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Patterns of antibiotics susceptibility of isolates and plasmid analysis of *Staphylococcus* from surgical site infections in Nigeria

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ABSTRACT

There has been a significant increase in resistance of common bacterial isolates from surgical site infections in our community resulting in prolonged hospital stay, disability and deaths of patients. In this vein, we surveyed the antibiotic susceptibility profiles of aerobic bacterial isolates from postoperative wound infections and determined whether resistance in *Staphylococcus aureus* was genetically mediated. A total of 161 isolates were obtained from 153 swab samples of infected wounds using cultural, morphological, and biochemical characteristics. The predominant bacterial isolates were: *S. aureus* (53.4%), *Escherichia coli* (23.0%), *Staphylococcus epidermidis* (11.2%), *Pseudomonas aeruginosa* (5.0%), and species of *Klebsiella* and *Proteus* 3.7% each. On the whole: *Escherichia coli*, *Klebsiella* and *Proteus* showed similar antibiotic susceptibility patterns viz: 66.7-100% for ciprofloxacin, 66.7-100% gentamicin and 50-80% augmentin; and less than 50% for amoxacillin, erythromycin, tetracycline, cotrimoxazole, cloxacillin and chloramphenicol. *S. aureus* showed percentage susceptibility of 50-100% and *Staphylococcus epidermidis* (50-100%) for cloxacillin and augmentin, and less than 60% for amoxacillin, erythromycin, tetracycline, cotrimoxazole, gentamicin and chloramphenicol. Multi drug resistance (MDR) of *S. aureus* strains to at least three classes of the antibiotics used was about 70.5%. Four out of the 11 MDR *S. aureus* strains were found to harbor plasmids with varying molecular weights that ranged from 3.114 to 6.509 kb. One of the multi-drug resistant isolates still exhibited resistance even after curing. This showed that other genetic elements may also be involved in the acquisition of these forms of resistance other than plasmid elements.

Key Words: Postoperative –Wounds-Aerobic bacteria-*Staphylococcus aureus*.

INTRODUCTION

Surgical site infections are common postoperative complications that result in a significant morbidity and mortality, prolonged hospital stay, and add hospital costs from 10% to 20%. Although the total elimination of wound infection is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both patient comfort and medical resources (Haley et al., 1981). Any purulent discharge from a closed surgical incision, together with signs of inflammation of the surrounding tissues should be considered as wound infection, irrespective of whether micro-organisms can be cultured. Infection can occur at an incision site within 30 days of an operation, but wounds that are closed and primarily healed are not considered infected (Horan et al., 1992).

There are many factors that are thought to affect the susceptibility of surgical site infection, some of which strongly predispose to wound infection. These factors include pre-existing illness, length of operation, wound class, and wound contamination. Other factors such as extremes of age, malignancy, metabolic diseases, malnutrition, immunosuppression, cigarette smoking, remote site infection, emergency procedures, and long duration of preoperative hospitalization are not considered. They are regarded as independent risk factors for surgical site infections (Sawyer and Pruett, 1994). However, hospitals worldwide are facing an unprecedented crisis due to the progressive, rapid emergence and dissemination of antimicrobial-resistant microorganisms of surgical site (Tenover, 1991; Bonhoeffer et al., 1997; Weinstein, 2001; Mah, 2003; Woo et al., 2003). The combination of highly susceptible patients, intensive and prolonged antimicrobial usage and cross-infection have resulted in nosocomial infections with high resistant bacteria pathogens (Shaes et al., 1988; Weinstein, 1991; Strausbaugh et al., 1991; Tenover, 1991; Larry et al., 1992; Bonhoeffer et al., 1997; Weinstein, 2001; Mah, 2003; Woo et al., 2003). Larry et al. (1992) suggested that antimicrobial resistant pathogens in Long Term Care Facility (LTCF) have 3 possible origins. Firstly, they might have arrived with a colonized or infected patient. Secondly, resistant pathogens might have been selected for or more rarely, might have arisen through mutation as a consequence of antimicrobial agent use for a given patient or for a facility as a whole (Rice et al., 1990; Strausbaugh et al., 1991). Thirdly, the resistant pathogens might have arisen from the transfer of genetic materials from one species or genus of bacteria to another within the facility. Recent studies reveal that most of these isolates demonstrate a high frequency of antimicrobial resistance to commonly prescribed antibiotics due to heterogeneous population of plasmids (Larry et al., 1992; Yah et al., 2007a).

