East African Medical Journal Vol. 90 No. 12 December 2013
CHARACTERISATION OF BACTERIAL ISOLATES FROM PATIENTS WOUNDS AND ENVIRONMENTAL FACTORS PREDICTIVE OF POST-SURGICAL INFECTIONS AT THE ORTHOPAEDIC WARD IN ILE-IFE, NIGERIA
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ABSTRACT

***Objective*: To determine the pattern of orthopaedic wound infection and the influence of environmental factors on the distribution of the etiologic bacterial agents.**
***Design*: A prospective observational study.**
***Setting*: Department of Orthopaedic Surgery and Traumatology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.**
***Subjects*: Sixty patients with orthopaedic wound infections and orthopaedic ward environment.**
***Results*: Thirty- nine males (mean age 33.31+2SD) and 21 females (mean age 27.47+2SD) with orthopaedic wounds. Three hundred and ten bacteria (190 from patients and 120 from ward environment) were isolated. The pattern of bacterial isolates from patients' wounds was different from that of the airborne bacterial isolates irrespective of the length of stay on the ward. There was a significant difference in the distribution and resistance pattern of bacterial isolates from the patient's wounds and ward environment.**
***Conclusion*: There is a high incidence of antibiotic resistance of bacterial isolates from samples cultured from patients compared with isolates from ward environment at this centre. The extensive use of pre-operative prophylactic and post-surgical antibiotics in various combinations at this centre needs to be re-examined to reduce the preponderance of antibiotic resistance.**

INTRODUCTION

The threat of infection remains a major challenge to a patient undergoing surgery and is a major contributor to morbidity in Orthopaedic wards. Depending on surgical condition, patient's immune status and type of wound sustained , infectious agents, the patient may remain in the hospital for one or a few days, weeks and even months(1). Many studies support the view that a reduction in post-surgical wound infections is directly related to increase in knowledge and awareness of its causes and prevention (2). Studies have shown that microbial virulence and host resistance are crucial determinants of surgical infections (3). Besides, the environmental conditions that may militate against delay in healing and subject the patient to prolonged hospitalisation must be monitored in the ward to reduce infection and financial cost (4). Several studies have been undertaken regarding contamination of surgical wounds in this environment (5, 6). These studies have primarily emphasised on bacterial contamination (5). Our study is different in that it monitored factors in the patient and orthopaedic ward environment predictive of infection at this centre for short and long term basis.

This six month prospective study purposely determined the prevalence of post-surgical wound infection, isolated and identified bacterial agents recovered from patients samples and compared them with airborne bacteria obtained on settle media plates exposed in the orthopaedic ward. The study also

compared the antibiotic resistance pattern of these bacterial isolates from host wounds and airborne sources of the orthopaedic ward. To our knowledge, mechanisms of indoor transmission in buildings has not been studied in our setting. Determination of these factors would assist in recommending effective strategies in reducing wound infections on the wards.

MATERIALS AND METHODS

Setting and recruitment of participants: The participants recruited for this study were consecutive patients that had their surgeries performed at the Department of Orthopaedic Surgery and Traumatology of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, between April 2012- September, 2012. They were 60 in number consisting of 23 (38.3%) clean wounds, 17(28.3%) contaminated wounds, 13 (21.7%) infected wounds and 7(11.7%) dirty wounds.

The adult orthopaedic ward occupies a 3030 m^2 floor area. The orthopaedic ward consists of 34 beds shared by both sexes. The ward is provided with standing fans only. The unit has 3 toilets located at the far-end of the ward and used primarily by patients who are mobile. Non-mobile patients constrained to their beds are supplied with bedpans and assisted by ward maids. There are four separate rooms on the west side of the unit each of which can occupy two patients. Each room contains facilities for standing fans. One hundred and four windows are located throughout the ward facilitating cross ventilation. The nurses' station is located in the middle of the unit. There is only one entrance to the unit. On the east side of the unit sunlight rays can directly enter the ward during daylight and the same is applicable to west side of the ward typical of a tropical environment.

