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EFFECTS OF SELECTED HOST, BACTERIAL AND ENVIRONMENTAL FACTORS ON OUTCOME OF CLEAN ORTHOPAEDIC WOUNDS

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ABSTRACT

Background: This study determined the effects of host, bacteria and environmental factors on the outcome of clean orthopedic wounds, antibiotic resistance profiles of selected bacterial isolates recovered from host and the environment, the operating room and the ward at Obafemi Awolowo University Hospitals Teaching complex, Ile-Ife. This was with a view to identify the factors associated with post-operative wound infections. **Material and method:** Seventy five (75) subjects with clean orthopedic wounds were recruited. Swabs were obtained from the surgical site before skin preparation, at incision, post incision and from the surgical site at the emergence of infection using sterile cotton- tipped applicators. Each applicator was inoculated into freshly prepared thioglycolate broth and incubated aerobically at 37^{O}C for 48h. A loopful of culture was streaked on different bacteriologic media and isolates characterized by conventional methods. Settle plates containing different bacteriologic media were also exposed at strategic locations in the theatre for the period of each surgical procedure as well as under the patient's bed on return of patient to the ward after the surgical procedure. Randomly selected bacterial isolates were screened by disk diffusion for antibiotic susceptibility using seventeen different antibiotics. Data generated from the study were analyzed using student's t-test (paired and unpaired), ANOVA and CHI square. Correlation analyses were used to show linear relationship between resistance patterns of various bacterial isolates to the different antibiotics. **Results:** Altogether, 162 bacterial isolates were recovered; one hundred and fifty six (156) from the operative site comprising of 68 from pre incision, 36 at incision and 52 from post incision sites while the last six (6) were from the three (3) patients with post-operative infection. The predominant isolates from both operative site and the infected site were *Staphylococcus aureus*, and *Corynebacterium pseudodiphtheriticum*. Two hundred and ninety one (291) bacterial isolates were recovered from the settle media plates exposed at four strategic locations in the operating room. Sixty-one (61) from location 1, 73 from location 2, 79 from location 3 and 78 from location 4. The predominant isolates were *Bacillus subtilis*, *Corynebacterium jeikeium*, *Staphylococcus aureus* and *Corynebacterium pseudodiphtheriticum*. Fifty (50) bacterial isolates were recovered from the ward environment; mainly *B. subtilis*, *C. pseudodiphtheriticum* and *S. aureus*. **Conclusion:** The study revealed high level of multiresistance in bacterial isolates from both the host and the environment. The results also identified some factors that predict post-operative infection thoμgh some were not statistically significant in this study. Six *Staphylococcus aureus* strains were subjected to RAPD – PCR to determine relatedness while thirty of the isolates were screened to detect resistance and virulence genes. The incidence of post-operative infection in clean orthopaedic wound in this study was 4%. *Staphylococcus aureus* was the commonest pathogen in the study.

KEYWORDS: Orthopedic wound**,** Bacterial isolates, Environmental factor, Surgical Site Infection.

INTRODUCTION

Wound is often referred to as injury to the skin or underlying tissues or organs. It could result when the operative barrier of the skin is breached by traumatic or surgical invasion of the skin and its adjacent tissues (Kaur, 2009). Whether a wound is caused accidentally by trauma or intentionally by surgery, the open area is extremely susceptible to microbial invasion and once a wound has become infected and pus is formed on the injured area, it results in wound abscess (Smith *et al.*, 2011). Surgeons classify wounds on the basis of gross appearances that is, either incised, lacerated or a confused wound in which the edges are crushed (Mangram, *et al.*, 1999). Surgical wound infection is used as an index of nosocomial infection which is a prototype of hospital acquired infection (HAI) and has been reported to constitute a serious problem especially in patients undergoing surgery (Kaur, 2009). According to Anguzu and Olila, 2007 all surgical wounds are contaminated by both pathogens and body commensals but the development of infection in the site depends on complex interplay of many factors (Olsen *et al.*, 2008).

These may be microbial virulence, operation-related risk factors of patient which include prolonged hospital stay before surgery, duration of the operation, tissue trauma, poor haemostasis, and foreign material in the wound and others (Bowler *et al.*, 2001).

The term for infections associated with surgical procedures was changed from surgical wound infection to Surgical Site Infection in 1992 by the Centers for Disease Control and Prevention (CDC) (Horan, 1992). These infections are classified into incisional, or organs and spaces manipulated during an operation; incisional infections are further divided into superficial (skin and subcutaneous tissue) and deep (deep soft tissue-muscle and fascia). Wound infection in orthopedic surgery carries high morbidity and mortality. Most of these infections are thought to originate from bacterial wound contamination at the time of operation, the incidence of which has been reported to be as high as 58% (Esler *et al*., 2003).