Thus, the continuing changing patterns of antibiotic susceptibility of common aerobic bacterial isolates from surgical site infections indicate the need for routine monitoring antimicrobial susceptibility. We, therefore, investigated the antimicrobial susceptibility patterns of common aerobic bacterial isolates from postoperative wounds to suggest timely recommendations for empirical antimicrobial therapy, if needed. However, we also looked at the curing and plasmid resistance markers of *S. aureus* strains to assess the potential ability of the resistances.

MATERIALS AND METHODS

Sample collections and identification of isolates

Deep swab specimens of patient surgical site infections from the University of Benin Teaching Hospital (UBTH), St. Philomena catholic hospital, Central (Specialist) Hospital and Faith Medical Centre, all in Benin City, Nigeria were obtained for the isolation and identification of aerobic bacterial isolates. The specimens were collected from May 2005 to June 2007. Samples collected were inoculated on Blood agar, MacConkey agar, and Chocolate agar. Characterization of the organisms was done using recommended methods (Cowan and Steel, 1974). These characteristics include colonial appearance, morphological characteristics, gram staining and standard biochemical tests. The following isolates were used as control: *S. aureus* control strain (ATCC 25923), *E. coli* control strain (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were obtained from Nigerian Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria

Antimicrobial susceptibility testing

The test was carried out by using commercially available antibiotic discs with known concentration of antimicrobial agents: erythromycin (10 µg), augmentin (10 µg), chloramphenicol (10 µg), cotrimoxazole (10 µg), amoxicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), cloxacillin (10µg), and gentamicin (10 µg) – (Abtek biological Ltd, UK). They were placed on a plate of sensitivity agar (Difco laboratories, Detroit, Mich, USA) that was uniformly inoculated with the test organism. The plates were then incubated overnight at 37 ^{o}C for 24 hours and the zones of inhibition were then recorded as sensitive (S) or resistant (R) strains according to the criteria of the National Committee of Clinical Laboratory Standards (2000). Macrodilution (Test tube) broth susceptibility testing method (CLSI/NCCLS, 2006) was used in the determination of the isolates susceptibility to antibacterial agents.

Curing of MDR *Staphylococcus aureus* isolates

To isolate the cured *S. aureus* strains, modifications of Yah et al. (2007a) method was used. This was carried out by treating the cells with sodium dodecyl sulfate (SDS). The colonies were then sub cultured onto Mueller Hinton agar (Difco Laboratories, Detroit, Mich) plates and test run for their respective antibiotic sensitivity patterns as previously described (NCCLS, 2000). Some of the bacteria were sensitive while some were still resistant. Absence of growth on Mueller Hinton agar was indicative of plasmids-mediated resistance while growth in Mueller Hinton agar was indicative of chromosome-mediated resistance.

Plasmids analysis of MDR *Staphylococcus aureus*

Plasmids isolation *S. aureus* strains was carried out based on rapid alkaline extraction procedures for screening of recombinant plasmid DNA, according to Birnboim and Doly (1979) and Zhou et al. (1990) methods. Agarose gel electrophoresis was carried out to resolve the extracted plasmids with standard DNA molecular weight marker II (0.12-23.1 kbp; bacteriophage lambda HindIII Roche Diagnostic GmbH).

RESULTS

One hundred and sixty-one isolates were recovered from 153 swab samples of infected wounds of patients. *S. aureus* ranked highest with an isolation rate of 53.4%, followed by *E. coli* (23.0%), *S. epidermidis* (11.2%), *P. aeruginosa* (5.0%), *Klebsiella* and *Proteus spp* (3.7% each). There were cases of mixed aerobic growth. There were a total of 85 *S. aureus* isolates in pure culture and one mixed growth with *Escherichia coli*. Single infections by *E. coli* were observed in 33 patients and polymicrobial infection 4 patients by *S. aureus*, *P. aeruginosa* and *Proteus* (Tables 1 and 2).

