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Aetiological agents of surgical site infection in a specialist hospital in Kano, north-western Nigeria

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Abstract: Despite the advances made in asepsis, antimicrobial drugs, sterilization and operative techniques, surgical site infections (SSI) continue to be a major problem in all branches of surgery in the hospitals. The objective of this study was to establish the incidence of SSI, the type and frequency of various pathogens and their antibiotic susceptibility pattern in Murtala Mohammed Specialist Hospital (MMSH), Kano, Nigeria. A total of 2,920 consecutive patients who underwent different surgical procedures between January 2009 and December 2010 at Murtala Mohammed Specialist Hospital were enrolled in this study. Samples of pus were collected from infected cases and screened for bacterial and fungal pathogens by standard microbiological methods. Antibiotic susceptibility tests were done by disc diffusion technique. A total 878 patients were found to be clinically infected and 919 isolates were obtained in all. This gave an incidence of 30.1% infection rate. While 783(89.2%) cultures yielded single organism, 77(9.8%) yielded mixed growth and 18(8.1%) cultures yielded no growth. The most frequently isolated organism was *E. coli* (25.5%) followed by *Staphylococcus aureus* 20.6%. The incidence of Methicillin resistant *Staphylococcus aureus* was 35.7%. Ceftriaxone, ceftazidime and ofloxacin showed good results against most isolates while ampicillin and cotrimoxazole which are commonly used drugs were ineffective. An understanding of the various types of pathogen involved in SSI and their antibiotic susceptibility pattern will reduce indiscriminate prescription of antibiotics and help in infection control.

Keywords: aetiology, surgical site, infection, antibiotics, susceptibility, Nigeria

Introduction

Despite the advances made in asepsis, antimicrobial drugs, sterilization and operative techniques, surgical site infections (SSI) continue to be a major problem in all branches of surgery in the hospitals (Linani *et al*., 2005). SSIs are responsible for the increasing costs and the morbidity and mortality which are related to surgical infections (Anvikar *et al*., 1999). The organisms which cause surgical infections vary from time to time and from place to place (Kumar *et al*., 1985). The pathogens that infect surgical wounds can be part of the patient's normal flora or they may be acquired from the hospital environment (Angue & Olila, 1999).

In a study on occurrence of *Pseudomonas aeruginosa* in postoperative wound infection in Lagos, it was found that *Pseudomonas aeruginosa* was the most frequently isolated followed by *Staphylococcus aureus* (Oguntubeju & Nwobu, 2004). The least isolated organism was *Streptococcus Pyogenes* and *Enterococcus faecalis*. Ejikeme (2004) also in a study of the bacteriology of postoperative wound infection in the surgical wards of a federal medical Centre, in Umuahia, South-Eastern Nigeria, found *Pseudomonas aeruginosa* as the most frequently isolated organism followed by *Proteus* spp. The least isolated organism was also *Streptococcus pyogenes*. Some other authors (Jan *et al*., 2010; Shah *et al*., 2010) reported *E. coli* as the most frequently isolated bacteria in their study

Collated data on the incidence of wound infections probably underestimate the incidence because some wound infections occur when the patient is discharged, and these infections may be treated in the community without hospital notification (Waqar *et al*., 2010). However, the incidence of infection varies from surgeon to surgeon, from hospital to hospital, from one surgical procedure to another and most importantly from one patient to another (Nichols, 2001).

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The objective of this study was to determine the incidence and various types of pathogens involved in SSI in Murtala Mohammed Specialist hospital, Kano, Nigeria including their antibiotic susceptibility pattern which will be of clinical relevance in Kano City, there is dearth of current and relevant information on this subject

Materials and Methods

Study area
Murtala Mohammed Specialist hospital (MMSH) is a tertiary health care facility in the city of Kano North-western Nigeria with about 1000 beds. It provides a high level Medicare to a large population of people in a highly populated state. The study was carried out in male and female surgical wards, maternity, gynaecology and paediatric surgical wards where both emergency and elective procedures were performed.

