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PREVALENCE OF PATHOGENIC BACTERIA ISOLATED FROM SURGICAL SITE AND WOUND INFECTION AMONG PATIENTS ADMITTED IN SOME SELECTED HOSPITALS IN SOKOTO METROPOLIS, NIGERIA

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Abstract

Surgical and open wounds are commonly encountered in clinical practice. This study was aim to determine the prevalence of pathogenic bacteria in surgical and open wound infection among patients admitted in some selected hospitals in Sokoto metropolis. A total of one hundred and fifty one (151) isolates were obtained from two hundred (200) surgical site and wound samples collected from patients in this study. The result showed that Usmanu Danfodiyo Teaching Hospital Sokoto (UDUTH) had the highest number of clinical isolates with 64 gram positive and gram negative bacteria followed by Specialist Hospital Sokoto (S.H.S) with 57 gram positive and gram negative bacteria and then Maryam Abacha Women and Children Hospital (MAWCH) with 30 gram positive and gram negative bacteria. Gram positive cocci 108 (71.5%) were more predominant pathogen isolated in the hospitals than gram negative bacilli 43 (28.5%). *Staphylococcus aureus* had the highest number of occurrence with 54(35.76%) followed by Coagulate negetive *Staphylococci* with 47(31.1%) while *Citrobacter freundii* had the lowest number of occurrence with 2(1.32%) isolates. Also, the susceptibility of the isolates to antimicrobial agents were carried out using Amoxacillin, Ampicillin, Erythromycin, Chloramphenicol, Ampiclox, Ciprofloxacin, Gentamycin, Tetracycline, Pefloxacin and Cotrimoxazole. The mean zone of inhibition recorded against *Staphlococcus aureus* by using Amoxacillin antibiotic is 2.20mm while with *Citrobacter freundii* is 1.00.

Key words: Surgical site, pathogenic bacteria, Susceptibility, UDUTH, MAWCH and S.H.S

Introduction

Surgical and open wounds are commonly encountered in clinical practice. They may arise post-operatively, following trauma, in association with heamoglobinopathy, or could be primarily of infective origin. Wound infection could be defined as the presence of pus in a lession, as well as other general or local features of sepsis including pyrexia, pain and induration. Wound infection is an important cause of morbidity and mortality among surgical patients. Apart from causing discomfort to patient, the effect may vary from being a simple nuisance; to a delay in wound healing and other major disasters (Anyiwo, 1995). Post-operative wound infections can be caused through two major sources: exogenous and endogenous bacteria. The probability of wound infections largely depends on the patients' systemic host defences, local wound conditions and microbial burden.

All surgical wounds are contaminated by both pathogens and body commensals ranging from bacteria and fungi to other parasites (Bowler *et al.,* 2001: Anguzu and Olila, 2007). The common gram positive organisms are the β – haemolytic streptococcus – *Streptococcus pyogenes* and *Staphylococcus aureus*. The gram negative aerobic rod is *Pseudomonas aeruginosa*. The facultative anaerobes include *Enterobacter* species, *Escherichia coli*, *Klebsiella* species and *Proteus* species. The fungi are *Candida* species and *Aspergillus* species (Mordi and Momoh, 2009: Sani *et al.,* 2012), but the development of infection in the site depends on complex interplay of many factors (Olsen *et al.,* 2008). These may be microbial virulence patient risk factors like diabetes, cigarette smoking, obesity, and coincident remote site infections or colonization and operation-related risk factors including prolonged hospital stay before surgery, duration of the operation, tissue trauma, poor homeostasis, and foreign materials in the wound. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria (Sani *et al.,* 2012). Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Certain antibiotic classes are highly associated with colonization of superbugs (Goossens *et al,* 2005; Motta *et al,* 2003). Now days, about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and they can only treat with experimental and potentially toxic drugs (Todar *et al.,* 2008). Infection of a wound is the successful invasion and proliferation by one or more species of microorganisms anywhere within the body's sterile tissues, sometimes resulting in pus formation (Calvin, 1998). Development of wound infection depends on the interplay of many factors. The breaking of the host protective layer, the skin, and thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response (Collier, 2003). 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 from patient's own bacterial flora such as *S. aureus* from skin and anterior nares or coliforms. Many infections are exogenous; skin and anterior nares are important sources of *Staphylococci*, spread of organisms from hospital staff and visitors occur by direct and indirect airborne routes.