The result in Table 3 shows the antibiotic susceptibility pattern of the isolates from different postoperative wounds. On the whole, *E. coli*, *Klebsiella* sp, and *Proteus* sp demonstrated similar antibiotic susceptibility patterns viz**:** 66.7-100% for ciprofloxacin, 66.7-100% gentamicin and 50-80% augmentin; and less than 50% for amoxacillin, erythromycin, tetracycline, cotrimoxazole, cloxacillin and chloramphenicol. *S. aureus* and *S. epidermidis* show percentage susceptibility of 50-100% for cloxacillin and augmentin, and < 60% for amoxacillin, erythromycin, tetracycline, cotrimoxazole, gentamicin and chloramphenicol. *P. aeruginosa* showed % susceptibility of 83.3-100% for ciprofloxacin, and < 50 for cloxacillin, erythromycin, tetracycline, cotrimoxazole, gentamicin and chloramphenicol.

The result in Table 4 shows the varied percentage distribution of *S. aureus* strains isolated from surgical site infections while Table 5 shows resistant markers of *S. aureus* strains before and after curing. Four out of the selected eleven multidrug resistant isolates of *S. aureus* had plasmids with molecular weights ranging from 3.114- 6.509 kb. Only one of the isolate was successfully cured; while the other three isolates resisted curing exercise. The *S. aureus* strains were highly resistant to tetracycline, amoxicillin, cotrimoxazole and least resistant to gentamicin and erythromycin after curing.

The agarose gel electrophoretographs of the extracted plasmids from the MDR resistant strains of *S. aureus* before curing is shown in figure 1, while figure 2 shows the bands of the test strains of the cured cells.

DISCUSSION

Despite advances and improvement in aseptic techniques in surgical procedures, wound infections still constitute common occurrences (Rosok et al., 1990). The incidence of wound infection varies from one surgical procedure to another and most importantly from one patient to another (Nichols, 2001).

The distribution of organisms was: *S. aureus* (53.4%), *E. coli* (23.0%), *S. epidermidis* (11.2%), *P. aeruginosa* (5.0%), *Klebsiella* sp. and *proteus* sp. (3.7%) each. The spectrum of the organisms recovered in this study is similar to those earlier reported by Wemambu (1981).

The results showed that *S. aureus* and *E. coli* were the most predominant causes of

Table 1: Frequency of bacteria isolates from Surgical Site Infections.

Wound types	No. of Cases	No. of isolates	Types of isolates					
			E coli	*S. aureus*	*S. epidermidis*	*Ps aeruginosa*	*Klebs* spp	*Prot* spp.
Exploratory Laparotomy	7	8	1	5	1	-	-	1
Herniorhaphy	23	22	6	8	4	-	1	2
Appendicectomy	66	69	19	29	5	6	3	2
Caesarian Section	39	37	7	23	6	-	2	1
Hysterectomy	2	3	1	3	-	-	-	-
Amputation	16	22	3	18	2	2	-	-
Total	153	161(100%)	37(23.0%)	86(53.4%)	18(11.2%)	8(5.0%)	6(3.7%)	6(3.7%)

Table 2: Distribution of Bacterial Isolates in Mixed Growth in Surgical Site Infections

Organisms isolated	No. of Single growth	No. of Mixed Growth	Total
Staphylococcus aureus	85	1	86
Staphylococcus epidermidis	18	-	18
Escherichia coli	33	4	37
Pseudomonas aeruginosa	6	2	8
Klebsiella spp.	6	-	6
Proteus spp.	5	1	6

Table 3: Antibiotic susceptibility patterns of isolates from infected surgical Site Infections.