Participation was on voluntary basis after the attending orthopaedic surgeons had explained the purpose of the research undertaking. Information relating to each participant was obtained from prepared questionnaire responses, personal interview and relevant information from each patient's case file. Inclusion criteria include all patients operated upon in the unit and those who later developed post-surgical infections after their discharge. Participants were admitted to study if they registered in the hospital and developed sepsis (pus) after their surgeries. Wound infection is defined as a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue (i.e. muscle, bone, nerves) following injury to the skin or underlying tissues/ organs caused by surgery, a blow, a cut, chemicals, heat/ cold, friction/ shear force, pressure or as a result of disease, such as leg ulcers or carcinomas (2,3). Approval for the study was obtained from the hospital's Research and Ethics Committee.

Collection of samples from patients contaminated wounds/ dressings

Processing of samples: Each sample(pus, purulent discharge, or blood stained dressing) was collected from infected wound/ dressing initially with sterile moistened cotton-tipped applicator into freshly prepared thioglycolate broth test tubes and thereafter incubated aerobically at 37ºC for 48-72 hour. When turbidity was observed in each test-tube, the sample was further processed. A loopful of sample was thereafter streaked onto conventional media such as: mannitol salt agar (MSA) tryptone soy agar (TSA) supplemented with nystatin, blood agar (BA), eosin methylene blue agar (EMB) and others and incubated for 24-48 hours at 37ºC. Colonies appearing on the media plates were picked and studied.

Identification of bacterial isolates from patients' pus/ dressings: Initial identification of each bacterial colony was based on cultural, colonial morphology and Gram reaction. Cocci that appeared in clusters on slide preparations were considered as staphylococci. Those staphylococci that fermented mannitol on (MSA), produced coagulase on slide and tube tests in human pooled plasma were considered as *S. aureus*. Staphylococci that did not ferment mannitol on MSA and did not produce coagulase in human pooled plasma were deemed coagulase negative staphylococci (CONS) which were further speciated by sugar fermentation (7). Gram negative rods were categorised into lactose fermenters and non-lactose fermenters on EMB agar, Triple sugar iron (TSI) agar and other conventional media (8).

Collection of airborne bacteria from orthopaedic ward (unit): Settle media plates consisting of TSA supplemented with nystatin , BA, MSA and EMB were exposed at strategic locations in the adult orthopaedic ward at specific time and period at window level. These plates were subsequently incubated aerobically 37 ºC for 24-72 hours short term 0- 120 minutes and long term for 0-8 hours. weekly for eight consecutive weeks. Colonies of airborne bacteria that appeared on settle media plates were counted as colony forming unit per plate (CFU/plate) picked, gram stained and characterised as described above for patients 'exudate or/and contaminated dressings.

Antibiotic susceptibility test: The antibiotic sensitivity test was carried out on all isolates encountered by the method of (9); using antibiotic discs (ABTEK, Biological Limited Liverpool, UK) containing: cotrimoxazole (Cot), 25μg; cloxacillin (Cxc), 5μg; erythromycin (Ery), 5μg; gentamicin (Gen), 10μg; agumentin (Aug), 30μg; streptomycin (Str), 10μg; tetracycline (Tet), 10μg; chloramphenicol (Chl), 10μg; ofloxacin (Ofl), 5μg; nalidixic acid (Nal), 30μg;

amoxycillin (Amx), 25μg; nitrofurantoin (Nit), 200μg. *S. aureus* ATCC 25923 and *Enterobacter aerogenes* will be used as control organisms.

STATISTICS ANALYSIS

Data analysis were done by descriptive method using one-sample t-test and analysed by SPSS version 16.0 package (Chicago, SPSS Inc., 2007). Significant difference is taken as $p<0.05$.