Post-operative wound infections are major global problem in the field of surgery leading to many complications, increased morbidity and mortality (Anguzu and Olla, 2007; Raza *et al.*, 2013). The infection rate vary from one hospital to the other (Isibor *et al.*, 2008) and could be affected by many factors such as the state of carrier, infection sources and conduct in the operation room (Bratzler, 2012). Many countries suffer high postoperative infection rates (PIR) in their hospitals, especially in developing countries. Brazil for instance reported a 15% PIR while in Europe and USA the highest PIR was 5%. (OSPA and OMS, 2001; CDC, 2003 and PREZIES, 2004).

It has been reported that the widespread uses of antibiotics, together with the length of time during which they have been available have led to the major problems of resistant organisms contributing to high morbidity and mortality. The most important function of an antibiotic drμg sensitivity test is the detection of clinically relevant antimicrobial resistance organisms causing an infection. This has made the management and treatment of post-operative wound infections difficult (Andhoga *et al.*, 2002). It is known that the situation has become serious and intolerable in developing countries due to irrational prescriptions of antimicrobial agents (Fadeyi *et al.*, 2008).

The hospital environment is known to be uniquely suited to the spread of infections as it houses both susceptible patients and patients with difficult-to-treat infections. There is a great risk that some patients may contract hospital-associated infections other than those they were admitted for because of nosocomial pathogens around them (Esposito and Leone, 2007; Lockhart et al., 2007).

Antimicrobial resistant pathogens that cause healthcare-associated infections (HAIs) pose an on-going and increasing challenge to hospitals, both in the clinical treatment of patients and in the prevention of the cross-transmission of these problematic pathogens, Esposito and Leone (2007) reported that selective pressure favouring resistant strains arises from misuse and over use of antibiotics. In recent years, bacterial resistance to different antibiotics has left physicians with few therapeutic options. It is known that patients infected with drμg-resistant organisms are more likely to have longer and more expensive hospital stays, and may be more likely to die as a result of the infection (Chaudhary and Aggarwal, 2004).

The burden of health-care associated infection (HAI) is relatively substantial in developed countries, where it affects from 5% to 15% of hospitalized patients in regular wards and as many as 50% or more of patients in intensive care units (ICUs) (Vincent *et al.*, 2009; WHO, 2009). The economic impact of prolonged hospital stay following an infection could be used in determining the level of productivity especially in developing countries where a relatively small amount is allocated to the Health sector (Guadagnoli and Cleary, 1995; Eli *et al.*, 2003).

Studies have shown that cultured microorganisms from surgical site infections may contain or produce toxins and other substances that increase their ability to invade a host, produce damage within the host or survive on or in host tissue leading to the morbidity and mortality among hospitalized patients, results of this work we hope will enable clinicians to be knowledgeable as well as understand the mechanisms of predominant pathogenic bacteria/ organisms of surgical site infections in this environment and their pathogenic potential.

MATERIALS AND METHODS

Study Center

The study was carried out prospectively at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife between (December 2013 to May 2016) The hospital is a referral center for over 1 million people in the contiguous states of Osun, Ondo, Oyo, Ogun and Lagos in Southwestern Nigeria.

Subjects

Subjects recruited for the study were all in-patients of male and female undergoing clean elective orthopedic surgical operation. All infected or open fractures were excluded from the study.

Ethical Approval

The investigators proposals were reviewed and approved by the Ethical Committee of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (Protocol number IPHOAU/12/507). Informed consent was obtained from each participant and parental consent from parents of subjects aged 13 years.

Collection of samples

Recruited patient sample was collected at pre-incision (before the skin preparation), at incision and post

incision (after the closure of the site) with clean wound on admission for surgical operation at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife. Simultaneously, a structured questionnaire indicating patient demographics such as age, gender, diagnosis, duration of operation and other relevant information was to collect data. All patients had had peri -operative antibiotic prophylaxis consisting of i.v. cefuroxine, 1.5g at induction of anesthesia followed by 750 i.v. 12 hourly x 2 doses as required by ward unit policy/directive.

Brief description of operating suite and orthopedic ward

The operating suite is located on the second floor of the hospital building designated as phase 2. The theater consists of four operating theaters equipped with two split air conditioning systems with the back opening into a free space where the system can easily be ventilated from the outside. The theater has two double corridors into which wing exit doors from the operating rooms open. Each theater has a separate scrub room and where anesthesia can be administered to patients.

The orthopedic ward consists of thirty-four beds occupied by both sexes. There are four separate rooms on the west side of each unit each of which can occupy two patients as a time with standing fans. There are one hundred and four windows (104) located throμghout the ward facilitating cross ventilation and where sunlight penetrates the ward. Three toilets are located at the far end of the ward meant for patients only. While patient that crippled can use bed pans for comfort.

Assessment of Orthopedic Ward and Theatre

Settle plates containing freshly prepared blood agar, EMB agar, Tryptone soy agar (supplemented with nystatin) and mannitol salt agar were exposed and placed in strategic locations in the operating room and in the ward.

