp*. This resistance pattern may be due to the increasing development of extended spectrum beta lactamases (ESBLs) in the *Klebsiella spp*.

*Staphylococcus aureus* showed a good response to the use of amoxicillin/clavulanic acid, cephalosporins and quinolones. Jido et al. [29] working from Kano, North west, Nigeria had earlier reported a similar profile. Our observations tend to be in agreement with a study from Nepal [30] but inconsistent with that of another Nigerian study from Niger State where a high resistant profile was observed [31]. Although, in an Indian study by Sonawane et al. [28] *Staphylococci* showed complete (100%) susceptibility to vancomycin. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and other multidrug resistant patterns in some Nigerian centres is a pointer to the magnitude of the problem in our environment [32,33].

**Table 1. Profile of antibiotic susceptibility of the monomicrobial organisms isolated from the patients with wound infection**

Tested antibiotics	<i>Klebsiella spp</i>		<i>Staphylococcus aureus</i>		<i>Proteus spp</i>		<i>Providencia spp</i>		<i>E. coli</i>	
	T [S] (% <sub>S</sub> )	[R] (% <sub>R</sub> )	T [S] % <sub>S</sub>	[R] (% <sub>R</sub> )	T [S] % <sub>S</sub>	[R] (% <sub>R</sub> )	T [S] % <sub>S</sub>	[R] (% <sub>R</sub> )	T [S] % <sub>S</sub>	[R] (% <sub>R</sub> )
Gentamicin	19 [19] (100.0)	[0] (0.0)	17 [2] (11.8)	[15] (88.2)	11 [11] (100.0)	[0] (0.0)	7 [7] (100.0)	[0] (0.0)	2 [2] (100.0)	[0] (0.0)
Ceftriaxone	19 [19] (100.0)	[0] (0.0)	17 [15] (88.2)	[2] (11.8)	11 [11] (100.0)	[0] (0.0)	7 [7] (100.0)	[0] (0.0)	2 [2] (100.0)	[0] (0.0)
Amoxicillin/clavunate	19 [5] (26.3)	[14] (73.7)	17 [17] (100.0)	[0] (0.0)	11 [8] (72.7)	[3] (27.3)	7 [3] (42.9)	[4] (57.1)	2 [0] (0.0)	[0] (0.0)
Penicillin	19 [2] (10.5)	[17] (89.5)	17 [14] (82.4)	[3] (17.6)	11 [4] (36.4)	[7] (63.6)	7 [3] (42.9)	[4] (57.1)	2 [0] (0.0)	[0] (0.0)
Cefuroxime	19 [16] (84.2)	[3] (15.8)	17 [17] (100.0)	[0] (0.0)	11 [11] (100.0)	[0] (0.0)	7 [7] (100.0)	[0] (0.0)	2 [1] (50.0)	[1] (50.0)
Ciprofloxacin	19 [15] (78.9)	[4] (21.1)	17 [16] (94.1)	[1] (5.9)	11 [9] (81.8)	[2] (18.2)	7 [4] (57.1)	[3] (42.9)	2 [2] (100.0)	[0] (0.0)
Ceftazidime	19 [19] (100.0)	[0] (0.0)	17 [3] (17.7)	[4] (82.3)	11 [11] (100.0)	[0] (0.0)	7 [7] (100.0)	[0] (0.0)	2 [2] (100.0)	[0] (0.0)
Sparfloxacin	19 [14] (73.7)	[5] (26.3)	17 [17] (100.0)	[0] (0.0)	11 [11] (100.0)	[0] (0.0)	7 [7] (100.0)	[0] (0.0)	2 [2] (100.0)	[0] (0.0)
Cotimoxazole	19 [15] (78.9)	[4] (21.1)	17 [14] (82.4)	[3] (17.6)	11 [4] (36.4)	[7] (63.6)	7 [3] (42.9)	[4] (57.1)	2 [2] (100.0)	[0] (0.0)
Ampicillin	19 [7] (36.8)	[12] (63.2)	17 [11] (64.7)	[6] (35.3)	11 [1] (9.1)	[10] (90.9)	7 [2] (28.6)	[5] (71.4)	2 [0] (0.0)	[0] (0.0)

Key: T- Number of tested isolates, S- number of tested isolated sensitive to the antibiotic used, %<sub>S</sub> – Percentage of tested isolate sensitive to antibiotic used, R- Number of resistant isolates, %<sub>R</sub>- Percentage of resistant isolates to tested antibiotic

**Table 2. Profile of antibiotic susceptibility of polymicrobial organisms isolated from the patients with wound infection**

Antibiotics	T	S	% <sub>S</sub>	R	% <sub>R</sub>
Ciprofloxacin	21	17	81.0	4	19.0
Sparfloxacin	21	14	66.7	7	33.3
Ceftriaxone	21	21	100.0	0	0.0
Cefuroxime	21	21	100.0	0	0.0
Ceftazidime	21	19	90.0	2	10.0
Amoxicillin/ calvulanate	21	11	52.4	10	47.6
Gentamicin	21	21	100.0	0	0.0
Cotrimoxazole	21	3	14.3	18	85.7
Penicillin	21	2	9.5	19	90.5
Ampicillin	21	7	33.3	15	66.7

Key: T- Number of tested isolates, S- Number of tested isolates sensitive to the antibiotic used, %<sub>S</sub> – Percentage of tested isolate sensitive to antibiotic used, R- Number of resistant isolates, %<sub>R</sub>- Percentage of resistant isolates to tested antibiotic

*Proteus spp* demonstrated a good response of > 80% to the cephalosporins, quinolones and gentamicin. A resistance pattern of >63% was observed with the use of penicillin and cotrimoxazole. Our finding is consistent with that of Iregbu et al. [26] in Abuja, Nigeria and that of Mama et al. [27] in Ethiopia. *Providencia* also demonstrated a good response similar to that of *Proteus spp*. *Escherichia coli* had a sensitivity rate of > 95% to cephalosporins, quinolones and gentamicin but not penicillin and amoxicillin/clavulanic acid. Reports by workers from Uyo, South south Nigeria [34] revealed a resistant profile of 100% to ceftriaxone, gentamicin and quinolone and this is similar to what Sonawane and his colleagues [27] had earlier reported.

The poor responses of these organisms to the tested antibiotics were borne out of factors that have been identified to be peculiar to our environment. These include injudicious use of such drugs, poor patient compliance, sub-standard drugs and self medication [35-37]. The emergence of multi-drug resistant organisms is a nightmare for clinicians and the patients and the management of such may entail the use of newer generation but expensive antibiotics like meropenem and vancomycin as already reported from different climes [26,28,34]. Other newer agents that have been found useful in resistant cases include the glycopeptides (Dalbavacin, Oritavacin) and quinupristin/dalfopristin combination which have been found particularly useful in cases of vancomycin resistant organisms as well as methicillin resistant staphylococcus aureus (MRSA). Considering the peculiar problem of poverty in our environment, many of our patients might not be able to eventually afford the more potent, newer generation drugs when the need arises as is the case when dealing with multidrug resistant organisms. A change in policy direction and enforcing antibiotic stewardship might be a necessary way of combating this problem.

## 5. CONCLUSION

It is evident that there is an emerging problem of multidrug resistant organisms. Collaborative efforts are required among clinicians in order to curtail this trend. Well-structured antibiotic stewardship programmes in our institutions will be of judicious benefit. Government policies should strengthen and restrict the prescription of antibiotics in our hospitals to clinicians at appropriate levels while measures to curb the

over the counter sale of antibiotics are put in place.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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