RESULTS

One hundred and sixteen (116) surgeries were performed between April – September, 2012 but 60 formed the subject of the study. A total of 39 males with mean age of 33.31±10.50 (SEM= 1.70) and 21 females 27.47±6.53 (SEM=1.46). The types of surgeries at the OAUTHC show 41.38% were from open reduction internal fixation (ORIF) plate screw, intramedullary interlocking nail external fixation femoral shaft, 11.21 % from fracture (tibia/fibular) and bone grafting contributed 10.34%. Amputations and osteotomy / pop application accounted for 7.76% each. Implantation and arthrosplasty each accounted for 4.31%. Removal of foreign body was 3.45 % and diabetic foot ulcers contributed 2.59%. Skin grafting and ankle arthrodesis both accounted for 1.72 % each while gangrene of the leg and biopsy of ring finger were 0.86 % each and wound debridement was 1.72%. The distribution of bacterial isolates from patients samples and airborne on settle media plate is shown in Table 1. The pattern of bacterial isolates from patients' wounds was different from that of the airborne bacterial isolates irrespective of the length of stay on the ward. The presence of co-morbidities (hypertension, obesity, diabetics mellitus) did not influence the pattern of bacterial isolates from patients wound.

Table 1

Distribution of bacterial groups cultured from patients and the air environment of the orthopaedic ward

Bacterial group	Patients wounds	Orthopaedic ward environment		
	Total No. involved	No & % of total	Total no. involved	No & % of total
Gram+ coccci	82 (*71%)	43.2	34(*29 %)	28.3
Gram -rods	48(*72%)	25.3	19(*28%)	15.8
Gram + non spore formers (bacilli)	41(*79%)	21.6	11 (*79%)	9.1
Gram + spore formers (bacilli)	19(*25%)	10	56 (*75%)	46.7
Total	190 310(*61%)	100	120 (*39%)	100

*= (%) of isolates in the group

Table 2

Resistant profile of predominant bacterial isolates recovered from patients and settle media plates of the orthopaedic ward

Bacterial isolates	Patients(Pus/Purulent and dressings)		Airborne bacterial of the orthopaedic ward	
	Total No of Isolates involved	No (%) resistant	Total No of Isolates involved	No(%) resistant
S. aureus	40	37(92.5)	8	4(50)
S. saprophyticus	6	5(83.3)	6	4(66.7)
S. haemolyticus	6	5(83.3)	-	-
S. epidermidis	5	4(80)	-	-
B. cereus	13	13(100)	30	12(40)
B. subtilis	6	4(66.7)	26	13(50)
Arcanobacterium haemolyticum	13	13(100)	-	-
Corynebacterim diptheriae	10	10(100)	7	1(14.3)
Listeria monocytogenes	18	17(94.4)	3	0

Table 3

Pattern of multiple antibiotics resistant of bacterial isolates cultured from patients with post-surgical sepsis.

Bacterial isolates	Total No. of isolates involved	No (%) of isolates	Classes of antibiotics			
			6	5	4	3
S. aureus	40	37(92.5)	15	7	11	4
S. saprophyticus	6	5(83.3)	2	1	1	1
S. haemolyticus	6	5(83.3)	3	-	1	1
S. epidermidis	5	4(80)	-	2	1	1
Micrococcus luteus	14	14(100)	1	1	5	7
B. cereus	13	13(100)	5	6	-	2
B. subtilis	6	4(66.7)	-	-	2	2
Arcanobacterium haemolyticum	13	13(100)	3	3	5	2
Corynebacterim diptheriae	10	10(100)	2	3	2	3
Listeria monocytogenes	18	17(94.4)	4	6	5	2
E.coli	17	16(94.1)	2	6	6	2
Citrobacter freundii	5	5(100)	1	2	2	-
K. pneumoniae	3	2(66.7)	1	-	1	-
P. aeruginosa	16	16(100)	3	5	6	2
Proteus mirabilis	5	5(100)	1	2	1	1
TOTAL	177	166(93.8)	43(25.9)	44(26.5)	49(29.5)	30(18.1)

Table 4

Pattern of multiple antibiotics resistance of airborne bacteria on settle media plates exposed the orthopaedic ward.

