AFRICAN JOURNAL OF CLINCAL & EXPERIMENTAL MICROBIOLOGY JAN. 2002 ISSN1595-689 Vol 3 No1
AJCEM/200115/2205

BACTERIAL PATHOGENS ASSOCIATED WITH INFECTED WOUNDS IN OGUN STATE UNIVERSITY TEACHING HOSPITAL, SAGAMU, NIGERIA.

*[1]Sule, A.M., [2]Thanni, L.O.A., [3]Sule Odu, O.A., [1]Olusanya O.
[1]Department of Medical Microbiology; [2]Department of Surgery; [3]Department of Obstetric and Gynaecology
Obafemi Awolowo College of Health Sciences, Ogun State University, Sagamu, Nigeria.

A prospective study was conducted at Ogun State University Teaching Hospital (OSUTH) between August 1999 and July 2000 in the Orthopaedics, Obstetrics and Gynaecological units to identify the bacterial pathogens associated with infected wounds as well as their antibiotic sensitivity profile.

A total of 1670 patients were seen in these units, out of which 130 (7.78%) developed wound infections. There was a statistical difference (P < 0.05) between the septic wounds associated with the non-operative cases (11.9%) and those of post-operative cases (6.41%). Amongst the 186 bacterial agents isolated from all the samples examined, Klebsiella species (25.3%) accounted for the most common isolates while the least was Enterococcus faecalis (5.4%). Klebsiella species was observed to be most prevalent in the Obstetrics and Gynaecological wounds while Pseudomonas aeruginosa was the commonest in the Orthopaedic wounds.

The sensitivity profile of the isolates to the commonly used antibiotics including those used as pre-operative prophylactic agents ranged between 1.67-46.8%, the range for the aminoglycosides was between 61.8-75%, while the fluoroquinolones had a range of 82.8%-89.2%.

The high level of bacterial resistance to the common antibiotics in this study, re-emphasized the need to properly monitor the use of antibiotics including those used as pre-operative prophylactic agents in this country.

INTRODUCTION

Wounds are commonly encountered in clinical practice. They may arise post-operatively, following trauma, in association with haemoglobinopathy, or could be primarily of infective origin. The contamination or the mere presence of pathogenic organism in a wound without local or systemic tissue involvement may not result in infection. [1] Wound infection could be defined as the presence of pus in a lesion, as well as other general or local features of sepsis including pyrexia, pain, and induration. [2] Wound infection is an important cause of morbidity and mortality among surgical patient. Apart from causing discomfort to the patient, the effect may vary from being a simple nuisance; to a delay in wound healing and other major disasters, such as wound dehiscence, gas gangrene or tetanus. This often result in prolonged hospitalized, thereby adding appreciably to the cost of treatment and management of the patient. [3] Factor such as the type of wound, nature of surgery, the dose and virulence of infecting organism, host resistance and use of antibiotic have been reported to be important in the establishment of wound infection. [1,2,4] The bacterial agents often incremianted in wound infections include *Staphylococcus, Pseudomonas, Klebsiella, Proteus* species and *Escherichia coli* as well as anaerobes such as *Clostridium* and *Bacteroides species*. [4,5]

The management of infected wounds is a challenge in terms of rational antimicrobial use, especially with the presence of a wide array of antimicrobial drugs and their unrelenting promotion by pharmaceutical companies. Similarly, a lot of concern has been generated world-wide over bacterial drug resistance, in view of the fact that cheap drugs have to be replaced with more effective and expensive ones. [6] Sule and Olusanya [7] have reported the increasing prevalence of bacterial resistance to most of the commonly used antibiotics in this environment. Otokunefor et al [5], have also suggested the need for an in-depth knowledge of the current predominant strains of bacterial agents and their pattern of antibiotic sensitivity in hospital units, as such information could assist in the blind treatment of bacterial infections when facilities are inadequate for laboratory diagnosis.