Study participants
This prospective cross sectional study was carried out between January 2009 and December 2010. Patients who underwent various surgical procedures available in the hospital in the wards mentioned above were included in the surgery after informed consent. Orthopaedic surgical patients including those with implants were not included. Also infants and children between 0 – 10 years were also not included.

Sampling and Sample size
Samples of pus were carefully collected from clinically confirmed infected cases after cleaning the surrounding areas in eight hundred and seventy eight (878) patients out of a total number of 2920 patients who actually underwent surgery. A surgical site infection (SSI) case was identified using Centre for Disease Control (CDC) definition which states that infection would be regarded as an SSI if it occurs within 30 days of procedure and has at least one of the following purulent drainage from the wound, pain or tenderness, localized swelling, redness, malodour and fever. Patients that underwent procedure with implants were not enrolled in the study (Emori & Gaynes, 1993).

Laboratory sample and test
A total number of 2,920 consecutive patients who underwent different surgical procedures between January 2009 and December 2010 at Murtala Mohammed Specialist Hospital (MMSH) were enrolled in this study. Samples of pus were collected from infected cases and screened for bacterial and fungal pathogens by standard microbiological methods. Antibiotic susceptibility tests were done by disc diffusion technique.

Eight hundred and seventy eight consecutive swab samples from post-operative infections were aseptically collected from patients in the male and female surgical ward, obstetrics and gynaecology including paediatric ward. Sterile swab sticks used were manufactured by Evepon Nigeria Limited and all the samples were processed at the Microbiology laboratory in MMSH. The samples were inoculated on MacConkey, manitol salt, Blood and saboraud's dextrose agar for bacterial and fungal screening. Aerobic incubation was done at 37°C for 18-24hrs. Culture plates for fungal isolates were incubated at room temperature for upwards of 72hrs. Isolates were identified by standard microbiological methods including gram stain, motility, oxidase, indole tests and other biochemical tests according to Cheesebrough (1993). Enterotube technique – Microbiology systems (BD BBL Enterotube II, UK) was used to identify isolates to species level. The control strains used were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

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Sensitivity tests

Sensitivity tests were carried out by the disc diffusion methods of Kirby-Bauer and compared with standard isolates (Bauer *et al*., 1996). The swab stick sample for anaerobes were inoculated immediately after aseptic collection onto neomycin sulphate blood agar and cooked meat medium for anaerobic bacteria and incubated at 37°C for 48 – 72 hrs. The cooked meat medium was sub-cultured on neomycin sulphate blood agar. The culture plates for anaerobes were put into an anaerobic jar (BBL gar pack system, Oxoid) and procedures carried out according to the manufacturer's instruction. Antibiotic disc identification tests with vancomycin 1mg, collistin 10µg and vancomycin 5µg (Oxoid) were used for the preliminary grouping of anaerobes. Bile disc was used for *Bacteroides fragilis* while penicillin and novobiocin discs (oxoid) were equally used for anaerobic cocci according to the methods of Baron *et al*. (1994).

The susceptibility of consecutive isolates of *S. aureus* to oxacillin was determined on Mueller-Hinton agar supplemented with 2% NaCl. Plates were inoculated by dipping sterile cotton swabs into the suspension of the overnight growth of the organism prepared to a density of McFarland No.0.5 standard; expressed excess liquid from the swabs and inoculated the surface of the agar by spread method. The 1µg oxacillin discs (Oxoid) were aseptically placed on the surface of the inoculated plates and incubated aerobically at 35°C for 18-24hours. The zones of inhibition were measured and compared with CLSI (2010). The isolates that were resistant to oxacillin (≤ 10mm diameter) were termed methicillin resistant *S. aureus* (MRSA) (CLSI, 2010).

Data analysis

Data obtained from the study were analysed using EPI INFO Version 6 for chi-squared test. Comparisons of other parameters were done by simple percentages.

Ethical considerations

Ethical clearance was approved for the research study by the management of Murtala Mohammed Specialist Hospital, Kano Northwestern Nigeria.

