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Original Research Article

Incidence and Antibiotic Susceptibility Pattern of Bacterial Isolates from Wound Infections in a Tertiary Hospital in Nigeria

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Abstract

Purpose: *To investigate the incidence of different bacteria isolates in 150 wound infections in Aminu Kano Teaching Hospital, Kano, Nigeria and their antibacterial susceptibility patterns.*
Methods: *Wound swab samples were collected from general culture bench of the Microbiology Department, after obtaining consent from the hospital's Medical Advisory Committee, and cultured for bacterial isolates. The isolates were characterized and identified by standard microbiological methods. Antibiotic susceptibility testing was carried out using Kirby-Bauer-CLSI modified Disc Agar Diffusion technique.*
Results: *Out of the 150 specimens collected, 82 % were infected with bacteria made up predominantl of Staphylococcus aureus (22 %), Pseudomonas aeruginosa (19.9 %), Citrobacter spp (15 %), Escherichia coli (14.7 %) and Proteus mirabilis (14.5 %). In vitro antibiotic susceptibility tests showed that Pseudomonas aeruginosa was susceptible to ceftazidime, ciprofloxacin and gentamicin while the enteric bacteria were generally more resistant to ceftazidime, gentamicin and ciprofloxacin.*
Conclusion: *The findings show that there is a high rate of wound infection in Kano, Nigeria and that antibiotic-resistant bacteria are present in the wound sites.*

Keywords*: Wound infection , Antibiotic, Susceptibility, Bacterial resistance*

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INTRODUCTION

The introduction of antibiotics has reduced mortality rates which were on the high side due to life-threatening diseases, especially infections. Enlightenment on the use and management of antibiotics cannot be overemphasized as it will continue to be the way out of many infections which occur frequently in plants and animals including humans. Increase in the misuse and mismanagement of antibiotics which are now leading to drug resistance is creating a lot of concern in medical practice. The current spread of multi-drug resistant bacterial pathogens has added a new dimension to the problem of wound infections [1]. This is particularly worse in resource-poor countries where sale of antibiotics is poorly controlled [2].

Infection of wound is the successful invasion, proliferation by one or more species of microorganisms anywhere within the body's sterile tissues, sometimes resulting in pus formation. Development of wound infection depends on the interplay of many factors. Wound infections may occur following accidental trauma and injections, but post-operative wound infections in hospital are most common. Some infections are endogenous in which infection occurs by patient's own bacterial flora such as *Staphylococcus aureus* from skin and anterior nares or coliforms.

Many infections are exogenous, with skin and anterior nares of the nostrils being important sources of *Staphylococci.* Spread of organisms from hospital staff and visitors occur by direct and indirect airborne routes. Certain parasite (e.g., *Hookworm larvae*) and bacteria (*Treponema pallidum*) can penetrate intact skin, but certain primary skin infections like impetigo is caused by *Streptococcus pyogenes* or *Staphylococcus aureus*, or both gaining access to abrasions as minor trauma to skin is a part of everyday life [3].

Organisms commonly found in infected wounds include Gram positive cocci such as *S. aureus, Streptococcus spp,* Gram negative bacilli mostly *Acinetobacter, Enterobacter*, *E. coli*, *Proteus spp*, *Ps. aeruginosa* and anaerobic bacteria such as *Propionibacterium spp.* and *Klebsiella spp*. [4]. This study therefore aims at investigating the incidence of wound infection in Kano, Nigeria and the antibiotic susceptibility of the possible bacterial isolates to commonly prescribed antibiotics in the locality.

EXPERIMENTAL

Study area

This study was carried out at Aminu Kano Teaching Hospital (AKTH), Kano in Northwestern Nigeria. It is the largest tertiary health institution in Kano State with a bed capacity of four hundred and twenty two.

Ethical considerations

In accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects [5], ethical approval for this study with number NHREC/21/08/2008a/ AKTH/EC/210 was obtained from the Medical Advisory Committee of the Aminu Kano Teaching Hospital, Kano, Nigeria.