Methods of sample collection

Each of the sample was collected from the site of the surgery before skin preparation, during and after wound closure from subject using sterile cotton –tipped applicators. Each of the sample was thereafter inoculated on the spot into freshly prepared McCartney bottles containing thioglycollate fluid medium ™ and brain heart infusion (BHI) broth. Each culture tube was transferred to the laboratory and incubated at 37^{o}C. A loopful of the growing culture was thereafter stained and based on the gram stain reaction inoculated on conventional microbiological media such as mannitol salt agar (MSA), eosin methylene blue agar and chocolate agar (CA). Bacterial colonies appearing on these on these plates were then studied and categorized as cocci and rods. Cocci that fermented mannitol on (MSA) were considered staphylococci and confirmed as *Staphylococcus aureus* by the isolate's ability to produce coagulase both on slide and tube tests using human pooled plasma API kit was also used. If such colonies were unable to produce coagulase were deemed coagulase negative (CONS).

However, cocci that grew on chocolate agar as tiny pin-point colonies appearing in short chains were tested for hemomolytic ability and their actives on Taxo A (0.04 units of bacitracin) and Taxo P (5μg) ethylhydrocuprene (optochin).

Rods that grew on EMB agar plates were also categorize as lactose fermenters on no-lactose fermenters. Each colony of bacteria was further tested on convention media such as citrate, urea agar, triple iron agar (TSI), sulphide indole motility (SIM).

Antibiotic sensitivity testing

The sensitivity testing was carried out by the Bauer et al; (1966) method. Four to five well-isolated colonies of 18-24 agar plate culture of the same morphological type were selected by touching the tip of each colony with a wire loop and transferring them to a tube containing 4-5 ml of tryptic – soy broth (TSB). Such tube was then incubated at 35^{0}C for 2-5h to produce moderately cloudy suspension that was standardized by diluting in TSB visual equivalent to the McFarland standard 0.5 (a turbidity standard prepared by adding 0.5 ml of 1% Barium chloride solution to 99.5 of 1% sulphuric/H_2S0 4). This equates to approximately 10^8 organisms per milliliter ( ) A sterile cotton –tipped applicator was dipped onto the adjusted suspension and incubated onto a dried Mueller- Hinton agar(MHA) plate by streaking the swab over the entire agar surface. The multidisc containing Ceftazidime (CAZ) 30 μg, Ceftriaxone (CRO) 30μg, Ampicillin (AMP) 10μg, Trimethoprim (W) 5μg, Gentamicin (CN) 30μg, Streptomycin (S) 10μg, Amoxicillin/Clavulanic acid (AMC) 3μg, Imipenem (IPM) 10μg, Ofloxacin (OFX) 5μg, Cefuroxime sodium (CXM) 30 μg, Oxacillin (OX) 1μg, Vancomycin (VA) 5μg, Cefoxitin (FOX) 30μg, Ciprofloxacin (CIP) 5μg, Kanamycin (K) 30 μg, Erythromycin (E) 15 μg and Cephalothin (KF) 30 μg was then placed onto the MHA plate using a sterile forceps. The plate was then inverted and placed in the incubator at 37^{0} C for 16-18 h and thereafter examined. The diameter of the growth inhibition was then measured with a transparent ruler and recorded. The zone of inhibition was interpreted by referring to manufacture's provided standard table and the isolate was scored susceptible or resistant. *S. aureus* ATCC 25923 was employed as a Control organism.

RESULTS

A total of two hundred and ninety one bacterial isolates were cultured from the settle media plates exposed at different locations in the operating room. The isolates cultured consisted of Gram positive cocci accounting for 27.8% of the total isolates, Gram positive rods were spore and the non-spore formers (64.3%) while Gram negative rods only (7.9%). The Gram positive cocci were mainly coagulase positive cocci (*Staphylococcus aureus*) (51.85%) of the positive cocci while coagulase negative

staphyloccus (46%) and micrococcus species only (1.2%). The spore formers of the Gram positive rods accounted for 28.9% of the positive rods while the non spore formers accounted for 71.1%. (Table 1).

Table 1: Distribution of Bacterial Isolates from Different Locations of the Operating Room.

