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Aetiology and antimicrobial studies of surgical wound infections in University of Uyo Teaching Hospital (UUTH) Uyo, Akwa Ibom State, Nigeria

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Abstract

The prevalence of surgical wound infections and antibiotic sensitivity patterns of bacterial isolates at the University of Uyo Teaching hospital was studied using standard microbiological techniques. From the 1370 surgeries, 120 (8.8%) patients had infected surgical wounds. Out of a total of 150 bacterial isolates, 100 (66.7%) were Gram-negative bacteria and 50 (33.3%) were Gram-positive bacteria. The most prevalent was *Proteus* spp (33.3%) followed by *Staphylococcus aureus* (20.0%), *Escherichia coli* (20.0%), Coagulase-negative Staphylococcus (13.3%), *Klebsiella* spp (6.7%) and *Pseudomonas* spp (6.7%) the least. Methicillin-resistance in *Staphylococcus aureus* was 100%. Extended spectrum beta-lactamase (ESBL) production was seen in 50% of Gram-negative bacteria (*Proteus spp, E.coli* and *Klebsiella spp*) and they were predominantly sensitive only to Imipenem. Both gram-negative and gram-positive bacteria were resistant to routinely used antibiotics. The gram-negative bacterial isolates were mainly resistant to Cefoxtazime, Cefpodoxime and Levofloxacin. Adequate preventive measures should therefore be enforced to prevent the spread of antibiotic resistant organisms since surgical wound infections constitute a major cause of mortality and morbidity.

Keywords: ESBL; MRSA; Drug resistance

Introduction

Surgical wound infection is clinically defined as purulent discharge from the surgical wound or the insertion wound of the drain, or spreading cellulitis from the wound [1]. Surgical wound is characterized by inflammation around periwound area. Surgical wound infections are the second most common cause of nosocomial infections [2,3]. The high rate of surgical wound infections is associated with higher mobidity, mortality and increased medical expenses [3,4]. In spite of the new antibiotics available today, surgical wound infection still remains a threat due to secondary bacterial contamination and widespread use of prophylactic antibiotics that lead to emergence of multi-drug resistant bacteria [3].

Surgical wounds are expected to heal within a predictable time depending on the type, wound, and extent of surgery. The primary closure of a clean, surgical wound would be expected to require minimal intervention to enable healing to progress naturally and quickly without complication in the surgical wound [1]. This may however be complicated by infection or existing co-morbidity. The control of wound infection has become more challenging due to wide spread bacterial resistance to antimicrobials, therefore, knowledge of the aetiological agent of wound infection has proven to be helpful in the selection of the empirical antimicrobials to be used in treating the infection. Beta-lactamases are bacterial enzymes that inactivate beta-lactam antibiotics. Beta-lactamases that inactivate all the penicillins and cephalosporins including the extended spectrum cephalosporins are termed Extended Spectrum Beta-Lactamases, (ESBLs). There are approximately 500 different ESBLs described, all of which are mutations of the classical broad-spectrum beta lactamase enzymes that were initially named TEM and SHV (TEM-1, TEM-2, SHV-1). The presence of ESBL-producing Enterobacteriaceae complicates therapy, especially because these organisms are often multidrug resistant. Therefore infections caused by ESBL-producing Enterobacteriaceae are of serious concerns in the current environment. Many ESBLs are frequently expressed in gram-negative bacteria and they confer resistance to ampicillin, amoxicillin, and other penicillins, as well as to early but not later-generation cephalosporins.

This study was carried out as part of the hospital infection surveillance and control. It seeks to prospectively evaluate the frequency of surgical wound infections, the bacterial aetiologic agents and their antimicrobial susceptibility patterns.

Materials and Methods

The study was carried out from January to October 2011 at the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, South-South Nigeria. It is a tertiary hospital, with >250 beds and served as a referral centre. Patients who had surgery during the study period were followed up until discharge. The wounds were aseptically swabbed and the exudates cultured on Blood agar, MacConkey agar (LabM limited, Lancashire, UK) and Manitol salt agar plates. Yellowish colonies from the Manitol salt agar plates were sub-cultured on blood agar base supplemented with 5% sheep blood. Plates were incubated aerobically at 35°C for 24-48 hours. The bacterial isolates were identified using bacteriological procedures involving morphology, microscopy and biochemical tests [5]. Antimicrobial susceptibility testing was carried out using the modified Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines [6].