Bacterial isolates	Total No. of isolates involved	No (%) of isolates	Classes of antibiotics			
			6	5	4	3
S. aureus	8	4(50)	1	-	-	3
S. xylosus	7	2(28.6)	-	-	-	2
S. scuri	6	1(16.7)	-	-	-	1
S. saprophyticus	6	4(66.7)	-	-	-	4
S. cohnii	3	2(66.7)	-	-	-	2
S.capitis	3	0	-	-	-	-
B.cereus	30	12(40)	-	1	2	9
B. subtilis	26	13(50)	-	2	2	9
Corynebacterim diptheriae	7	1(14.3)	-	-	1	-
Listeria monocytogenes	3	0	-	-	-	-
K. pneumoniae	8	4(50)	-	-	2	2
P. aeruginosa	7	4(57.1)	-	-	1	3
Proteus mirabilis	4	3(75)	-	-	1	2
TOTAL	120	50(41.7)	1(2)	3(6)	9(18)	37(74)

The resistance profile of the predominant bacterial isolates recovered from patients and on settle media plate in the orthopaedic ward is shown in Table 2. Table 3 also shows the pattern of multiple antibiotic resistance of bacterial isolates cultured from patients with post surgical wound sepsis. The pattern of multiple antibiotics resistance on settle media exposed in the orthopaedic ward is shown in Table 4. The results also show bacterial count increased as the exposure time increased. The mean bacterial count per plate (CFU / plate) was highest with Tryptone soy agar (230.8) followed by blood agar (223), mannitol salt agar 98 and eosin methylene blue agar 12.3 respectively. The bacterial count per settle media plate increased as time of exposure increased. When settle media plates were exposed for 30 min., they yielded 60.4 CFU / plate, for 45 min. 74 CFU / plate, 60 min. 125.4 CFU / plate and for 120 min. 136.8 CFU / plate. The airborne bacterial count per plate was highest in the morning period compared with afternoon period probably due to the influence UV radiation filtering through the windows also number of health care personnel in ward at these periods. Gram positive spore-formers (bacilli) represented 56 (46.7%), Gram positive cocci in clusters (staphylococci) 34 (28.3%) Gram negative rods, 19 (15.8%) and gram positive non- spore formers (bacilli) accounted for 11(9.2%).

Twelve (20%) of the 60 patients with post-surgical infections were not administered pre-surgical antibiotics. 48 (80%) of these patients were however administered 20 different antibiotics in various combinations : These antibiotics were applied in various combinations to the patients as follows: Six different antibiotics combinations to four (15.8%) patients, five different antibiotics to 5(13.2%) patients, 4 different antibiotics given to 15(39.5%) patients, three different antibiotics to 14(36.8%) patients. There seems to be no antibiotics policy in the treatment of orthopaedic patient in the study population as varying classes of antibiotics were used for the patients.

DISCUSSION

In spite of the increasing use of prophylactic antibiotics in most surgeries, (10) infections still remain a real risk and constitute a substantial burden of disease for both the patient and health care services. (11). Our study was designed to investigate prevalence of post-surgical wound infection, bacterial factors that constituted to delay in wound healing and possibly prolonged patient hospital stay, analysed the preponderance of bacteria in the orthopaedic ward . We also tested the bacterial isolates recovered from patients' wound samples and airborne bacteria on exposed settle media plates in the orthopaedic ward to commonly employed antibiotics in this environment.

Our study show altogether 116 surgeries were conducted in this centre between April to September 2012 and 31 (26.72 %) developed infection. The data show a high incidence of post-surgical infection in this centre despite administration of pre- surgical prophylactic antibiotics to other studies in East Africa and Italy (12).

