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Full Length Research Paper

Studies on multiple antibiotic resistant bacterial isolated from surgical site infection

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Surgical Site Infection (SSI) has been difficult to manage due to bacterial resistance. The study aim was to determine multiple antibiotic resistance by bacterial pathogens from SSI. Purulent materials were aseptically collected from postsurgical in-patients (n=64) and assessed for sensitivity to antibiotic of frequent use in the study area. A total of 80 bacterial isolates were obtained. Forty eight (75%) of the samples yielded monomicrobial growth while 16 (25%) yielded polymicrobial growth. *Staphylococcus aureus* was the predominant bacteria (25%); followed by *Pseudomonas aeruginosa* (20%), *Escherichia coli* (15%), *Klebsiella oxytoca* (10%), *Proteus mirabilis* (10%), *Klebsiella aerogenes* (5%), *Coagulase-negative Staphylococcus* (5%), *Streptococcus pyogenes* (5%) and *Proteus vulgaris* (5%). The infection was most prevalent in the age group of 21 (65%), least prevalent in the age group of '41 years and above' (5%); more in males (76.5%) than females. This was statistically significant (P<0.01). The results of the antibiotics susceptibility showed that the Gram-positive isolates were highly resistant to penicillin, cloxacillin, chloramphenicol and ampicillin but all except *Staphylococcus aureus* were highly sensitive (70 - 90%) to streptomycin and gentamycin and erythromycin. The Gram-negative isolates showed resistance (70 - 100%) to clotrimoxazole, ampicillin, streptomycin and tetracycline and moderately sensitive to nitrofurantoin, nalidixic acid, but highly sensitive to gentamycin and colistin except *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. The result showed that 56 (70%) of the isolates were multi drug resistant. This calls for abstinence from antibiotic abuse.

Key words: Multi drug resistant, susceptibility, isolates, surgical, wound.

INTRODUCTION

All surgical wounds are contaminated by both pathogens and body commensals (Davis et al., 1999; Anguzu and Olila, 2007), but the development of infection in the site depends on complex interplay of many factors (Olsen, 2008). These may be microbial virulence (Bowler et al., 2001), patient risk factors like diabetes, cigarette smoking, obesity, and coincident remote site infections or colonization (Reichman and Greenberg, 2009) and operation-related risk factors including prolonged hospital stay before surgery, duration of the operation, tissue trauma, poor hemostasis, and foreign material in the wound, with these last greatly increasing the risk of serious infection despite a relatively small bacterial inoculum (Rubin, 2006).

Meanwhile, the organisms that would invade the tissue depend on the location of the wound (Oluwatosin, 2005). This is because of close correlations between microorganisms present in wounds in close proximity to those sites (Brook, 1987). Superficial incisions are mostly colonized by endogenous bacterial flora (commensals) or pathogens, of which *S. aureus* is frequently observed (Lilani et al., 2005). The microbial pathogens are therefore brought to wound site by direct contact, self contamination and airborne (Prakash, 2010). Whichever organism(s) is involved depends on the locations of wound, exposure of the patients and the hospital hygiene. Nosocomial infection poses a great treat to surgical wound management

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especially when the microbe involved is resistant to conventional antibiotics in wound management.

Davis et al. (1999) observed that 106 (14%) of the surgical equipment yielded positive culture containing *Staphylococcus* spp*., Pseudomonas* spp*., Bacillus* spp*. among* others. Such isolates have been implicated as causing multiple antibiotic resistant infection of wound and soft tissue by Misra et al. (2001). *Pseudomonas aeruginosa* on its own has been of great threat in surgical site infection due to its records of staggering resistance to extended spectrum antibiotics (Brown and Izundu, 2004; Rajput et al*.,* 2008; El-Souny and Magaam, 2009). The organism has defied therapy even with many drugs in the last line of defense.

MATERIALS AND METHODS

Study location

The study was carried out in oil rich state of Uyo, Akwa Ibom State, Nigeria. The University of Uyo Teaching Hospitals was chosen as the sampling hospital because it is a referral centre for about 5 million people within and outside the state. Only the conventional antibiotics available for frequent use by patients in the area were considered for this study.

Sample collection

During the study, a total of 64 surgical wound samples were collected from in-patients at the University of Uyo Teaching Hospitals and Saint Luke's Hospital, Uyo using sterile cotton-tipped applicators. The swab samples were collected before wound dressing. They were inoculated aseptically into sterile nutrient broth as transport medium and were transported to the laboratory within 48 h for analysis. The samples were analyzed using the standard bacteriological media like blood agar, heated blood agar, mannitol salt agar, MacConkey agar, etc. All the bacterial isolates thus obtained were characterized and identified by studying their cultural and morphological features from the results of Gram staining reaction, serological and biochemical tests such as catalase, coagulase, motility, oxidase, indole, citrate utilization, urease, carbohydrate oxidation/fermentation etc described by Cowan (1974).