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicilin resistance was tested using 1 µg oxacillin disc (Oxoid

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Ltd, Cambridge, UK) according to CLSI guidelines. Mueller Hinton Agar were inoculated with 0.5 McFarland preparation of the inoculum using the spread plate technique. 1 µg Oxacillin disc was placed on the agar and incubated aerobically at 35°C for 24 hours. Organisms with zones of inhibition ≤ 12 mm were interpreted as methicillin resistant while those with zones of inhibition ≥ 13 mm were interpreted as methicillin susceptible (Figure 1).

Inducible Macrolide Lincomycin Streptogramin B (iMLS$_B$)

This was done for all *Staphylococcus* isolates which were resistant to Erythromycin but susceptible to Clindamycin as described by the CLSI [6]. Mueller Hinton Agar plates were inoculated with 0.5 McFarland preparation of the inoculum using the spread plate technique. Then a 15 µg Erythromycin (Oxoid ltd, Cambridge, UK) and 2 µg Clindamycin discs were placed at 15-26 mm apart. The plates were incubated for 18-24 hrs at 35°C aerobically. Isolates that showed flattening of the Clindamycin zone of inhibition adjacent to the erythromycin disc (referred to as a D-zone) was regarded as exhibiting inducible Clindamycin resistance.

Extended spectrum β-Lactamase (ESBL)

Enterobacteriaceae isolates with cefotaxime, ceftazidime and cefpodoxime zones of inhibition less than 27 mm, 22 mm, 17 mm respectively were suspected to be ESBL producing . A confirmatory test using the Double Disc Synergy technique was carried out according to CLSI guidelines [6]. Mueller Hinton Agar plates were inoculated with 0.5 McFarland preparation of the inoculum using the spread plate technique. Then amoxicillin-clavulanate (20 µg-10 µg) disc was sandwiched by 30 µg ceftazidime, 30 µg cefpodoxime and 30 µg cefotaxime discs placed 15 to 20 mm edge to edge from the amoxicillin-clavulanate disc (Figure 2). The plates were incubated aerobically for 16-18 hrs at 35°C. Isolates which showed increase in the inhibition zone of the cephalosporin adjacent to the amoxicillin-clavulanate disc was considered to be ESBL- producing (Figure 3).

Staphylococcus aureus ATTC 25923 and *Escherichia coli* ATTC 25922, and locally isolated MRSA and ESBL producing E. coli designated ASU11 and AFU11 respectively were used as controls during the study (Figure 4).

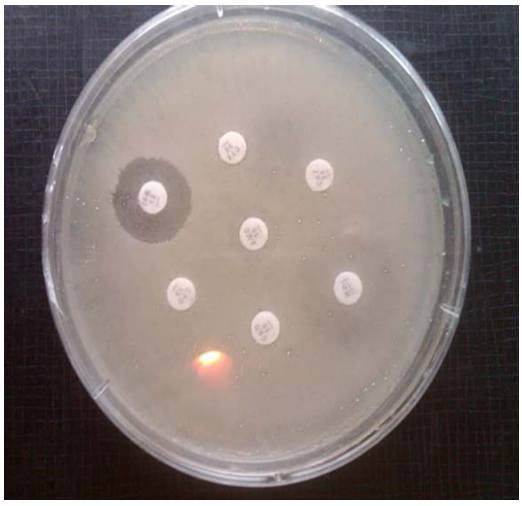

Figure 1: A sensitivity plate showing multidrug resistant isolate of *S. aureus*.