	Bacterial isolates	Total No Cultured	L1	L2	L3	L4	P value
Gram positive Bacteria							
Gram positive cocci							
Pathogenic Staphylococci (coagulase positive)	*Staphylococcus aureus*	42	15	8	9	10	0.94
Coagulase negative Staphylococci	*Staphylococcus saprophyticus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Staphylococcus schleiferi* *Staphylococcus sciuri* *Staphylococcus capitis* *Staphylococcus simulans* *Staphylococcus haemolyticus* *Staphylococcus cohnii*	1 2 1 16 5 9 2 1 1	1 1 1 1 2 2 1 0 0	0 1 0 2 1 3 0 1 0	0 0 0 3 2 4 1 0 1	0 0 0 10 0 0 0 0 0	
Micrococci	*Micrococcus luteus*	1	0	1	0	0	
Gram positive rods (spore formers)	*Bacillus subtilis* *Bacillus cereus*	46 8	19 0	14 0	12 4	1 4	
Gram positive rods (non spore formers)	*Arcanobacterium haemolyticum* *Corynebacterium jeikeium* *Corynebacterium xerosis* *Corynebacterium pseudodiphtheriticum* *Corynebacterium ulcerans* *Corynebacterium pseudotuberculosis*	9 43 29 38 4 10	1 9 3 1 1 0	1 17 10 1 0 3	5 8 7 14 0 4	2 9 9 22 3 3	
Gram negative Bacteria							
Lactose fermenters	*Enterobacter aerogenes* *Escherichia coli*	14 1	2 0	5 1	4 0	3 0	
Non lactose fermenters	*Pseudomonas aeruginosa* *Proteus vulgaris*	6 2	1 0	2 2	1 0	2 0	
Total		**291**	**61**	**73**	**79**	**78**	

Legend: L1= A.C Vent 1, L2= A.C Vent 2, L3= Air filter, L4= surgical table

Identification of Bacterial Isolates Recovered From the Site of Orthopaedic Patients with Clean Wounds

A total of one hundred and fifty–six (156) bacterial isolates were recovered from the operative site of orthopedic patients with clean wounds. Sixty eight (43.6%) were from the pre – incision site, 36 (23.1%) incision site and 52 (33.3%) post - incision site. The bacterial isolates recovered were broadly classified into Gram positive cocci, Gram positive rods and Gram negative rods based on their Gram's reaction (Table 2).

Identification of Bacterial Isolates Recovered from the Operating Room Air of Orthopaedic Patients with Clean Wounds

Table 2: Distribution of Bacterial Isolates from the Pre – Incision, Incision and Post Incision of the Operative Site of Orthopaedic Patients with Clean Wounds.

	Bacterial isolates	Total No. cultured	Pre incision	Incision	Post incision	P value
Gram positive Bacteria						
Gram positive cocci						
Pathogenic Staphylococci (coagulase positive)	*Staphylococcus aureus*	31	17	6	8	0.001
Coagulase negative Staphylococci	*Staphylococcus sciuri* *Staphylococcus cohnii* *Staphylococcus capitis*	5 1 1	2 0 0	2 0 0	1 1 1	
Micrococci	*Micrococcus varians* *Micrococcus luteus*	1 2	1 0	0 1	0 1	
Gram positive rods						
Spore formers	*Bacillus subtilis* *Bacillus cereus*	9 1	4 0	3 1	2 0	
Non spore formers	*Arcanobacterium haemolyticum*	8	5	0	3	
	Corynebacterium pseudotuberculosis	19	6	3	10	
	Corynebacterium ulcerans	4	3	0	1	
	Corynebacterium jeikeium	14	8	0	6	
	Corynebacterium diphtheria	2	2	0	0	
	Corynebacterium pseudodiphtheriticum	25	10	9	6	
	Corynebacterium xerosis	15	6	3	6	
Gram negative Bacteria						
Gram negative rod						
Lactose fermenters	*Escherichia coli* *Enterobacter aerogenes*	4 1	1 0	1 0	2 1	
Non lactose fermenters	*Pseudomonas aeruginosa* *Proteus vulgaris*	11 2	2 1	6 1	3 0	
		156	**68**	**36**	**52**	

Table 3a: Total Heterotrophic Count of Bacteria colonies Recovered from the Operating Room Air on Settle Media Plates at Different Locations.

Media	No of times settle plates were exposed	Bacterial count at different locations (CFU/plate)				Mean	P value
		L1	L2	L3	L4		
Tryptone soy agar(supplemented with Nystatin)	75	216.29	206.91	138.23	196.6	189.51	
Mannitol salt agar	75	124.89	119.96	81.04	125.48	112.84	
Eosin methylene blue agar	75	14.75	10.55	5.11	15.56	11.49	
Blood agar	75	221.36	214.84	161.91	78.44	169.14	
Mean	75	144.32	138.07	96.57	104.02	120.75	(0.84)

Legend: L1=A.C Vent 1, L2=A.C Vent 2, L3= Air filter, L4= Surgical table

Table 3b: Total Heterotrophic Count of Bacteria colonies Recovered from the Operating Room Air and Ward Environment on Settle Media Plates.