Antibiotic susceptibility test

Only the conventional antibiotics regularly available for frequent use in the study area were considered for the study. The diffusion technique was employed to determine the antibiotic susceptibility pattern of the isolates to the selected antibiotics such as penicillin (11.u), ampicillin (10 µg), tetracycline (10 µg), streptomycin (25 µg), cotrimoxazole (25 µg), cloxacillin (10 µg), colistin (10 µg), erythromycin (10 µg), gentamycin (10 µg), Cloramphenicol (30 µg) and Nalidixic acid (30 µg). The multi-antibiotic discs were commercially prepared by Abtek. The antibiogram was performed in accordance with standards described by the National Committee for Clinical Laboratory Standards (1987).

Standardization of inoculums

Four pure colonies of each isolate on a 24 h plate culture were randomly selected and inoculated into 2 mL of sterile peptone water broth in Bijou bottles. This was incubated at 37°C for 6 h and the turbidity was adjusted by serial dilution in phosphate buffer saline (pH 7. 2) to match an opacity tube containing 0. 5 mL of 1% barium chloride in 1% suplhuric acid (a Mc Farlands 0.5 barium sulphate standard containing 10^5 cfu/mL of the inoculums). One milliliter (1 mL) of the culture dilution (bacteria suspension) was transferred into a well dried surface of diagnostic sensitivity test agar (DST) medium and tilted to spread evenly over the entire surface of the agar plate.

The excess fluid was drained off and dried in incubator for less than 15 min. Multi-antibiotic discs were then placed on the surface of the inoculated plate, placed in a refrigerator to allow proper diffusion of the antibiotics and incubated aerobically at 37°C for 18 to 24 h (over-night). *S. aureus* NCTC 6751 and *E. coli* NCTC 10418 were used as control organisms for the sensitivity test. The diameter of the zone of inhibition was measured in millimeter. The result of each antimicrobial agent tested was reported as susceptible or resistant when the test organism was compared with the control and manufacturer's manuals for interpretation.

Multiple antibiotic resistant (MAR)

The percentage of the isolates that showed multiple antibiotic resistance was estimated and recorded.

RESULTS

PATIENTS' CLINICAL DATA AND ORGANISMS RECOVERY FROM SAMPLES

The patients had records ranging from acute post surgical wound sepsis to septicaemia after surgery. They all have high body temperature and fever. The patients were made up of 40 males and 24 females. The ages ranged from 21 to 40. Just 2 patients were about 80 years old ("about" because they did not know their actual age). Wound infection was observed in all the patients (100%) as all cultures were positive with 80 isolates. *S. aureus* was the most frequently isolated organism (25%), followed by *P. aeruginosa* (20%), *E. coli* (15%), *Klebsiella oxytoca* (10%), *Proteus mirabilis* (10%). *Klebsiella aerogenes* (5%), coagulase negative *Staphylococcus* (5%), *Streptococcus pyogenes* (5%) and *Proteus vulgaris* (5%) which were less frequently isolated (Table 1). Samples from forty-eight patients (75%) had monomicrobial growth while the remaining 16 patients (25%) had polymicrobial growth (Summary on Table 2). Surgical wound infection was most prevalent in the age group of 21-30 and less prevalent in the age group of 41 and above (Table 3).

In relation to sex, surgical wound infection was more prevalent in males than in females (Figure 1). The antibiotic susceptibility pattern of the isolates revealed that the Gram-positive organisms (coagulase-negative *Staphylococcus* and *S. pyogenes*) were highly susceptible to gentamycin, erythromycin, streptomycin and tetracycline, with percentage ranging from 70-95%. Majority of the Gram-negative organisms were moderately sensitive to nitrofurantoin, nalidixic acid and highly sensitive to gentamicin with percentage ranging from 70 - 90%. The result equally showed that 14 (70%) of the

Table 1. Prevalence/occurrence of bacteria isolates in surgical wound infection.

Bacteria	Number of isolates	Percentage of isolates (%)
Escherichia coli	12	15
Klebsiella oxytoca	8	10
Klebsiella aerogenes	4	5
Staphylococcus aureus	20	25
Coagulase-negative Staphylococcus	4	5
Pseudomonas aeruginosa	16	20
Streptococcus pyogenes	4	5
Proteus vulgaris	4	5
Proteus mirabilis	8	10
Total	80	100

Table 2. Categorizing patients according to clinical source of isolation, age/sex and organisms isolated from patients.