The current spread of multi-drug resistant bacteria pathogens has added a new dimension to the problem of wound infections (Sule and Olusanya, 2000). This is particularly worse in resource poor countries where sale of antibiotics is under poor control (Onile, 1997). A regular bacteriological review of infected wounds is therefore a necessity if affected patients must receive qualitative health care, particularly when blind treatment is a necessity, as in underdeveloped and developing nations (Fadeyi *et al.,* 2008). The conditions of antimicrobial therapy, both prophylactically and therapeutically, can only be defined when these factors are under control. Hence, an on-going surveillance could play a significant role in the early recognition of a problem and hence, there is a need for early intervention for better management of post-operative wound infections. Therefore, the aim of this study is to determine the prevalence of pathogenic bacteria in surgical and open wound infection among patients admitted in some selected hospitals in Sokoto metropolis.

Materials and methods

Study area

Sokoto is the capital of Sokoto state, located at north western part of Nigeria. It covers approximately an area of 56,000 square kilometer and lies roughly between 3^0E and 15^0E of Greenwich and between 4^oN and 14^0N of the equator (Yakubu, 1989). It share border with Niger republic toward north, Zamfara state to east and Kebbi state to the south and west. It has population of 3,702,676 (NPC, 2006). Sokoto State has a land area of about 28,232.37 km^2, with a mean annual rainfall ranging between 500mm to 1, 300mm. The mean annual temperature is about 28.30◦C; the maximum daytime temperatures are under 40 0C for most months of the year. The warmest months are usually between February and April, when daytime temperatures can exceed 45◦C (Ikuomola, 2010). There are two major ethnic groups namely, Hausa and Fulani. Also, there are Zabarmawa as minority in the border of the local government areas.

Samples collection

Patients with surgical and open wounds attending Specialist hospital Sokoto (SHS), Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Maryam Abacha Women and Children Hospital Sokoto (MAWCH) were sampled in this study. The hospitals were situated in Sokoto metropolis; the choice was based on the fact that the hospitals were one of the centers which attend to large number of patients from different socio economic backgrounds in the state. Consent of the hospital management and patient were sought for prior to sampling. A total of 200 samples of patient were collected, of which 80 samples were from SHS, 80 also from UDUTH and 40 samples from MAWCH. From the total samples, 123 were males and 77 were females with surgical and open wounds using sterile commercial swab sticks.

Inoculation and isolation of samples

The swabs collected from each patient were inoculated on macconkey agar, blood agar and chocolate agar by spreading, and the plates were incubated aerobically and anaerobically (Gaspack anaerobic jar) for 24 hours at 37^0C (Cheesbrough, 2006; Holt *et al.*, 1994). The colonies that emerge were subcultured continuously to obtain pure culture as described by Cheesbrough, (2006).

Antibiotic susceptibility test

Antibiotics used in Sensitivity test include the following: Amoxacillin (30µg), Ampicillin (10µg), Erythromycin (15µg), Chloramphenicol (25µg), Ampiclox (30µg), Ciprofloxacin (10µg), Gentamycin (30µg), Tetracycline (30µg), Pefloxacin (30µg) and Co-trimoxazole (25µg). A well dried plate of Mueller-Hinton agar was inoculated using a sterile swab; the swab was streaked evenly over the surface of the medium. Using a sterile forceps the antimicrobial discs were placed evenly on the inoculated plates. Each discs was firmly pressed to ensure its contact with the agar, 30 minutes of applying the discs, the plates were inverted and incubated aerobically at 35^{0}C for 18 hours (Cheesbrough, 2006).