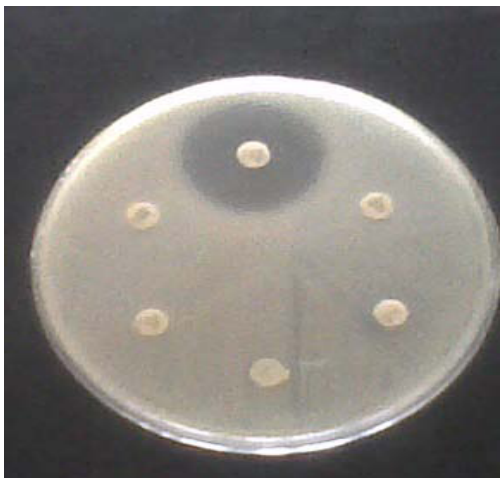

Figure 2: Sensitivity test plate of *E. coli* showing resistance to Ceftrixone and Ceftazidime.

Figure 3: Isolate from (plate 2) confirmed to be ESBL positive with double disc synergy test.

Figure 4: ESBL positive control of *E. coli*.

Results

Out of a total of 1370 surgeries carried out in University of Uyo

Teaching Hospital from January to October 2011, obstetrics ward was found to have the highest 730 (53%) while the female surgical ward had the lowest 40 (2.9%). Surgical wound infections occurred in 120 (8.8%) of the patients (Table 1).

One hundred and fifty bacterial isolates were obtained from the 120 wound samples which were infected out of the 1370 samples. Single aetiological agent was identified in 90 wound while two aetiological agents were found in 30 samples. The isolates were *Proteus* spp 50 (33.3%), followed by *E.coli* 30 (20.0%), *Staphylococcus aureus* 30 (20.0%) coagulase negative staphylococcus 20 (13.3%), *Klebsiella* spp 10 (6.7%) and *Pseudomonas* spp 10 (6.7%) (Table 2).

Extended Spectrum Beta-lactamase (ESBL) production was seen in 50 out of the 100 Gram-negative isolates. All three *E.coli* isolates were ESBL producing. Twenty of the *Proteus* isolates were also ESBL-producing (Table 3). All *S. aureus* isolates were methicillin resistant. Inducible Clindamycin resistance was however not observed on any isolate.

Table 4B shows that all the 30 (100%) *Staphylococcus aureus* isolates were sensitive to Clindamycin, 20 (66.7%) were sensitive to Erythromycin and 10 (33.3%) sensitive to Trimethoprim-sulfamethaxole. Table 4A; E.coli, 10 (33.3%) were sensitive to Amoxycillin-clavulanate, 20 (66.7%) were sensitive to Ceftazidime and 30 (100%) were sensitive to imipenem. For *Proteus* spp, 20 (40%) were sensitive to Gentamicin and Amoxycillin-clavulanate while ten isolates (20%) showed sensitivity to Cefotaxime, Cefpodoxime, Ceftazidime and Levofloxacin. For *Klebsiella* spp, all the isolates were sensitive to Imipenem and Gentamicin. In the case of *Pseudomonas* spp, the ten isolates were sensitive to Imipenem, Ceftazidime

Wards	Surgeries done (%)	No of SWI (%)
Obstetrics	730(53.3%)	50(41.7%)
Male Orthopedics	90(6.6%)	20(16.7%)
Female Orthopedics	130(9.5%)	10(8.3%)
Pediatrics	50(3.6%)	10(8.3%)
Male Surgical ward	220(16.1%)	20(16.7%)
Female Surgical ward	40(2.9%)	10(8.3%)
Gynecologic ward	110(8.0%)	0
TOTAL	**1370**	**120(8.8%)**

Table 1: Distribution of surgeries done and Surgical Wound Infections (SWI) according to surgical units in UUTH in the month of October 2011.

Organisms	No (%)
Staphylococcus aureus	30(20.0%)
Coagulase-negative *Staphylococcus* spp	20(13.3%)
Escherichia coli	30(20.0%)
Proteus spp	50(33.3%)
Klebsiella spp	10(6.7%)
Pseudomonas spp	10(6.7%)
TOTAL	**150**

Spectrum Beta-lactamase (ESBL).

Table 2: Prevalence of individual bacterial isolates from wound infection.