Isolates	No. of Isolates	% Susceptibility								
		CIP	AUG	C	TE	CXM	AMX	GN	CLO	E
E coli	37	97.6	37.5	61.9	55.5	46.4	50.4	96.7	43.2	00
Ps aeruginosa	8	91.7	00	00	00	8.3	00	50	8.3	00
S aureus	86	78.8	66.7	26.4	10.7	20.4	50.5	66.9	100	10.7
S epidermidis	18	84.3	81.0	10.7	00	00	32	82	91	64.5
Klebsiella spp.	6	100	55.6	72.2	100	100	61.1	100	33.3	00
Proteus spp.	6	100	40	100	00	50	37.5	100	25	00

Key: Ciprofloxacin = CIP, Augmentin = AUG, Chloramphenicol = C, Tetracycline = TE, Cotrimoxazole = CXM, Amoxacillin, =AMX, Gentamicin = GN, Cloxacillin = CLO, Erythromycin = E

Table 4: Distribution of multidrug resistant (MDR) strains of *S. aureus* in various Surgical Sites

Site/total No. of isolates	No. of MDR isolates	% of MDR isolates
Exploratory Laparatomy(5)	5	100.0
Herniorrhaphy (8)	5	62.5
Appendicectomy (29)	17	58.6
Caesarian Section (23)	19	82.6
Hysterectomy (3)	3	100.0
Amputation (18)	12	66.7
Total (86)	61	70.9%

Table 5: Resistance markers of *S. aureus* strains from Surgical Site Infections before and after curing.

Organism	Code	Source	No. of Plasmids	Resistance spectrum before curing	Resistance spectrum after curing
S. aureus	C5	C/S	1	AMX,TE,CLO,CIP	AMX,TE,CLO,CIP
S. aureus	M1	Amputation	1	CXM,TE,AMX,E,GN,CLO,AUG	CXM,TE,AMX,E,GN,CLO,AUG
S. aureus	A17	Appendectomy	1	C,GN,CXM,AMX,TE,E	C,GN,CXM,AMX,TE,E
S. aureus	A26	Appendectomy	2	TE,E,AMX,C,CXM,GN	TE,E,CXM,C

Key: Ciprofloxacin = CIP, Augmentin = AUG, Chloramphenicol = C, Tetracycline = TE, Cotrimoxazole = CXM, Amoxacillin, =AMX, Gentamicin = GN, Cloxacillin CLO, Erythromycin = E, C/S = Caesarian Section

surgical site infections. This was in agreement to some extent with those earlier reported by Wemambu (1981) and Yah et al. (2004) who found *S. aureus* predominating followed by *Proteus* sp. However, in this study *S. aureus* constitutes 53.4% of the total isolates. In relation to other organisms isolated the results were statistically significant (P< 0.05). Earlier work done have shown that *S. aureus* constitutes about 65% of the common isolates in wound samples (Beiner et al., 2003; Bhatia et al., 2003).

There were cases of polymicrobial infection of surgical sites indicating whether monomicrobial or polymicrobial were in preponderance. Earlier studies have shown that about 8-10% of surgical site infections have mixed growth in cultured specimens (Beiner et al., 2003; Bhatia et al., 2003). The mixed organisms could be due to contamination from hospital staff or other patients. This may account for the unusually high level of gram negative organisms recovered in this study.

The antibiotic susceptibility pattern of the isolates from different postoperative wounds showed varied patterns. On the whole, *E. coli, Klebsiella* sp, and *Proteus* sp showed similar antibiotic susceptibility patterns viz**:** 66.7-100% for ciprofloxacin, gentamicin (66.7-100%) and augmentin (50-80%) and less than 50% for other antibiotics. *S. aureus* and *S. epidermidis* show percentage susceptibility of 50-100% for cloxacillin and augmentin, and < 60% for other antibiotics. *P. aeruginosa* showed a percentage susceptibility of 83.3-100% for ciprofloxacin, and < 50% for cloxacillin, erythromycin, tetracycline, cotrimoxazole, gentamicin and chloramphenicol. Finland (1984) isolated from staff nurses *S. aureus* and found that methicillin resistant *S. aureus* (MRSA) was relatively high in surgical wound patients, followed by *Pseudomonas aeruginosa* while Wemambu (1981) found that *S. aureus* isolated from noses of surgeons, theatre nurses and wounds were resistant to penicillin, streptomycin and tetracycline.