Overall, 310 bacterial isolates were cultured from both sources. 190 (61.3%) from patients' wound samples and 120 (38.7%) from airborne bacteria cultured on settle media plates in the orthopaedic ward. When we compared the distribution of and types bacteria isolates recovered from patients' wounds specimens and those on settle media plates. Our data show Gram positive cocci made of staphylococci and micrococci accounted for 43.2% of total bacterial isolates compared with 28.3% of airborne bacteria on settle media plates exposed in the orthopaedic ward. Gram negative rods constituted 25.3% from patients' wounds samples compared with 15.8% of airborne bacteria recovered on settle media plates. 21.6% of bacterial isolates were Gram positive non-spore formers cultured from patients wounds compared with 9.1 % from airborne bacteria on settle media plates in the orthopaedic ward. In addition, Gram positive spore formers accounted for only 10 % of patients bacteria compared with 46.7% of airborne bacteria on settle media plates. Our data show bacterial isolates cultured from patients wounds samples and settle media plated varied in types (10). The results show bacterial isolates recovered from patients' post-surgical wound infection were predominantly staphylococci (36%) of which *S. aureus* isolates accounted for 21% and coagulase negative staphylococci (15%). Many investigators have reported similar findings of the predominance of staphylococci in suppurative and in hospital acquired infections (11) Gram negative non- spore formers and enteric rods followed in preponderance (23%) followed by gram positive non- spore formers (22%) and spore -formers interestingly constituted only (10%).

When we analysed airborne bacteria on settle media plates, 46.7 % were spore-formers. The prevalence of *S. aureus* isolates from patients samples may probably be due to auto-infection from patients' flora and those of hospital personnel. Studies previously carried out in this centre had reported similar phage types *S. aureus* strains recovered from mothers and infant pairs and among nurses and medical students in the neonatal ward of the hospital (13,14,2). The study and those reported by other investigators' also found nasal carriage rates of *S. aureus* strains of 20- 56 % in this centre among hospital staff including nurses and nurses' aides (13). Coagulase negative staphylococci are known to be normal flora of the skin of most individuals which have been reported to be transferrable intra-operatively (15,16). In contrast, aerobic spore–formers predominated among airborne bacteria recovered from the settle media plates 46.7% made of *B. cereus*

and *B. substilis* both of which have been identified as aerial contaminants. It is therefore not surprising these airborne isolates may contaminate patients' wounds if their personal hygiene is compromised.

Other bacterial isolates encountered in patients' samples were *L. monocytogenes*, A*rcanobacterium haemolyticum*, and *Corynebacterium diphtheriae* 9.8%, 7.4% 4.7% respectively while lactose fermenters like *E. coli* accounted for 8.9%, *C. freundii* 2.6% and *K. pneumoniae* 1.6%. In addition, enteric non-lactose fermenters *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella sp* contributed8.4%, 2.6% and 1.1% respectively.

Our results also show 93.2% of bacterial isolates cultured from patients' wounds were resistant to antibiotics tested compared to 41.7% of airborne bacteria isolates on settle media plates of the orthopaedic ward. Studies of bacterial isolates from Italy (12), India and East Africa, have reported extensively on resistance of bacteria isolated from wounds to various antibiotics. In many of these studies (12,16, 17,). *S. aureus* isolates predominated and other bacterial isolates they reported are similar to those cultured in our study. The high incidence of antibiotic resistance (93.2%) recorded for bacterial isolates cultured from patients in this study at least *in-vitro* is worrisome and portends serious challenge in the management of patient care and can also create unwanted economic burden. The widespread incidence of multiple antibiotic resistance among bacterial isolates recovered from patients samples in particular, underscores the infectiveness of most antibiotics used in this centre and may delay patients' healing and enhance prolonged hospital stay. Our data show the mean hospital stay for patients with post-surgical infection at the centre was four weeks. One patient was hospitalised for 32 weeks, another two patients for 24 and 20 weeks each respectively. 11 patients hospital duration was 16 weeks while seven stayed 12 weeks. Six patients hospital stay lasted for eight weeks and ten others stayed for four weeks. The duration of stay for seven patients was three weeks each and four patients two weeks. Only one patient stayed for one and a half weeks. Prolonged hospital stay is often related to patients immunity, resistance of bacterial agents to various antibiotics which usually are ineffective as seen in this study. The pathogenic potential of *S. aureus* strains to elaborate enzymes capable of degrading tissue thereby compromising wound healing in diabetic foot ulcers have been reported in this centre (3).