Age (yrs)/sex	No. of patients in the category	Culture source	Organisms isolated
22 / F	3	Abdomen	a, d
25 / M	5	Leg	c, b
28 / M	6	Arm	f
27 / M	4	Thigh	a
31 / M	1	Leg	c, e
28 / M	7	Knee	b
80 / F	2	Leg	a
21 / F	2	Arm	b, g
30 / F	2	Abdomen	i
28 / M	7	Head	h
32 / M	4	Leg	a
40 / M	4	High	i
26 / M	6	Knee	c
39 / M	5	Arm	a
23 / F	3	Arm	b
36 / F	4	Neck	d

Key: a, *Staphylococcus aureus;* b, *Pseudomonas aeruginosa;* c, *Escherichia coli;* d, *Klebsiella oxytoca;* e, *Klebsiella aerogenes;* f, Coagulase-negative *Staphylococcus;* g, *Streptococcus pyogenes;* h, *Proteus vulgaris;* I, *Proteus mirabilis.*

Table 3. Occurrence of bacterial isolates in surgical wound infection in relation to age.

Age	No. of samples	No. of organisms isolated	% of isolates
0-20	-	-	-
21-30	45	52	65
31-40	17	24	30
41 and above	02	04	05

isolates were multidrug resistant (Table 4 and 5).

DISCUSSION

Surgical site infection was observed to be on the high side among the volunteers in patients. There have been notable differences in various report of the incidence of surgical site infection. Observation of infection in all the patients (100%) in this study differs considerably from the report of various workers (Lilani et al., 2005). As low as 3.7% was observed in Iran (Bahar et al., 2010) while higher infection rates were noted after colon resection (32.1%), gastric and oesophageal operations (21.1%), cholesystectomy (17.2%),

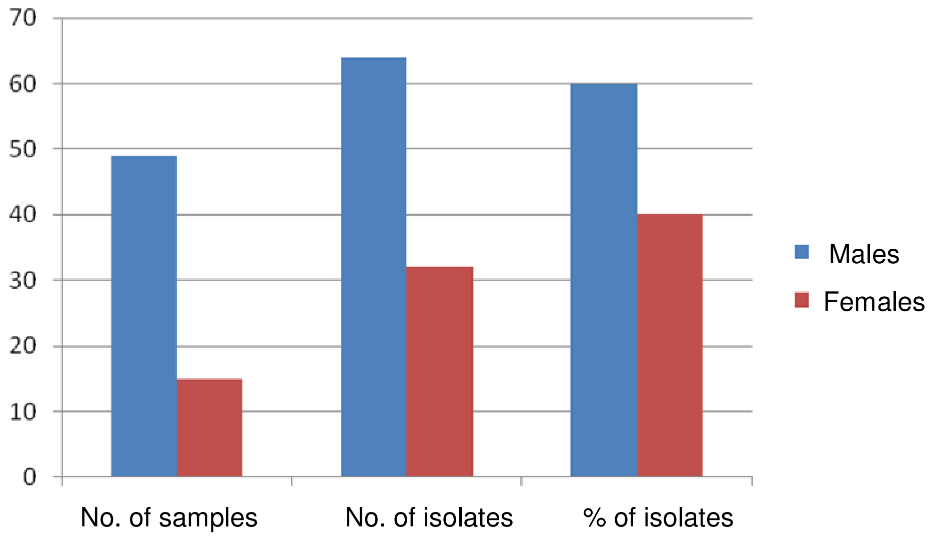

Figure 1. Occurrence of bacterial isolates in surgical wound infection in relation to sex.

Table 4. Occurrence of bacterial isolates in surgical wound infection in relation to sex.

	Male	Female
No. of samples	49	14
No. of isolates	48	32
Percentage of isolates	60	40

Table 5. Percentage antibiotics susceptibility pattern of gram positive isolates from surgical wounds.

Antibiotics	*Staphylococcus aureus*			Coagulase-negative *Staphylococcus*			*Streptococcus pyogenes*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Tetracyclin	10	0	90	80	0	20	80	0	20
Streptomycin	20	0	80	70	0	30	90	0	10
Penicillin	10	0	90	10	0	90	0	0	100
Gentamicin	20	0	80	70	0	30	95	5	0
Erythromycin	20	0	80	80	0	20	80	5	15
Cloxacillin	30	0	70	20	0	80	30	0	70
Cloramphenicol	30	0	70	30	0	70	30	0	70
Ampicillin	15	0	85	30	0	70	25	0	75

Key**:** S %, percentage sensitivity; I%, percentage intermediate**;** R%, percentage resistance.

and splenectomy (10.2%) by Yalcin et al. (1995). The observation made in our study was repertory of poor nursing service and poor hospital hygiene.