Results

A total number of one hundred and fifty one (151) isolates were obtained from two hundred (200) surgical site and wound samples collected from patients in this study. The result of the bacteria isolated from wound and surgical site samples in the three selected Hospitals showed that Usmanu Danfodiyo Teaching Hospital Sokoto (UDUTH) had the highest number of clinical isolates with 64 gram positive and gram negative bacteria followed by Specialist Hospital Sokoto (SHS) with 57 gram positive and gram negative bacteria and then Maryam Abacha Women and Children Hospital (MAWCH) with 30 gram positive and gram negative bacteria (Table 1). Gram positive cocci 108 (71.5%) were more predominant pathogen isolated in the hospitals than gram negative bacilli 43 (28.5%). However, high statistically significant difference was observed between the gram positive and gram negative organisms ($t = 4.2402$, $df = 5$, $p = 0.008168$) as shown in Table 1.

The frequency of occurrence of the isolates in the three hospitals was shown in Table 2. *Staphylococcus aureus* had the highest number of occurrence with 54(35.76%) followed by Coagulate negetive *Staphylococci* with 47(31.1%), then *Proteus mirabilis* with 13(8.61). *Citrobacter freundii* had the lowest number of occurrence with 2(1.32%) isolates.

Table 3 shows the susceptibility of the isolates to antimicrobial agents were carried out using Amoxacillin, Ampicillin, Erythromycin, Chloramphenicol, Ampiclox, Ciprofloxacin, Gentamycin, Tetracycline, Pefloxacin and Cotrimoxazole. The mean zone of inhibition recorded against *Staphlococcus aureus* by using Amoxacillin antibiotic is 2.20mm, against Coagulate negative *Staphylococci* is 2.21, against *Streptococcus pyogenes* is 2.14, against *Proteus vulgaris* is 1.22, against *Proteus mirabilis* is 1.15, against *Klebsiella* spp is 2.80, against *Pseudomonas aeroginosa* is 1.66, against *Eschericia coli* is 1.50 and *Citrobacter freundii* is 1.00. This was done to all the antibiotics used. The isolates were highly resistant to Amoxacillin with the mean total of 1.76mm, followed by Ampicillin with mean total of 1.82mm, and Ampiclox with 2.04mm. Pefloxacin has the highest number of sensitive bacteria with 6.16mm which means Pefloxacin is highly sensitive to the isolates.

Table 1: Bacteria isolated from surgical sites and wound infection

HOSPITALS	TOTAL SAMPLES	NO. OF ISOLATES (%)	GNB (%)	GPC (%)
S.H.S	80(40.0)	57 (37.7)	15 (26.3)	42 (73.7)
UDUTH	80(40.0)	64 (42.4)	21 (32.8)	43 (67.2)
MAWCH	40(20.0)	30 (19.9)	7 (23.3)	23 (76.7)
TOTAL	200(100)	151(100)	43 (28.5)	108 (71.5)

GNB – Gram negative bacilli, GPC – Gram positive cocci (t = 4.2402, df = 5, p = 0.008168)

Table 2: Frequency of occurrence of the identified bacteria

Bact. Isolated	S H S	UDUTH	MAWCH	No. Of isolateas (%)
Staph aureus	20 (37.0)	26 (48.2)	8 (14.8)	54(35.8)
Coag neg Staph	19 (40.4)	15 (31.9)	13 (27.7)	47(31.1)
Strept pyogene	3 (42.8)	2 (28.6)	2 (28.6)	7(4.6)
Proteus vulgaris	4 (44.4)	4 (44.4)	1 (11.2)	9(6.0)
Proteus mirabilis	6 (46.2)	5 (38.5)	2 (15.3)	13(8.6)
Kleb spp.	1 (20.0)	3 (60.0)	1 (20.0)	5(3.3)
Pseudomonas	2 (22.2)	5 (55.6)	2 (22.2)	9(6.0)
E. coli	1 (20.0)	3 (60.0)	1 (20.0)	5(3.3)
Citrobacter.	0(0.00)	2 (100.0)	0 (0.00)	2(1.3)
Total	57 (37.7)	64 (42.4)	30 (19.9)	151(100)