Organisms	ESBL Positive No (%)
Escherichia coli n=30	30(100)
Proteus spp n=50	20(66.7)
Klebsiella spp n=10	0(0.0)
Pseudomonas aeruginosa n=10	0(0.0)
TOTAL	**50**

Table 3: Distribution of Gram-negative bacteria producing Extended.

A. Gram-negative bacilli (% sensitivity) n=100

Antibiotics	*E.coli* (n=30)	*Proteus* spp(n=50)	*Klebsiella* spp(n=10)	*Pseudomonas* spp(n=10)
Cefoxitin	ND	ND	ND	ND
Carbenicillin	ND	ND	ND	0
Erythromycin	ND	ND	ND	ND
Clyndamycin	0	0	ND	ND
Gentamicin	0	20(40%)	10(100%)	10(100%)
Levofloxacin	0	10(20%)	0	0
Imipenem	30(100%)	50(100%)	10(100%)	10(100%)
Amoxycillin-clavulanate	10(33.3%)	20(40%)	0	ND
Ceftazidime	20(66.7%)	10(20%)	0	10(100%)
Ceftriaxone	0	ND	ND	ND
Cefpodoxime	0	10(20%)	0	0
Cefoxtazime	ND	10(20%)	0	0
Trimethoprim-sulfamethaxole	0	10(20%)	0	ND

Key: ND- Not Determined, 0 - 100% resistant

B. Gram-positive organisms (% sensitivity) n=30.

Antibiotics	*S.aureus* (n=30)
Cefoxitin	0
Erythromycin	20(66.7%)
Clyndamycin	30(100%)
Levofloxacin	0
Amoxycillin-clavulanate	10(33.3%)
Ceftriaxone	20(66.7%)
Trimethoprim-sulfamethaxole	10(33.3%)

Key: 0 - 100% resistant

Table 4: Antimicrobial Sensitivity Pattern of bacterial isolates from Surgical Wounds.

Discussion

Surgical wound infection has been a major concern among health care practitioners, not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost effective management within the health care system [1]. The study revealed surgical wound infection occurred in 8.8% of the patients investigated.

Obstetrics unit had the highest number of surgical wound infections (41.7%) followed by male orthopaedic/male surgical ward. However, in the gynecologic ward, there was no reported case of surgical wound infection. Sonawane et al. [7] showed that surgical wound infections were higher in general surgery (73.86%) than obstetrics/gynecology (10%). The incidence of sternal wound infection reported by different studies range from 0.9% to 20.0% [8].

The risk of developing surgical wound infection depends on the number of bacteria that colonise the surgical wound [8]. While the operating wound following surgery is considered to be "clean", the surgical wound may be contaminated by air-borne bacteria in the operating room and intensive care units, by bacteria from endogenous sources such as the patient's mucous membrane, the hands of theatre personnel or by direct contamination by the patient's normal skin microflora [9]. The effect of specific types of microrganisms on wound healing has been widely published, and although the majority of wounds are polymicrobial involving both aerobes and anaerobes, aerobic pathogens such as *S. aureus*, *P. aereginosa* and beta-hemolytic streptococci have been most frequently cited as the cause in delayed

wound healing and infections. In the study, 150 bacterial isolates comprising 66.7% gram negative and 33.3% gram positive were isolated. *Proteus* spp (33.3%), *Staphylococcus aureus* (20%) and *E.coli* (20%) were the three predominant isolates. This result is in conformity with the findings of Oguachuba [10] who found *Proteus* spp to be the most common isolate (41.9%) followed by *Staphylococcus aureus* (25.6%). This finding is different from Gayne et al. [11] in which *Pseudomonas* spp had the highest prevalence of 33.3%. Onchne [12] found that *Staphylococcus aureus* accounted for 71.4% of the total isolates; while Mbamali [13] isolated *Staphylococcus aureus* in 60% of the patients.

Shittu et al. [14] reported that *S. aureus* was predominant (25%) followed by *E. coli* (12%), *Pseudomonas aereginosa* (9%) and *S. epidermidis* (9%). Also Sonawane et al. [7] showed that *Staphylococcus aureus* (29.26%) the commonest isolate followed by *Escherichia coli* (18.7%) and *Pseudomonas* spp (15.37%).