S. aureus found as the most prevalent organism (25%) is a commensal of the skin and nasal passages (Adegoke and Komolafe, 2008). Poor wound management allows the organisms to invade the inner tissue and bring aboutchronic systemic infection (Komolafe and Adegoke, 2008). *E. coli* invasion of the wound is a clear case of poor hospital hygiene, just like other implicated organisms are frequent agent of nosocomial infection (Samuel et al., 2010). *P. aeruginosa* was observed as the second most prevalent organism (20%) and this tallies with the report of Lilani et al. (2005) where the organism occurs 4 out of 17 times.

When the observed infection rate was categorized with respect to age, sex, location of the surgery and the organisms, it was discovered that deeply infected limb related surgery could be attributed to young men (Table 2). This is attributable to the fact that the age range

Table 6. Percentage antibiotics susceptibility pattern of gram negative isolates from surgical wounds.

Antibiotic	I			II			III			IV			V			VI		
	S (%)	I(%)	R (%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)
TET	30	0	70	0	0	100	80	0	20	0	0	100	40	0	60	80	0	20
STREP	20	0	80	0	0	100	70	0	95	5	0	95	30	0	70	30	0	70
COL	80	0	20	5	0	95	80	0	20	0	0	100	90	0	10	90	0	10
NIT	10	0	90	0	0	100	75	0	25	0	0	100	80	0	20	90	0	10
NAL	0	0	100	0	0	100	85	0	15	0	0	100	75	0	25	75	0	25
GEN	75	0	25	0	0	100	80	5	15	10	0	80	70	0	30	90	0	10
COT	10	0	90	5	0	95	0	0	100	5	0	95	0	0	100	0	0	100
AMP	0	0	100	0	0	100	10	0	90	0	0	100	5	0	95	0	0	100

Key: I (*Escherichia coli*); II (*Klebsiella oxytoca*); III (*Klebsiella aerogenes*); IV (*Pseudomonas aeruginosa*); V (Proteus *vulgaris*); VI (*Proteus mirabilis*); TET (TETRACYCLINE); STREP (STREPTOMYCIN); COL (COLISTIN); NIT (NITROFURANTOIN); NAL (NALIDIXIC ACID); GEN (GENTAMYCIN); COT (COTRIMOXAZOLE); AMP (AMPICILIN).

21-30 years is termed as the leisurely active age group (Tanaka et al., 2000). Largest number of bacterial pathogens isolated from the same age range might also be due to their agility as we observed (during sample collection) that many of them hardly stayed on their beds. This observation was peculiar to male patients and might explain higher infection in them than their female counterparts (Table 4).

A study of *in vitro* antimicrobial susceptibility profile of the aetiological agents of surgical site infection has revealed that there is a growing emergence of multi-drug resistant microbes. Forty six percent (46%) of *S. aureus* isolated were resistant to cloxacillin which is a drug often used for initial and empirical treatment of Staphylococcal infections. This high level of resistance to cloxacillin may pose problems in the treatment of SSI. The increasing resistance of *S. aureus* to cloxacillin is 70% observed in SSI as against 40% documented by Angyo et al. (2001) in septicaemia. This may be due to the widespread abuse of the drug which is usually available in combinations with ampicillin for the treatment of infections in our society and can be obtained over the counter without a prescription. About seventy to ninety percent (70 – 90%) of *S. aureus* isolates were resistant to other commonly used antibiotics like penicillin, ampicllin, tetracyline and cloramphenicol. The consequences of using an ineffective drug in severe bacterial infections could be disastrous as this can complicate management and increase morbidity and mortality. In adults of all ages, SSI is associated with increase in mortality, longer days of hospitalization (Kirkland et al., 1999) and adverse impact on clinical outcomes.

A general overview of the antibiogram of all the bacterial isolates indicated that Gram positive bacteria exhibited a greater level of antimicrobial susceptibility (ranging between 10 - 95%) than Gram negative bacteria (5 – 90%) to various antibacterial agents employed during the study period (Table 6). This situation raises serious concern. This suggests a very high resistance gene pool due perhaps to gross misuse and inappropriate usage of the antibacterial agents. The upsurge in the antibiotic resistance noticed in this study is in agreement with an earlier report by Obseiki-Ebor et al. (1987) where antibiotic abuse and high prevalence of self medication with antibiotics were identified as being responsible for the selection of antibiotic resistant bacterial strains. This piece of work has demonstrated vividly the urgent need for management strategies designed for specific groups of patients with infections in order to maximize therapeutic benefits, cost reduction and possible reduction in the incidence of adverse drug reactions. There is therefore need for usage policy that would be made applicable to the different tiers of our health care providers at the primary, secondary and tertiary levels. This can be done concurrently with sustained enlightenment and media publicity focusing attention on the dangers of high incidence of bacterial resistance to antibacterial agents in general and the ultimate consequences.

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