Sample size

A total of 150 wound swabs submitted at the general culture bench from in-patients in different wards of the hospital, 76 of which were male and 74 female. Inclusion criterion was patients with purulent wounds.

Culture of specimen

The specimens were inoculated on blood, chocolate and MacConkey agar plates (Oxoid, Basingstoke, U.K) . The plates were incubated aerobically at 37 ^{0}C for 24 to 48 hours. Pure colonies were kept in nutrient agar slants. The nutrient agar slants were incubated at 37 ^{o}C for 18 – 24 h before storage in the refrigerator at 4 °C pending biochemical analysis.

Identification of bacterial pathogens

Pure cultures were characterized using morphological appearances on selective and differential media. Motility test and biochemical tests such as catalase, coagulase, oxidase, Voges Proskauer, hydrogen sulphide production, urease, methyl red, indole, citrate and sugar utilization tests were carried out according to standard techniques [6].

Antibiotics

A total of five (5) antibiotics, which represent the most commonly prescribed antibiotics for treatment of wound infections in the study area, were used in the study. Oxoid antibiotic discs used were amoxicillin (AMX, 10 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), ciprofloxacin (CIP, 10 µg) and gentamicin (CN, 30 µg).

Antibiotic susceptibility test

This was carried out using Kirby-Bauer-CLSI modified disc agar diffusion technique (DAD) [7]. One milliliter (1.0 ml) of standardized overnight culture of each isolate (containing 10^{6} CFU/ml) was used to flood the surface of Mueller Hinton Agar (MHA) plates and the excess drained off. The plates were left to dry. The standard antibiotic discs were then aseptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1 h. The plates (prepared in duplicates for each isolate) were then incubated at 37°C for 18 h. The diameter of the zones of inhibition produced by each antibiotic disc was measured and recorded after incubation.

Determination of the multiple antibiotic resistance index (MARI):
Multiple antibiotic resistance index (MARI) was determined for each isolate by dividing the number of antibiotics to which the organisms is resistant by the total number of antibiotics tested [8].

Statistical analysis

Data were processed with Microsoft Excel 2010. and general descriptive analysis, and correlation coefficient was used to analyze occurrence and extent of factors using Microsoft Excel 2010. $P < 0.05$ was considered significant.

RESULTS

Relatively higher proportion of samples from male patients yielded isolates. Ten (10) of the positive samples yielded two isolates on culturing. Gender distribution of patients with wound infections from October 2010 – December 2010 showed that out of seventy-six wound samples from male patients sixty-five (85.5 %) yielded bacterial isolates while fifty-eight (78.4 %) out of the seventy-four samples from the female patients yielded bacterial isolates. The difference was not statistically significant.

Surgical sites were the most frequently infected, accounting for 35 % of overall infection Burns and wound sepsis accounted for a cumulative of about 40 %. Diabetic ulcers were the least sites with infection (Figure 1).

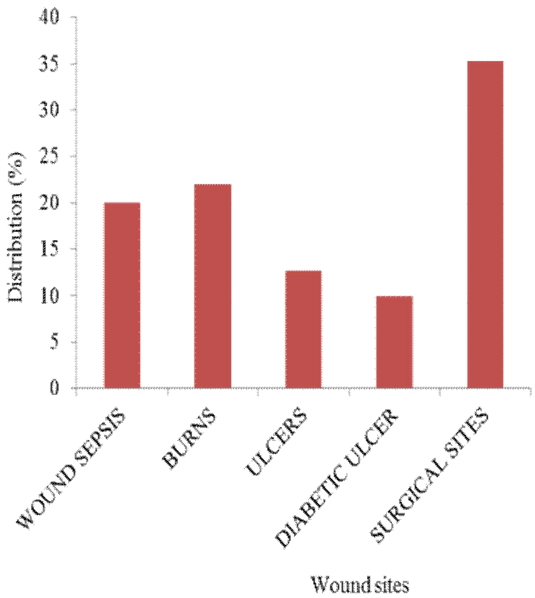

Figure 1: Distribution (%) of bacteria isolates from different wound sites of patients attending Aminu Kano Teaching Hospital from October – December 2010