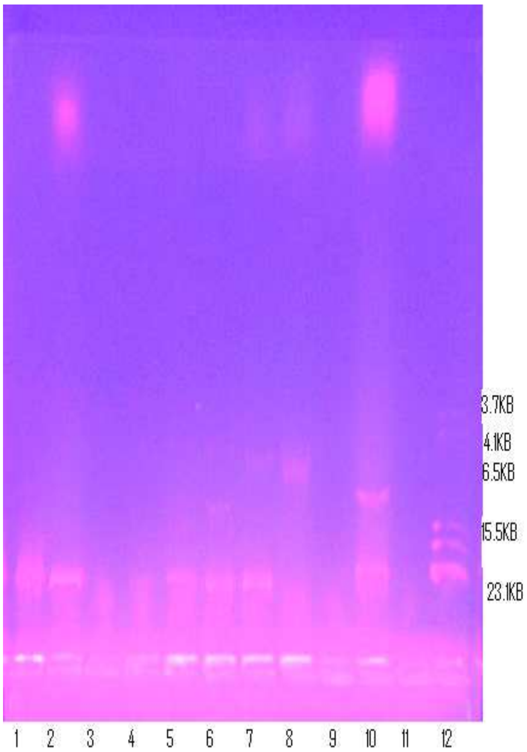

Fig.1: Agarose gel electrophoretograph of extracted plasmids from MDR Staphylococcus aureus from postoperative wounds.

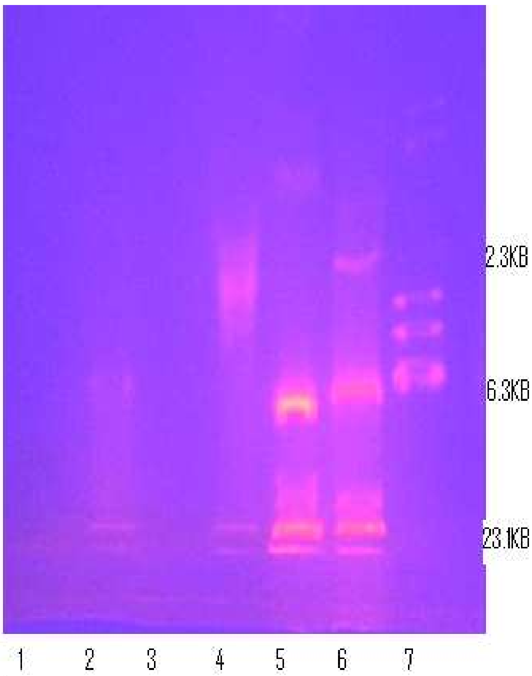

Fig. 2: Bands of Cured Staphylococcus aurues strains.

The excessive use of antibiotics particularly in hospitalized patients, have led to the suppression of drug susceptible organisms and favours the persistent growth and spread of drug resistant bacteria (Yah et al., 2007a). Close hospital environment also have favoured the transmission and spread of antibiotic resistant organisms through personnel, fomites as well as by direct contact. Some organisms produce the β-lactamase enzyme, which binds and cleave the β-lactam ring of penicillin's and cephalosporins (Rice et al., 1990; Weinstein, 2001).

Gentamicin, one of the commonest and less expensive antibiotics was found to be quite effective against the gram – negative bacterial isolates in this study; this pattern of course was not at variance with the result earlier obtained by Yah el al. (2004). This could be due to its mode of administration, which is via parenteral route; this makes it difficult for the drug to be seriously abused. About 100% of the isolates were sensitive to ciprofloxacin – a flouroquinolone; this may be due to the fact that the flouroquinolones are new generation of antibiotics that have not been subjected to much abuse and secondly they are expensive. The flouroquinolones are bacteriocidal and selectively inhibit bacterial DNA gyrase enzymes thereby preventing DNA production.