Media	*No of times settle plates were exposed*	*Bacterial count (CFU/plate)*		*Mean*	*P value*
		Operating room air	*Ward environment*		
Tryptone soy agar (supplemented with Nystatin)	75	189.51	168.91	179.21	
Mannitol salt agar	75	112.84	103.99	108.42	
Eosin methylene blue agar	75	11.49	34.64	23.07	
Blood agar	75	169.14	215.28	192.21	
Mean	75	120.75	130.71	125.73	(0.86)

Table 3a and 3b above represent the total Heterotrophic Count of Bacteria colonies Recovered from the Operating Room Air on Settle Media Plates at Different Locations.

Antibiotic Resistance Pattern of Bacterial Isolates

The *in vitro* antibiotic resistance test was carried out on bacterial isolates cultured from the operative site, infected site and the air borne bacteria of the operating room and the orthopaedic ward. A total of one hundred and fifty three isolates from these sources were involved in the analysis. They were tested against seventeen different antibiotics belonging to eight different classes. Some of the isolates showed multiresistance to some of the drugs. The antibiotic resistance pattern of the bacterial isolates from the operative site, the environment and the infected site is shown in tables 4-6. 53.7% of the bacterial isolates from the operative site were resistance to ampicillin as against 16.3% from air borne bacteria, 44.2% were resistance to Oxacillin as against 15.6% from the air borne bacteria, 27.2% were resistance to Amoxicillin/Clavulanic compared to 9.5% of the air borne isolates. The resistance pattern followed the same trend in other antibiotics. In the wound infected patients, a total of 6 Gram positive isolates were screened. 5 out of 6 (83.3%) were resistant to ampicillin, only one *S. aureus* strain was susceptible to ampicillin. 16.7% were resistant to Cephalothin, vancomycin and trimethoprim, 33.3% were resistant to amoxicillin-clavulanic, ceftriazone and kanamycin while 50% were resistant to Oxacillin, ceftazidime and erythromycin. None of them were resistant to any of gentamycin, cefoxitin, cefuroxime, imipenem, streptomycin, ciprofloxin and Ofloxacin.

Table 4: Pattern of Antibiotic Resistance on bacterial Isolates Cultured from the Pre - Incision Site of Orthopedic Patients.

Bacterial isolates	Total No. of isolates tested	Antibiotics to which isolates from pre – incision site of orthopaedic patients with clean wound were resistant															
		AMP	OXA	AMC	FOX	CAZ	CRO	CXM	KF	IPM	VAN	GEN	KAN	STR	ERY	CIP	OFX
Gram positive cocci																	
Staphylococcus aureus	10	8	4	3	3	10	7	1	1	0	7	0	5	2	3	4	4
Gram positive rods																	
Spore formers Bacillus subtilis	4	4	1	1	0	4	2	1	1	0	2	0	1	0	2	0	0
Non spore formers																	
Arcanobacterium haemolyticum	5	3	3	1	2	5	2	1	1	0	4	0	2	1	2	2	1
Corynebacterium pseudotuberculosis	6	6	4	1	0	6	1	2	1	0	4	1	4	4	4	1	1
Corynebacterium jeikeium	9	9	9	7	3	9	4	4	8	0	9	3	7	6	9	6	6
Corynebacterium pseudodiphtheriticum	6	5	5	1	1	6	1	2	1	0	5	1	4	4	4	4	3
Corynebacterium xerosis	5	5	4	4	4	5	4	4	4	0	5	3	3	4	5	3	5
Gram negative rods																	
Pseudomonas aeruginosa	2	2	2	2	2	2	1	2	2	0	2	0	2	2	2	2	2
Ttotal	47	42 (89.4)	32 (68.1)	20 (42.6)	15 (31.9)	47 (100)	22 (46.8)	17 (36.2)	19 (40.4)	0 (0)	38 (80.9)	8 (17.0)	28 (59.6)	23 (48.9)	31 (65.9)	22 (46.8)	22 (46.8)

Table 5: Pattern of Antibiotic Resistance on Bacterial Isolates Cultured from the Incision Site of Orthopaedic Patients.

Bacterial isolates	Total No. of isolates tested	Antibiotics to which isolates from the incision site of orthopaedic patients with clean wound were resistant																
		AMP	OXA	AMC	FOX	CAZ	CRO	CXM	KF	IPM	VAN	GEN	KAN	STR	ERY	CIP	OFX	TRI
Gram positive cocci																		
Staphylococcus aureus	2	2	2	2	2	2	2	2	2	0	2	0	0	1	0	0	0	2
Gram positive rods																		
Non spore formers																		
Corynebacterium pseudodiphtheriticum	4	3	3	3	2	4	1	2	3	1	3	1	2	1	3	0	1	3
Corynebacterium xerosis	3	3	3	1	1	3	2	1	2	0	2	2	3	2	2	2	1	3
corynebacterium pseudothberculosis	3	2	2	1	0	3	1	0	1	0	2	0	2	1	2	0	1	2
Gram negative rods																		
Pseudomonas aeruginosa	2	2	2	2	2	2	1	2	2	0	2	0	2	1	2	1	1	2
Total	14	12 (85.7)	12 (85.7)	9 (64.3)	7 (50)	14 (100)	7 (50)	7 (50)	10 (71.4)	1 (7.1)	11 (78.6)	3 (21.4)	9 (64.3)	6 (42.9)	9 (64.3)	3 (21.4)	4 (28.6)	12 (85.7)

Table 6: Pattern of Antibiotic Resistance on Bacterial Isolates Cultured from the Post - Incision Site of Orthopaedic Patients.