Furthermore, our data show 93.8% of bacterial isolates cultured from patients demonstrated multiple antibiotic resistance. Of the 37 *S. aureus* isolates tested, 15 were resistant to six different classes of antibiotics, seven to five classes and 11 to four classes. In contrast, of the eight airborne *S. aureu*s isolates encountered, four were multi-resistant with one strain only resistant to six different classes of antibiotics (Table 3) Our results show the employment of penicillin was ineffective as all the *S. aureus* isolates recovered from patients were resistant to cloxacillin, augment and amoxicillin. A similar trend of wide spread resistance was seen with spore forming gram positive bacilli such as *B. cereus* and *B. substilis* as well as other gram positive bacilli (Table 4). Many studies have reported the ineffectiveness of penicillin against bacterial isolates recovered from patients with post surgical wound sepsis (19) Relative success however, have been achieved with quinolones, aminoglycosides and third generation cephalosporins (19,20). Microbial virulence and host resistance have been reported to be important determinants of surgical infections (3).

An environment where over 90% of bacterial isolates recovered from patients were multi-resistant does not augur well for prompt wound healing. The widespread prevalence of resistant bacteria in this centre is worrisome and suggests urgent action and strategies to curb and reduce this phenomenon.

In conclusion, our results show more preponderance of bacterial isolates in samples cultured from patients with post-surgical wound infection than in airborne isolates on settled media plates exposed in the orthopaedic ward. We encountered wide spread antibiotic resistance among bacterial isolates from patients' samples compared with airborne bacteria samples on settled media plates. *Staphylococci* constituted 41% of bacterial isolates with *S. aureus* (21 %) being the single most predominant isolates encountered in patients wounds compared to aerobic gram positive spore formers *B. cereus* and *B. substilis* that both contributed 46.7% in airborne bacteria seen on settle media plates of the orthopaedic ward. The settle media plates had varied CFU/per plates according to the media type used with tryptone soy agar and blood agar having the highest bacterial count and eosin methylene blue agar the least CFU/ plate.

Although the merits of ventilation in operating theatres and isolation rooms are known, the benefits of cross ventilation of hospital ward remain contentious. Conditions contributing to aerial contamination of ward environment are complex and multifactorial and involve the interplay of unstable meteorological parameters such as humidity, temperature, and wind velocity which may vary momentarily and seasonally probably owing to global warming. Besides, the architectural design of buildings plays a major role in airborne bacterial contamination. The provision of many windows (104 in total) in the orthopaedic ward in this centre may have enhanced cross ventilation and dispersion of airborne bacteria. The fact that the majority of gram positive aerobic spore formers with high densities (bacilli) were recovered from settle media plates exposed in the ward underscores the importance of aerial dispersion of airborne microbes

in this ward. Studies done by Beggs *et. al* (2008)(21) tend to support this assumption. Similarly, provision of multiple windows in this orthopaedic ward may also have enhanced ultra violet rays into the ward thereby reducing population of airborne bacteria hence aerial decontamination in the ward. In spite of the increasing use of prophylactic antibiotics in most surgeries, infections still remain a real risk and constitute a substantial burden to the patient (10). If the results of our investigation are anything to go by, the extensive use of pre-operative prophylactic and post-surgical antibiotics in various combinations at this centre needs to be re-examined. A patient –to-patient determination of prior effectiveness to chemotherapy may be necessary. The unusually high incidence of antibiotic resistance of bacterial isolates from samples cultured from patients at this centre is of concern and requires urgent action on the part of policy makers to formulate effective strategies to reduce the preponderance of resistance at this centre.

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