It has been shown that majority of surgical wound infections are caused by *Staphylococcus aureus*, and other species of the patients own microflora [9]. Overall, wound infections caused by *S. aureus* in cardiac surgery patients vary between 12% and 36.4%.

In addition to *S.aureus*, and coagulate negative staphylococci, *E. coli*, *Klebsiella* spp, Propionibacterium acnes, Streptococcus, Enterococci and other less common pathogens have been isolated from infected sternal wounds [15].

In the present study 100% of *S. aureus* isolates were methicillin-resistant. This is higher than the 38.56% reported by Mohanty et al. [16], and 19.56% reported by Tahnkiwale et al. [17], Vellore, India (24%) [18]. Jones et al. [19] also reported 32.4% to 44.4% *S. aureus* isolates were methicillin-resistant. Sonawane et al. [7] also showed that Methicillin resistance accounted for 27.85% of *S. aureus* isolates.

The spread of ESBL producing bacteria has been strikingly rapid worldwide, indicating that continuous monitoring systems and effective control measures are absolutely required. In the study, expression of ESBL was demonstrated in 50% of the total Gram negative bacilli. Mohanty et al. [16] and Mathur et al. [20] obtained higher values of 66.75% and 68.0% respectively. Enterobacteriaceae may also express ESBLs that are not closely related to TEMor SHV-related species, including CTX-M- and OXA type ESBLs, among others. CTX-M-type ESBLs typically hydrolyze cefotaxime more efficiently than Ceftazidime [21]. The study showed that 70% of the ESBL in *E. coli* isolated from patients with community onset infections were of the CTX-M-type [22]. Other studies, too, have reported a high prevalence of CTX-M-type ESBLs in community-onset infections [23].

Metri et al. [24] working in India, the prevalence of ESBL–producing organisms was found to be 32.1% which was slightly higher than Vinodkumar and Neelagund [25] and Krishnan and Macaden [26].

The present study reveals that among the Gram negative isolates, 100% of *E. coli* isolates 66.7% and *Proteus* spp were ESBL-producing whereas Metri et al. [24] showed that *K. pneumonia* and *E. coli* were the major ESBL-producers.

However, Sonawane et al. [7] ESBL productions was seen in 71.72% of gram negative isolates notably *Enterobacter* spp (100%), *E. coli* (82.18%), *Citrobacter* spp (72.72%), *Klebsiella* spp (67.4%), *Pseudomonas* spp (66.26%) and *Proteus* spp (63.64%).

Antibiotic sensitivity testing showed that amongst the gram-negatives, *E. coli* and *Proteus* spp showed sensitivity to Amoxycillin-clavulanate and Ceftazidime while *Klebsiella* spp was completely resistant to Amoxycillin-clavulanate, Cefpodoxime, Cefotaxime, Levofloxacin Trimethoprim-sulfamethaxole and Ceftazidime. The organism was 100% sensitive to Imipenem. This could be due to the fact that Imipenem is a new drug and it is less predisposed to abuse because of its high cost. Also, Sonawane et al. [7] showed that the gram negatives were more sensitive to piperacillin-tazobactam and Imipenem while both Gram positive and Gram negatives were increasingly resistant to routinely used antibiotics.

The susceptibility data collected in this study suggests that multidrug resistance is a common problem in hospital pathogens such as *Proteus* spp, *Staphylococcus aureus*, *E. coli, Klebsiella* spp, *Pseudomonas* spp etc. Surgical Wound Infection isolates were found to be resistant to Ceftazidime, Levofloxacin and the aminoglycosides. This has important implication as patients in a tertiary care hospital like UUTH receive Cephalosporins, Aminoglycosides, Quinolones or combinations of these drugs as empirical therapy.

The susceptibility data suggests that multidrug resistance is a common problem in University of Uyo Teaching Hospital (UUTH). There is need for re-evaluation of pre, intra and post surgical procedures in the management of surgical patients and regular quality control of disinfections to re-establish their effectiveness.

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