Table 3: Susceptibility of bacteria isolates to selected antimicrobial agent (Mean zones of inhibition in milimeter)

Antimicrobial agent (mm)	*Staph aureus*	*Coag neg st*	*Strept pyog*	*P.vulg*	*P.mirab*	*Kleb*	*Pseudo aerugi*	*E.coli*	*Citro freundii*	Mean Total
Amoxacillin (µg)	2.20	2.21	2.14	1.22	1.15	2.80	1.67	1.50	1.00	1.76
Ampicillin (µg)	3.28	3.59	1.71	1.67	1.08	2.40	R	2.67	R	1.82
Erythromycin (µg)	3.67	4.34	2.29	3.44	1.31	3.80	0.78	2.60	R	2.46
Chloramphenicol (µg)	4.09	4.34	2.43	2.33	1.85	4.60	0.22	4.40	3.00	3.03
Ampiclox (µg)	3.11	3.19	1.57	4.22	0.92	2.00	1.11	2.20	R	2.04
Ciprofloxacin (µg)	4.91	5.64	3.71	2.89	3.46	5.00	0.22	3.60	R	3.27
Gentamycin (µg)	4.00	4.49	3.57	3.22	2.08	4.20	0.44	3.00	R	2.77
Tetracycine (µg)	3.11	3.85	3.00	2.11	1.77	5.40	0.22	1.20	R	2.29
Pefloxacin (µg)	6.33	6.72	6.29	6.33	4.54	7.40	2.89	6.00	9.00	6.16
Co-trimoxazole (µg)	3.24	3.68	2.29	3.11	1.69	3.20	0.44	2.00	4.00	2.62

Key
Coagu neg S. - Coagulate negative *Staphylococci*
P. *vulg* - *Proteus vulgaris*
P. *mirab* - *Proteus mirabilis*
Kleb - *Klebsiella* spp.
Pseudo - *Pseudomonas aeruginosa*
Citro – Citrobacter freundii
R – Resistant

Discussion

The result of this study showed that the bacteria isolated from wound samples of patient's attending three major health centres in Sokoto metropolis, a preponderance of Gram positive organisms 108 (71.5%) as compared to gram negative isolates 43 (28.5%). This could be due to the fact that the Gram positive bacteria are the most common organisms found on the skin (normal flora), which causes boils or wound infection if they get an access to the inner layer of the skin through a cut or an abrasion. Gram positive bacteria has been documented to be one of the most common cause of skin infection such as boils, abscesses as well as a great number of more serious infections such as osteomyelitis, acute endocarditis and septiceaemia (Cheesbrough, 2006). The implication is that, most wounds will be infected with Gram positive bacteria since they form the major commensals of the skin. This agrees with previous study of Cutting and White (2004), who reported that pathogens that infect the wound are primarily gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative bacteria such as *Acinetobacter baumannii-calcoaceticus* complex, *Pseudomonas aeruginosa*, and *Klebsiella* species. Bowler *et al*. (2001) and Branom, (2002) reported similar observations; they stated that wound infections are caused by both gram-positive and gram-negative bacteria, especially by *Staphylococcus aureus*, *Eschericia coli* and *Pseudomonas.* Similarly, studies conducted in Ethiopia had shown that wound infections are caused by *Staphylococcus aureus, E.coli, Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and Coagulase negative *Staphylococci* (Biadeglegne *et al*., 2009; Tesfahunegu *et al*., 2009; Gedebou *et al*., 1987). Similar results have been obtained in Ghana (Newman *et al*., 2006), Nigeria (Okesola and Oni, 2000) and Uganda (Anguzu and Olila, 2007).