Bacterial isolates	***Total No. of isolates tested***	***Antibiotics to which isolates from the post - incision site of orthopaedic patients with clean wound were resistant***																
		AMP	**OXA**	**AMC**	**FOX**	**CAZ**	**CRO**	**CXM**	**KF**	**IPM**	**VAN**	**GEN**	**KAN**	**STR**	**ERY**	**CIP**	**OFX**	**TRI**
Gram positive cocci																		
Staphylococcus aureus	7	4	4	3	3	7	5	4	3	0	4	2	2	2	3	3	1	5
Gram positive rods																		
Spore formers Bacillus subtilis	2	1	1	0	0	1	0	0	0	0	1	0	1	0	2	2	1	1
Non spore formers																		
Arcanobacterium haemolyticum	2	2	2	1	0	2	0	1	0	0	1	0	0	2	1	1	1	2
Corynebacterium pseudotuberculosis	9	5	3	1	1	7	0	1	1	0	4	0	3	1	5	1	0	4
Corynebacterium jeikeium	5	4	3	0	0	2	0	0	0	0	2	0	3	2	2	0	0	4
Corynebacterium pseudodiphtheriticum	4	3	4	2	1	3	0	1	3	0	4	0	3	1	3	0	0	4
Corynebacterium xerosis	6	6	4	4	5	6	2	2	4	0	4	0	2	0	4	1	0	3
Gram negative rods																		
Pseudomonas aeruginosa	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Total	36	25 (69.4)	21 (58.3)	11 (30.6)	10 (27.8)	28 (77.8)	8 (22.2)	9 (25)	11 (30.6)	0 (0)	20 (55.6)	2 (5.6)	14 (38.9)	8 (22.2)	20 (55.6)	8 (22.2)	3 (8.3)	23 (63.9)

1 DISCUSSION

Our study determined the effect of host (patient), bacteria and environmental factors on the outcome of clean orthopedic wounds of subjects at O.A.U.T.H.C, Ile – Ife. Bacterial isolates were cultured from the surgical site before incision, at incision, post incision and from the infected site. Bacterial isolates were also cultured from the operating room and ward air and were subsequently characterized by standard bacteriological methods. The antibiotic resistance profiles of the isolates were determined by the disk diffusion methods. The purpose of this study was to determine the sources of intraoperative bacterial colonization of clean surgical wound and subsequent development of infection.

Seventy five patients used in this study consisted of forty four males and thirty one females. Three (3) out of the seventy five patients included two males and one female developed post operative infection resulting in surgical site infection (SSI) rate of 4%. This SSI rate was similar to a previous study reported in one of the Nigerian hospitals (Enweani, 1991). The three infected cases occurred in patients with superficial incisional infections described superficial incisional infection as the most common surgical site infection (Ercole *et al*., 2011; Nichols, 2004; Oliveira and Carvalho, 2007). The SSI rate among the male patients was 4.5% while that of female was 3.2%. This observation corroborated similar report conducted in India where males had higher SSI rate than females (Anusha *et al*., 2010; Anand *et al*., 2013). Similar values were also reported in a Tanzanian study (Mawalla *et al*., 2011). Though males tended to have higher SSIs, there was no significant difference in SSI rate when compared with the females (p=0.08). This was consistent with a number of other studies (Ntsama *et al*., 2013; Nwankwo *et al*., 2013).

Infection was 38.85 ± 2.23 with maximum age of 80 years. The statistical analysis that compared average ages of these two groups showed that there was The mean age of the patients with post-operative infection was 44 ± 13.32 years with the maximum age of 70 years while those without post-operative no significant difference between them (p=0.65). This study of no significant relationship between gender and post operative infection was consistent with other studies (Ntsama *et al*., 2013; Nwankwo *et al*., 2013). Maksimovic *et al*., 2008 also reported no significant differences in age or gender between their case patients and matched controls.

In our study, a total number of 1,091 medical personnel participated. The result of this study altogether revealed a total of five hundred and three (503) bacteria isolates with one hundred and thirty one (26%) gram positive isolates from the surgical site of patients (pre incision, incision and post incision) see Table 2.