Results

A total of 2,920 subjects were involved in this study. The age group 31 - 40yrs was observed to have the least infection rate while >70yrs had the highest. The difference was statistically significant (χ^2 trend = 4.57 p = 0.03) (Table 1). The infection rate by service units among surgical patients shows that the least infection rate was observed in the female surgical ward while the highest was seen at the Male ward D (mostly urogenital patients) (Table 2). When all the wards were compared together there was a significant difference in the infection rate (χ^2 = 88.4 df = 4 p<0.0001). When the ward comprising only females were compared, a statistically significant difference in the infection rate was observed (χ^2 = 72.58 df = 2 p<0.0001). When the wards comprising only male was analyzed, there was also a significant difference observed (χ^2 = 10.17; df = 1 p<0.001).

Table 1: Age, sex distribution and infection rate among surgical wound infected patients in MMSH

Age	Male (%)	Female (%)	No. of subjects	No. infected	Infection rate(%)
11-20	68(21.5)	126(22.1)	587	194(22.1)	33.0
21-30	65(20.5)	257(45.7)	1100	322(36.7)	29.3
31-40	63(19.9)	93(16.5)	675	156(17.8)	23.1
41-50	44(13.9)	39(6.9)	257	83(9.4)	32.3
51-60	25(7.9)	31(5.5)	136	56(6.4)	41.2
61-70	30(9.4)	-	85	30(3.4)	35.3
>70	21(6.6)	16(22.8)	80	37(4.2)	46.2
Total	316	562	2920	878	30.1

χ^2_{trend} =4.57 p=0.03

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Table 3 shows the frequency of isolates from pure culture from surgical site infection patients in MMSH. *E. coli* (25.5%) was the most frequently isolated bacteria followed by *S. aureus* (20.6%) and *Proteus mirabilis* (13.5%) *Bacteroides fragilis* (2.0%) was the most frequently isolated anaerobe followed by *Peptococcus* spp. (1.4%). *Candida* spp. (1.0%) was also observed. Most common isolates from mixed culture were *E. coli+P. mirabilis* (18), *S. aureus+P. vulgaris* (10) and *Peptostreptococci+S. aureus* (6) (Table 4).

Table 2: Infection rate by service units among surgical patients at MMSH

Service units	Number (%) of patients	No. (%) of patients infected
Maternity ward	1161 (39.8)	446 (38.4)
Female ward A	428 (14.6)	94 (21.9)
Female surgical ward	294 (10.1)	50 (17.0)
Male surgical ward	668 (22.9)	163 (24.4)
Male ward D	369 (12.6)	125 (33.8)
Total	2920	878 (30.1)

χ^2 =88.4, df=4 p<0.0001

Polymicrobial cultures were frequently observed with *P. mirabilis* occurring 18 times together with *E. coli*, while *S. aureus* and *P. vulgaris* also occurred together for 10 times. Also two cultures were observed to yield three different organisms each. While one of the cultures yielded *P. mirabilis*, *E. coli* and *S. aureus*, the other yielded *P. vulgaris*, *E. coli* and *S. aureus*. The total number of isolates reported in this study was 919. While 603 (65.6%) were Gram negative, 316(34.4%) were Gram positive bacteria. A ratio of 2.9:1 was observed.

Table 3: Species and frequency of isolates in pure culture from surgical wound infection in patients

Isolate	No. of isolates	Total isolates
E. coli	197	197
P. mirabilis	95	95
P. vulgaris	33	33
Coagulase negative *Staphylococcus*	23	23
E. faecalis	32	32
S. aureus	164	164
Streptococcus spp.	28	28
P. aeruginosa	68	68
C. freundii	16	16
Candida spp.	9	9
K. pneumonia	45	45
M. morgana	3	3
B. fragilis	14	14
Clostridium spp	2	2
Peptostreptococcus spp	12	12
Peptococcus spp	13	13
Fusobacterium spp	4	4
Serracia marcescens	2	2
Enterobacter spp.	3	3
Total	840	919

The traditional wound classification showed the infection rate a follows; clean wound 8.4%, clean contaminated 38.6% contaminated 45.0% and dirty infected 70.0%. *S. aureus* and Coagulase negative *Staphylococcus* (COANS) were commonly isolated in clean wounds while bacteria of the enterobacteriacea and anaerobes were the isolates from the other types of wounds.