However, Obunge and Ekere (2002) reported *Pseudomonas* spp to be the most common bacteria isolated from wounds, others are *Staphylococcus aureus*, *Klebsiella* spp, *Escherichia coli* and *Proteus* spp. The prevalence is about 70% and 30% respectively, and is similar to the previously reported prevalence of 67% and 32% in a study conducted by Sule *et al*., (2002) on bacterial isolated from wound infection in Ogun state of Nigeria.

Similarly, a retrospective study by Agnihotri *et al*, (2004) revealed the major aerobic bacterial isolates from pus/wound swabs taken from patients admitted to the burn unit at Govt. Medical College Hospital, Chandigarh, India, over a period of 5 years (June 1997–May 2002). In the study, the pus/wound swabs yielded very high culture positivity (96%) for 665 total isolates. *Pseudomonas aeruginosa* was found to be most common isolate (59%) followed by *Staphylococcus aureus* (17.9%), *Acinetobacter* spp. (7.2%), *Klebsiella* spp. (3.9%), *Enterobacter* spp. (3.9%), *Proteus* spp. (3.3%) and others (4.8%).

The organisms isolated from wounds of patients in the three selected hospitals in Sokoto metropolis include *Staphylococcus aureus,* Coagulate negetive *Staphylococci, Streptococcus* spp, *Proteus vulgaris, Proteus mirabilis*, *Klebsiella* spp, *Pseudomonas aeruginosa*, *Escherichia coli* and *Citrobacter freundii*. The source of these organisms may probably be from endogenous (normal flora), exogenous (hospital environment) or health personnel. It may also be due to the fact that wounds are initially colonized predominantly by Gram positive bacteria, which are fairly quickly replaced by Gram negative organisms usually within a week of the wound injury (Weber, 1998). The implication of this finding is that, these organisms may play a role in at least one stage of the wound infection, and they can prevent healing and making treatment difficult to patient. This work can be compared to that of Shriyan *et al.*, (2010) carried out in India on post operative wound infections. These researchers were able to isolate *Staphylococcus aureus* Coagulate negetive *Staphylococci*, *Proteus vulgaris, Proteus mirabilis, Klebsiella* spp, *Pseudomonas aeruginosa*, *Escherichia coli* and others in their samples. Emele *et al.*, (1999) also reported *Staphylococcus aureus, Pseudomonas* spp and *coliform bacilli* in wound infection in Ekpoma. Nicoletti *et al.*, (2006) discovered that three bacterial species, *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli,* accounted for more than 50% among all Intensive Care Unit (ICU) isolates in a study carried out in Italy. Other prevalent bacterial isolates were *Staphylococcus epidermidis* and *Enterococcus faecalis*, while *Acinetobacter baumanii* ranked third.

Several studies have implicated these organisms in wound infection (Biadeglegne *et al.*, 2009; Tesfahunegu *et al.*, 2009; Gedebou *et al.*, 1987). Sule *et al*, (2002) discovered that *klebsiella* spp, *Pseudomonas aeruginosa* and *Proteus mirabilis* have the occurrence in a study carried out in Ogun state on bacteria associated with wound infection. Likewise, Nasser *et al*. (2003) revealed that the most frequent isolate was *Pseudomonas aeruginosa* (21.6%), followed by *Klebsiella pneumoniae* (15.2%), then *Escherichia coli* (13.6%), *Staphylococcus aureus*

(13.2%), Coagulase-negative *Staphylococci* (11.6%), *Streptococcus pyogenes* (8.3%), *Enterobacter* species (6.6%), and lastly *Streptococcus faecalis* and *Candida albicans* (5.9 and 3.6%, respectively) in a study carried out in Egypt.