Table 1 shows that the predominant contaminating organisms differ, depending on the wound type/site. The most predominant organism in wound sepsis was *S. aureus*, while in burns; the most predominant organisms were *S. aureus* and *Ps. aeruginosa*. In non–diabetic ulcers, the most predominant organisms were *Pr. mirabilis*, *E. coli*, and *S. aureus* as against *Ps. aeruginosa*, *Citrobacter spp*, *E. coli*, and *S. aureus* in diabetic ulcers. For surgical sites, there was a predominance of Enterobacteriaceae and *Ps. aeruginosa.*

High level of resistance against amoxicillin was observed among the organisms. Resistance to ciprofloxacin and gentamicin was observed to be the lowest among the organisms. Ceftazidime was observed to be the most active against *Ps. aeruginosa*. Resistance to gentamicin and ciprofloxacin was observed to be low amongst the *S. aureus* isolates. However, the Enterobacteriaceae especially *E. coli* isolates generally showed a high level of resistance against the test antibiotics (Table 2).

DISCUSSION

The distribution of wound contaminants in the prospective study which showed that *S. aureus*, *Ps. aeruginosa*, *Citrobacter spp, Pr. mirabilis and E. coli* are the predominant contaminating organisms is in agreement with the findings of Sani *et al* [10]

Surgical wounds sites with high bacterial contaminants constitute a serious problem in the hospital especially in surgical practice where clean operations can become contaminated and subsequently infected. The degree to which surface wounds are infected by surrounding bacteria contaminants have become clinically important [4]. The predominance of Gram negative bacilli in surgical site infection (SSI) in this study is similar to that of Mofikoya *et al*, [11]. In most cases of SSI, the organism is usually patient's endogenous flora. In abdominal surgeries, the opening of the gastrointestinal tract increases the likelihood of coliforms and Gram negative bacilli as agents of wound infection which was the finding in this study. These groups of organisms tend to be endemic in hospital environment by being easily transferred from object to object and they also tend to be resistant to common antiseptics, often difficult to eradicate in the long term [11]. This does not agree with the work of Jonathan *et al* [12] who reported *S. aureus* as the predominant isolate. Enterobacteriaceae are increasingly playing a greater role in the many hospital acquired infections [11].

Staphylococcus aureus was the predominant organism in wound sepsis. This finding is in agreement with the works of Mashita *et al* [13]. In burn wounds, both *S. aureus* and *Ps. aeruginosa* were the most prevalent as has been reported by others in the past [14]. The type of organisms isolated from ulcers and diabetic ulcers such as *Ps. aeruginosa*, *S. aureus*, *Citrobacter spp* and *E. coli* in a prospective study is similar to findings

Table 1: Distribution (%) of organisms at different infected wound sites

	Distribution at different wound sites				
Bacteria isolates	**Wound sepsis**	**Burns**	**Ulcers**	**Diabetic ulcers**	**Surgical sites**
E.coli (n = 18)	27.8	5.6	22.2	11.1	33.3
Salmonella spp (n = 1)	0.0	0.0	0.0	0.0	100
Providencia spp (n = 2)	0.0	0.0	0.0	0.0	100
Pr. vulgaris (n = 5)	0.0	0.0	0.0	20.0	80.0
Pr. mirabilis (n = 19)	5.3	21.0	26.3	0.0	47.4
Ps. aeruginosa (n = 28)	7.1	32.1	7.1	14.3	40.0
Citrobacter spp (n = 21)	23.8	14.3	4.8	9.5	47.6
Enterobacter spp (n = 7)	0.0	28.6	0.0	0.0	71.4
S. aureus (n = 31)	48.4	29.0	9.6	6.5	6.5
Klebsiella spp (n = 9)	11.1	33.4	22.2	11.1	22.2