The predominant bacteria species isolated from all the sources were mainly *Staphylococcus aureus* (16.1%), *Corynebacterium pseudodiphtheriticum* (15.1%), *Bacillus subtilis* (13.7%) and *Corynebacterium jeikeium* (12.1%). Interestingly, the bacteria isolates recovered from the infected site were mainly *Staphylococcus aureus, Corynebacterium pseudodiphtheriticum* and *Corynebacterium xerosis*. It has been reported that in clean surgical procedures, *S. aureus* from the exogenous environment or the patient's skin flora is the common pathogen in the cause of surgical site infection (Andhoga *et al*., 2002; Aishby, 2010). *Corynebacterium jeikeium* was confirmed as the most common coryneform from orthopaedics and other surgical site infections (Rizvi *et al*., 2011). *Corynebacterium sp* that were originally referred to as commensals or saprophytes in human, animal or the environment have now been associated with human or animal infections (Bernard and Funke, 2012). The isolation of *Corynebacterium sp* from the surgical site before infection is suggesting the presence of these non-diphtheiroids corynebacteria in the mucosa and normal skin flora of humans and animals as reported (Yassin *et al.,* 2003; Collins *et al*., 2004).

The study also determined the antibiotic susceptibility pattern of bacterial isolates collected from different sources. A total of 153 randomly selected bacterial isolates (97 from the host, 6 from the infected site and 50 from the environment) were subjected to antibiotic susceptibility testing using seventeen different antibiotics belonging to 8 classes (Tables 4 - 6). The results of antibiotic resistance of Gram positive isolates cultured from the surgical site of patients with no post operative infection showed 92.4% resistance to Ceftazidime; followed by Ampicillin (81.5%); Vancomycin (70.7%); Trimethoprim (70.7%) Oxacillin (66.3%) and Erythromycin (60.9%). The resistance pattern of isolates from the infected site revealed that 83.3% of the isolates were resistant to Ampicillin, followed by Oxacillin, Ceftazidime and Erythromycin with 50% each while Amoxicillin/Clavulanic, Ceftriazone and Kanamycin followed closely with 33.3% and Trimethoprim (16.7%). None of these isolates from the infected site were resistant to any of Gentamycin, Cefoxitin, Cefuroxime, Imipenem, Streptomycin, Ciprofloxin and Ofloxacin. Similarly, the resistance pattern of isolates from the operating room and ward environment revealed 77.3% resistance to Ceftazidime; Trimethoprim (56.8%); Ampicillin and Erythromycin with 52.3% each followed closely with oxacillin (41%). This correlates with previous studies carried out by Onche and Adedeji (2004) where all the isolates screened were resisitant to ampicillin and also by Akinkunmi *et al.,* (2014) where 99.1% of the total bacterial isolates screened were resistant to penicillin.

CONCLUSION

Our study revealed that the rate of SSI in clean Orthopedic operations is 4%. This value can still be reduced to 2% (Culver *et al*., 1991). According to our data, the major challenge of SSI is the infection caused by resistant bacteria and their virulence toxins produced during surgical procedures as well as the numbers of

personnel and mode of operation. The presence of large number in the operating rooms only happens in developing countries in contrast to the developed counties of North America and Europe where surgery is performed by technology, that is, in the presence of remote media that do not attract the presence of personnel.