The frequency of occurrence of the isolates from the three selected hospitals (SHS, UDUTH and MAWCH) shows that *Staphylococcus aureus and* coagulate negetive *Stahylococci* had the highest incidence of 35.76% and 31.1% respectively (Table 3). This can also be attributed to the fact that, since *Staphylococcus* spp forms the larger parts of normal flora on the skin, they could possibly be among the first bacteria to infect wounds. Another major reason could be that *Staphylococcus* spp is a successful pathogen that has combination of bacterial immuno-evasive strategies. One of these strategies is the production of carotenoid pigment staphyloxanthin, which is responsible for the characteristic golden colour of *S. aureus* colonies. This pigment acts as a virulence factor, primarily by being a bacterial antioxidant which helps the microbe evade the reactive oxygen species which the host immune system uses to kill pathogens (Clauditz *et al*., 2006; Liu *et al*., 2005). The implication of this result is that, both *Staphylococcus* spp has certain enzymes that can cause serious side effects like Scalded skin syndrome. This enzyme can loosens the "cement" holding the various layers of the skin together, and allows blister formation and sloughing of the top layer of skin. If it occurs over large body regions, it can be deadly. A study of bacteria associated with wound infection carried out in Ekpoma Edo state Nigeria, showed that out of the organisms encountered, *Staphylococcus aureus* was the most frequently occurring organism (39%), followed by (24%) coliform (Emele *et al.*, 1999). Similarly, studies conducted in Ethiopia have shown that wound infections are caused by *Staphylococcus aureus, E. coli, Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and Coagulate negative *Staphylococci* (Biadeglegne *et al.*, 2009; Tesfahunegn *et al.*, 2009; Gedebou *et al.,* 1987). A study on post-operative wound, in which 84 isolates were obtained from 100 pus samples / wound swabs which were collected from clinically suspected post-operative wound infections shows that *Staphylococcus aureus* was the most frequently isolated pathogenic bacteria from post-operative wounds (Shriyan *et al.,* 2010).

In terms of the susceptibility to amoxillin, Ampicillin, Erythromycin, Chloramphenicol, Ampiclox, Ciprofloxacin, Gentamycin, Tetracycline, Pefloxacin and Cotrimoxazole, Pefloxacin with 6.16mm was sensitive to almost all the isolates (Table 3). This may be due to the fact that the antibiotics belong to the third generation quinolones, being broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, which is an enzyme necessary to separate, replicated DNA, thereby inhibiting cell division . Pefloxacin possess excellent activity against gram-negative aerobic bacteria such as *E.coli* and *Neisseria gonorrhoea* as well as gram-positive bacteria including *S. pneumoniae* and *Staphylococcus aureus*. They also possess effective activity against *shigella, salmonella, campylobacter, gonococcal* organisms, and multi drug resistant *pseudomonas* and *enterobacter* (Drlica and Zhao, 1997).The isolates showed some degree of resistance to some of the antibiotics. The least active amongst the antibiotics were Amoxacillin (1.76mm) and Ampiclox (2.04mm), this could be due to fact that, these antibiotics belong to the first and second generation cephalosporins, which possess a β- lactam ring, since the organism are capable of producing β- lactamase enzymes which is capable of inactivating the β- lactam ring's in the drugs, rendering the drug inactive. This work can be compared to that of Sule *at el*, (2002) which shows the sensitivity profile of the isolates in his study to different antibiotics to have high in-vitro susceptibility to fluoroquinolones (Pefloxacin, Ciprofloxacin, Ofloxacin). The isolates however showed a low susceptibility to Ampicillin, Penicillin, Tetracycline and Cotrimoxazole. Similarly in a study done by Azene and Beyene, (2011), shows that Amoxicillin had the highest resistance rate 78.9%, followed by Tetracycline 76.1% and Erythromycin (63.9%). The sensitivity rates of flouroquinolones (Norfloxacin, Ciprofloxacin and Gentamycin) were 95.1%, 91.8% and 85%, respectively. The most frequently isolated bacteria were sensitive to ciprofloxacin, gentamicin, cloxacillin and norfloxacin. These antimicrobials are considered as appropriate antimicrobials for empirical treatment of wound infections.

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