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Out of a total of 193 isolates of *S. aureus* observed 69(35.7%) were methicillin resistant when tested. The cephalosporins, ceftazidime and ceftriaxone including the fluoroquinolone, ofloxacin showed above 70% sensitivity to most Gram negative bacteria while cotrimoxazole and ampicillin were ineffective (Table 5).

Table 4: Species and frequency of isolates in mixed culture from surgical wound infection in patients

Isolate	No. of isolates	Total isolates
E. coli + *P. mirabilis*	18	36
E. coli + *Streptococcus spp.*	5	10
E. coli + *Proteus vulgaris*	4	8
E. coli + *Staphylococcus aureus*	2	4
E. coli + *Enterococci* spp	3	6
P. aeruginosa + *P. mirabilis*	2	4
K. pneuminiae + *P. vulgaris*	2	4
P. mirabilis + *S. aureus*	7	14
P. aeruginosa + *E. Coli*	2	4
E. faecalis + *K. Pneumonia*	1	2
S. aureus + *P. vulgaris*	10	20
S. aureus + *P. aeruginosa*	3	6
Candida spp + *Escherichia coli*	2	4
P. mirabilis+E. coli+S. aureus	1	3
P. vulgaris+Enterobacter+E. Coli	1	3
Bacteriodes+E. Coli	4	8
Peptostreptococci+S. aureus	6	12
Fusobacterium+P. mirabilis	4	8

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Table 5: Antibiotic susceptibility pattern of isolates from surgical wound infection in patients from MMSH

Pathogens	**Total no. of isolates**	No. (%) of isolates sensitive to									
		CAZ	**OFX**	**AMC**	**CXC**	**CN**	**COT**	**CRO**	**ERY**	**CIP**	**AMP**
E. coli	239	192(80.3%)	180(75.3%)	155(64.8%)	ND	144(60.3%)	6(2.5%)	202(84.5%)	ND	128(53.5%)	18(7.5%)
P. mirabilis	127	93(73.2%)	99(77.9%)	72(56.6%)	ND	81(63.7%)	0(0%)	80(62.9%)	ND	65(51.2%)	10(7.8%)
P. vulgaris	50	36(72.0%)	35(60.0%)	28(56.0%)	ND	32(64.0%)	0(0%)	38(76.0%)	ND	26(52.0%)	8(16.0%)
CN- Staphylococcus	23	13(56.0%)	15(65.2%)	12(52.1%)	18(78.0%)	13(56.0%)	0(0%)	13(56.0%)	18(78.0%)	16(69.5%)	0(0%)
E. faecalis	36	22(61.1%)	21(58.3%)	18(50.0%)	15(41.6%)	15(41.6%)	4(11.1%)	24(66.6%)	18(50.8%)	20(55.5%)	5(13.8%)
S. aureus	193	156(80.8%)	160(82.9%)	120(62.1%)	164(84.9%)	119(61.6%)	0(0%)	150(77.7%)	124(64.5%)	120(62.1%)	0(0%)
Streptococcus spp.	33	12(36.4%)	14(42.4%)	12(33.3%)	19(57.5%)	12(33.3%)	2(6.1%)	17(51.5%)	15(55.5%)	12(33.3%)	3(9.1%)
P. aeruginosa	75	57(76.0%)	59(78.6%)	1(1.3%)	ND	50(66.6%)	0(0%)	56(74.7%)	ND	25(33.3%)	0(0%)
C. freundii	16	10(62.5%)	12(75.0%)	8(50.0%)	ND	8(50.0%)	0(0%)	11(68.8%)	ND	8(50.0%)	2(12.5%)
K. pneumoniea	48	35(72.9%)	34(70.8%)	25(52.1%)	ND	28(58.3%)	0(0%)	35(72.9%)	ND	30(62.5%)	4(8.3%)
M. morgana	3	2(66.6%)	3(100.0%)	1(33.3%)	ND	0(0%)	0(0%)	3(100%)	ND	0(0%)	0(0%)
S. marsescenes	2	2(100.0%)	1(50%)	1(50%)	ND	1(50%)	0(0%)	1(50.0%)	ND	1(50.0%)	0(0%)
Enterobacter spp	4	3(75%)	3(75%)	2(50.0%)	ND	2(50%)	0(0%)	3(100%)	ND	4(100%)	0(0%)