REFERENCES

1. Kaur, (2009). Wound infection overview, History. The ancient Egyptians to treat physical ailments. *Eastern Medical General Surgery*. Surgical Infection, 10: 323 – 25.
2. Smith M, Jacobs L, Rodier, L, Taylor A and White C (2011). Clinical quality indicators: infection prophylaxis for total knee arthroplasty. *Orthopaedic Nursing*, 30(5): 301 – 304.
3. Mangram A.J, Horan T.C and Pearson M.L. (1999): Guideline for prevention of surgical site infection. Hospital Infection Control Practices Advisory Committee. Infection Control Hospital Epidemiology, 20: 250–78.
4. Anguzu, J.R. and Olila D. (2007). Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *Afr Health Sci.*, 2007; 7: 148-54.
5. Olsen MA, Butler AM, and Willers D.M. (2008). Risk factors for surgical site infection after pinal surgery. *Journal of Neurosurgery*, 98: 149–55.
6. Bowler P.G, Duerden B.L and Armstrong D.G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Review*, 14: 244–269.
7. Horan T (1992): CDC definitions of nosocomial surgical site infections: a modification of CDC definitions of surgical wound infections. *Infection Control Hospital Epidemiology*, 13: 606–608.
8. Esler CN, Blakeway C, and Fiddian NJ (2003). The use of a closed- suction drain in total knee arthroplasty. A prospective, randomised study. J Bone Joint Surg Br., 85: 215 – 217.
9. Raza M.S, Chander A, Ranabhat A (2013). Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. OJMM., 3(3): 159–163.
10. Isibor and Candida albicans in Postoperative wound infections. African Journal of Microbiology Re, J.O., Osemi, A., Eyaufe, A., Rachael, O. & Turay, A. (2008). Incidence of aerobic bacteria search, 2: 288 – 291.
11. Bratzler D.W. (2012). Strategies for the prevention of Surgical site infections: Review of New Multi-specialty Society Guidelines. *University of Oklahoma Health Sciences Center*. 2012. | Website.
12. Centers for Disease Control and Prevention (CDC) (2003): "Guidelines for Environmental Infection Control in Health care facilities". U.S. Department of health and human services. Atlanta.
13. Andhoga J, Macharia A.G, Maikuma I.R, Wanyonyi Z.S, Ayumba B.R and Kakai R. (2002). Aerobic pathogenic bacteria in post-operative wounds at Moi teaching and referral hospital. *East African Medical Journal*, 79(12): 640–644.
14. Fadeyi A, Ismaila A.A, and Ganiyu A.R (2008). Bacteriological pattern of wound swab isolates in patients with chronic leg ulcer. Int. J. Health Res., 1(4): 183 – 188.
15. Esposito S and Leone S. (2007): Antimicrobial treatment for intensive care unit (ICU) infections including the role of the infectious disease specialist. *International Journal of Antimicrobial Agents*, 29: 494–500.
16. Lockhart S.R, Abramson M.A, and Beekmann S.E, (2007). Antimicrobial resistance among gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. *J Clin Microbiology*, 45: 3352–3359.
17. Chaudhary U and Aggarwal R (2004). Extended spectrum – lactamases (ESBL) – An emerging threat to clinical therapeutics. *Indian J. Med. Microbiol*, 22: 75 – 80.
18. Vincent J.L, Rello J, Marshall J, Silva E, Anzueto A and Martin C.D (2009). International study of the prevalence and outcomes of infection in intensive care units. JAMA, 302: 2323-9 doi: 10.1001/jama.2009.1754 pmid: 19952319.
19. WHO (2009). Guidelines on hand hygiene in health care. Geneva: World Health Organization.
20. Guadagnoli E and Cleary P.D. (1995): How consistent is patient-reported pre-admission health status when collected during and after hospital stay? Medical Care, 33: 106–12.
21. Eli N. P., Sands K. E and Cosgrove S. E. (2003): Health and Economic Impact of Surgical Site Infections Diagnosed after Hospital Discharge. *Emerging Infectious Diseases*, 9(2): 196-203.
22. Bauer A.W., Kirby W.W.M, Sherris J.C and Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 1966; 45: 493-5.
23. Enweani U.N (1991). Surgical wound sepsis in clean orthopaedic procedures: bacteriology and sensitivity pattern in a regional specialist centre. *Orient. Journal of Medicine*, 3: 1 – 6.
24. Ercole F.F, Chianca T.C.M, Duarte D, Starling C.E.F and Carneiro (2011). Surgical Site Infection in Patients Submitted to Orthopaedic Surgery: The NNIS Risk Index and Risk Prediction. Rev. Latino – Am. Enfermagem, 19(2): 269 – 76.
25. Nichols R.L (2004). Preventing Surgical Site Infections. Clin Med Res., 2(2): 115- 8.
26. Oliveira A.C and Carvalho D.V (2007). Evaluation of underreported surgical site infection evidenced by post – discharge surveillance. Rev. Latino – Am. Enfermagem, 15(5): 992 – 7.
27. Anusha S, Vijaya L.D, Pallavi K and Manavalan R. (2010). An Epidemiological Study of Surgical Wound Infections in a surgical unit of Tertiary care

Teaching Hospital *Indian Journal of Pharmacy Practice*, 4: 8 – 13.
28. Anand S, Mahendra P.S, Swagata B and Malay B (2013). Surgical Site Infection among postoperative patients of tertiary care centre in Central India – A prospective study. Asian Journal of Biomedical and Pharmaceutical Sciences, 17: 41 – 44.
29. Mawalla B, Mshana SE, Chalya PL, Imirzalioglu C, and Mahalu W. (2011). Predictors of Surgical Site Infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. BMC Surgery, 11: 21.
30. Ntsama E.C, Avomo J, Esiene A, Leme B.L, Abologo A.L, Masso M.P *et al*., (2013). Prevalence of surgical site infections and evaluation of risk factors after surgery, case of three public hospitals in Cameroon. *Journal of Medicine and Medical Sciences*, 6: 241 – 246.
31. Nwankwo E.O, Ibeh I.N, Enabulele O.I (2013). Incidence and Risk Factors of Surgical Site Infection in a Tertiary Health Institution in Kano, Northwestern Nigeria. http://www.ijic.info/article/download/10573/7621.
32. Culver D.H, Horan T.C, Gaynes R.P, Martone W.J, Jarvis W.R, and Emori T.G (1991).
33. Surgical wound infection rates by wound class, operative procedure and patient risk index. National Nosocomial Infections Surveillance System. Am. J Med., 91: 152S – 7S.