Biofilms associated organisms are known to be less susceptible to antimicrobial treatments thereby posing a public health concern (Tattawasat et al., 1999). Microorganisms commonly attach to living and non- living surfaces to form biofilms made up of extracellular polymers include *S. aureus*, *Enterococcus faecalis*, *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, and *Klebsiella pneumoniae (*Stickler, 1996). These organisms are similar to those isolated in this work. Electronmicroscopy of the surfaces of medical devices that are sources of device-related infections have shown the presence of large numbers of encased bacteria. Furthermore, tissues taken from such non-device related chronic infections also show the presence of biofilm bacteria surrounded by an exopolysaccharide matrix (Costerton et al., 1999). Therefore, biofilm formation may be one of the factors responsible for the multi-resistant strains isolated from these wounds. Also, some of the surgical wounds had drains, which communicate with the exterior; this may have favoured the development of biofilms.

The percentage distribution of multidrug resistant *S. aureus* strains isolated from surgical site infections before curing showed that most of the *S. aureus* (66.6 - 100%) were multi-drug resistant. They exhibited resistance to three or more classes of antibiotics. The plasmids molecular weights of the *S. aureus* strains ranged from 3.114-6.509 kb. The molecular weights were similar to those earlier reported by Glatman et al. (1984) and Yah et al. (2007b) who isolated plasmids that code for antibiotics resistance in *Escherichia coli, Klebsiella* sp*., Pseudomonas aeruginosa* and *Proteus* sp. from enteric sources. Bacteria can also transfer extrachromosomal elements (transposons and plasmids) within biofilms and indwelling devices (Ryan, 1990; Donlan, 2001). This might be a major source of spread of genes conferring antibiotics resistance as well as a selective pressure brought about by the increase and often indiscriminate use of antibiotics in humans and animals (Rotimi, 1984). Acquisition of mobile genetic elements is known to be the main mechanism for short term accumulation of resistance determinants in bacterial genomes (Yah et al., 2007a). Other studies have shown resistance to gentamicin, tobramycin and carbenicillin to be attributed to transferable plasmids (Tsakris et al., 1992). Glatman et al. (2001) in Greece, found plasmids (100Mda in size) isolated from multidrug resistant *Pseudomonas aeruginosa* strains, which encode high-level resistance to gentamicin and tobramycin; whereas resistant to other drugs such as ciprofloxacin and rifampicin were not transferable and all.

It is increasingly evident that many bacteria are pathogenic because of their plasmids. Some of these plasmids confer antibiotic resistance on them. Some typically have genes (R- factors) that code for enzymes capable of destroying the antibiotics (Shaes et al., 1988; Pfaller, 2001).

A multi-drug resistant isolate was found to exhibit resistance to some antibiotics even after curing. This shows that bacteria resistance to antibiotics can either be chromosomal or due to other genetic elements. The extracted plasmids DNA bands of the *S. aureus* were shown to range from 3.114 – 6.509 kb while that of cured cells were ≥ 23.1 kbp. This shows that molecular typing can be used to determine whether different isolates give the same or different results, as epidemiologically related isolates share the same fingerprints (Pfaller, 2001).

Conclusion and recommendations

This study reveals that multiple antibiotic resistant gram-positive organisms; *Staphylococcus aureus,* and gram-negative bacilli such as *Escherichia coli, Pseudomonas aeruginosa* are the bacteria commonly implicated in surgical site infections in some hospitals in Benin City, Nigeria. They are also known to be important nosocomial agents. As these organisms have relatively high occurrence among surgical patients, the issue of pre and postoperative antisepsis should be taken seriously. This may include continuous medical education for the medical team involved in pre and post surgical wound management. Also the combination of antibiotic chemotherapy may be the more appropriate method in the management of some infections where these organisms are isolated rather than the traditional single antibiotics therapy. Continued surveillance of antibiotic profile of pathogens is important and this trend needs to be watched so that the information derived from it can be communicated to the clinicians to help guide patient management. Plasmid isolation revealed that majority of the multiresistant strains harbored plasmids. These plasmids may have been a cause of the resistance and may have been acquired via transmission. It is necessary to have antibiotic policy in place in hospitals as an additional effort towards reducing this immense problem of MDR development in pathogens.

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