CAZ-Ceftazidime, OFX-Ofloxacin, AMC-Amoxicillin/clavulanate, CXC-Cloxacillin, COT-Cotrimoxazole, CRO-Ceftraxone, ERY-Erythromycin, CIP-Ciprofloxacin, AMP-Ampicillin, ND-Not done; CN= coagulase negative

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Discussion

Once a patient's major defence against infection, the intact skin, is breached by either trauma or surgical knife, a broad avenue is opened to introduction of virulent bacteria (Enhrenkranz & Meakins, 1992). Multiplicity of factors influence SSI rate in clinical practice. This could result from the patient undergoing surgery, members of the operating room or the operating room environment. The pathogens isolated from infections differ primarily depending on the surgical procedure. In clean surgical procedures, in which the gastrointestinal, gynaecologic and respiratory tracts have not been entered, *S. aureus* from the exogenous environment or the patient's skin flora is the usual cause of infection (Nichols, 1984). In other categories of surgical procedures, including clean-contaminated, contaminated and dirty, the polymicrobial aerobic and anaerobic flora closely resembling the normal endogenous microflora of the surgically resected organ are the most frequently isolated pathogens (Nichols, 1984). This is in agreement with the observation in this study.

In the present study *E. coli* was the most frequently isolated bacterium and also reflected in the reports of Jan *et al*. (2010) and Shah *et al*. (2010). However, the above observation is at variance with the findings from some other workers who observed *Staphylococcus aureus* as the most frequently isolated organism in their different centres (Isibor *et al*., 2008; Abu, 1990; Twum-Danso *et al*., 1992). The recovery of Candida spp. in this study also agrees with the finding of Isibor *et al*. (2008) in Nigeria.

Polymicrobial cultures were observed in this study where a mixture of two and three different bacterial isolates was obtained in significant proportions. Ganguly *et al*. (2000) reported a similar observation in their study. The high ratio of Gram negative bacteria observed over Gram positive bacteria in this study agrees with the report of Oni *et al*. (2001). Methicillin resistant *S. aureus* had a prevalence rate of 35.7% which compares favorably with the observations of Taiwo *et al*. (2005) though lower than the report from studies in Jos, Nigeria (Ikeh, 2003).

The overall infection rate of 30.1% observed in this study is in agreement with the findings of Kamat *et al*. (2008) but higher than in more developed countries (Dellinger & Enhrenkranz, 1998). A report from Tehran (Arabshahi & Koohpayezade, 2006) reported an infection rate of 8.4% while that from Tanzania (Eriksen *et al*., 2003) reported an infection rate of 19.4%. The hospital environment supports the acquisition of resistance to antibiotic agents by pathogens, complicating the treatment of infections due to drug resistant pathogens (Emori & Gaynes, 1993). Commonly used antimicrobial agents such as cotrimoxazole and ampicillin were found to be ineffective in the *in vitro* sensitivity. Cephalosporins such as ceftriaxone and ceftazidime including the fluoroquinolone ofloxacin gave encouraging results. These observations are in agreement with the reports of Mahesh *et al*. (2010).

Studies on surgical site infection should be carried out regularly in each locality to establish any changes in the pattern of antibiotic resistance as well as spectrum of prevalent pathogens. Baseline data obtained from such studies will be of clinical relevance in guiding antibiotic prescription policies for prophylaxis and therapeutic purposes. This will be very helpful in reducing SSI in this environment.

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