Table 2: Resistance (%) of bacterial isolates to selected antibiotics

	Resistance to selected antibiotics (%)				
Bacteria isolates	**Amx**	**Ctx**	**Ctz**	**Cip**	**Gen**
Ps. aeruginosa (n = 28)	100	46	4	18	21
S. aureus (n = 31)	100	52	84	29	29
Klebsiella spp (n = 9)	100	56	67	56	78
Enterobacter spp (n = 7)	100	86	86	57	71
Citrobacter spp (n = 21)	67	76	81	57	57
E. coli (n = 18)	94	89	89	89	89
Pr. mirabilis (n = 19)	68	32	37	26	21
Pr. vulgaris (n = 5)	100	20	20	0	50
Salmonella spp (n = 1)	100	100	100	0	0

Key: Amx = Amoxicillin, Ctx = Ceftriaxone, Ctz = Ceftazidime, Cip = Ciprofloxacin, Gen = Gentamicin Values presented in Table 3 shows that a high proportion of the wound bacterial isolates were multiple antibiotic resistant (i.e., had MARI > 0.2). While S. aureus, Salmonella spp, Enterobacter spp, Citrobacter spp, Klebsiella spp and E. coli isolates were multi – antibiotic resistant isolates. Proteus spp and to a lesser extent Providencia spp were less resistant to multiple antibiotics.

Table 3: Multiple Antibiotic Resistance Index (MARI) of bacteria isolates from wound infections (based on the antibiotics in Table 2 above)

Bacteria isolate	**% with MARI > 0.2**
Ps. aeruginosa (n=28)	60.7
S. aureus (n=31)	90.3
Salmonella spp (n=1)	100
Klebsiella spp (n=9)	77.8
Providencia spp (n=2)	50.0
Enterobacter spp (n=7)	100
Citrobacter spp (n=21)	85.7
E. coli (n=18)	88.9
Pr. mirabilis (n=19)	42.1
Pr. vulgaris (n=5)	40.0

of Fadeyi *et al* [15] and Vimalin and Growther [16]. *Pseudomonas aeruginosa* and *S. aureus* have been implicated in wound sepsis by a number of researchers [17,18].

Generally, inadequate antimicrobial treatment defined as ineffective treatment of infection is an important factor in emergence of antibiotic resistant bacteria. Factors that contribute to inadequate antimicrobial treatment of hospitalized patients include: the prior use of antibiotic, broad spectrum antibiotics, prolonged hospital stay and the presence of invasive medical devices. Other factors include the spread of resistant organisms through overcrowding and inadequate hospital infection control practices [19].

There was general resistance to amoxicillin by the wound bacterial isolates, which might be due to the pressure of prolonged usage and regular abuse in our society. This is not unconnected with the abuse of amoxicillin by the populace since antibiotics are still sold across the counter in some pharmaceutical and patent medicine stores in Nigeria. The recent surge in the widespread use of ceftriaxone, a broad spectrum cephalosporin, in this region for empirical treatment could explain the increase in the resistance level observed in this study [20].

The low level of resistance shown by the *Ps. aeruginosa* isolates to ceftazidime (a third generation cephalosporin), ciprofloxacin and gentamicin indicates that these three drugs are still effective in this region compared to other studies which showed, for example, that *Ps. aeruginosa* is highly resistant to gentamicin [21]. A high sensitivity profile to ciprofloxacin and gentamicin has also been reported by Taiwo *et al* [4]. It is gratifying to note that *S. aureus* isolates, which were the most predominant species among the wound bacterial contaminants were sensitive to gentamicin and ciprofloxacin. A similar observation was reported by Taiwo *et al* [4]. These antibiotics, therefore, are still relevant in the treatment of wound infection in this study area.

CONCLUSION

The study showed that *S. aureus*, *Ps. aeruginosa* and enteric bacteria are the major agents found in infected wound site in AKTH, Kano, Nigeria, Nigeria. The bacteria isolates are generally resistant to β-lactam antibiotics but gentamicin and ciprofloxacin are effective against the isolates. However, bacterial isolates which were not readily susceptible to commonly used antibiotics, such as amoxicillin and ceftriaxone, were present in the study environment.

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