This study is therefore aimed at determining the prevalence and type of bacterial pathogens in both post-operative and non-operative wound infections in this hospital. The antibiotic sensitivity profiles of the isolates is also to be determined so as to develop a policy for the chemotherapeutic management of wound infections.

MATERIALS AND METHODS

A prospective study was conducted at Ogun State University Teaching Hospital (OSUTH) Sagamu, between August 1999 and July 2000 in the Orthopaedics, Obstetrics and Gynaecological units to identify the bacterial pathogens associated with infected wounds. The sensitivity profile of such agents were also determined.

Patients seen in these units with clinically diagnosed cased of infected non-operative or infected wounds complicating a surgical operation were included in the study. All data on each patient examined were entered into a proforma used during the study. The information entered into the proforma included name, age, sex, whether the wounds were post-operative or non-operative, the size and site of the wound. Other information included admission and operation interval, type and duration of operative procedure as well as the type of pre-operative antibiotics used.

Two wound swab samples were collected from each patient. These were inoculated into Cooked Meat Medium and Glucose broth to preserve and maintain the anaerobic and aerobic organisms present respectively, during transportation to the

Corresponding Author

Medical Microbiology Laboratory of Obefemi Awolowo College of Health Science, Ogun State Unviersity, Sagamu. On receipt in the laboratory, all the turbid broth cultures were subcultured immediately while the non-turbid ones were incubated for 18-48 hours at 37oC before being subcultured. Each Glucose broth culture was inoculated onto MacConkey an Blood Agar and Kanamycin Blood Agar plates, and were incubated aerobically and anaerobically (using Oxoid gas generating kit for the generation of hydrogen and carbondioxide in an Oxoid anaerobic jar) respectively.

All the plates incubated aerobically were initially examined for growth after 24hours, the ones without growth were further incubated for up to 48 hours, while those incubated anaerobically were examined after 48 hours. Each isolate of the different colonies from both the aerobically and anaerobically incubated plates were picked for microscopic, biochemical and serological identifications using standard methods.

Sensitivity testing was carried out on each identified organism by touching 4-5 well isolated colonies with a sterile straight inoculating wire. This was inoculated into sterile peptone water which was then poured onto a previously dried sensitivity test agar plate. The excess culture fluid was decanted into a discard jar containing disinfection. The inoculated plate was left to dry and antibiotic disc was applied with a sterile forceps, allowed to and for 10-15 minutes for pre-diffusion of the antibiotics and incubated at 37oC overnight. The zone of inhibition produced after incubation was read using a meter rule[9]. The antibiotics used in this study were commercially produced single and multodiscs obtained from Oxoid Ltd. Basingstore Hampshire England; Interpharma Ltd, Lagos and Abtek Biological Ltd. Liverpool. The concentration of the different antibiotics used, were as shown in Table III. Statistical analysis of all the data were done by Chi-square method.

RESULTS

Among the 1670 patients seen in the different units, 130 (7.78) developed wound infections. Out of the total patients examined, 1248 had post-operative wounds while the remaining 422 had non-operative wounds. Table 1, showed that 80 (6.41) of the post-operative patients developed wound sepsis while 50(11.9) of the non-operative patients had septic wound. Statistical analysis of the data showed that the difference between the post-operative patients with septic wounds and non-operative patients with infected wounds were statistically significant (P<0.05).

Category of Patients	No	Those with infected Wounds	Percentage (%)
Post-operative	1248	80	6.4
Non-operative	422	50	11.9
Total	**1670**	**130**	**7.78**

Table I: The Prevalence of Post-operative and Non-operative Wound infections in Ogun State University Teaching Hospital.

Out of the 130 septic wound examined bacteriologically, 125 gave positive bacterial cultures while only 5 had no growth. Seventy three of the specimens were mono-bacterial while 52 were polybacterial cases two or three bacterial agents. A total of 186 bacterial agents were isolated from all the septic wound examined. *Klebsiella* species 47 (25.3) accounted for the most frequently isolated organisms in all the 3 units while *Enterococcus faecalis* 10(5.4) accounted for the least isolates (Table II). Interestingly, *Klebsiella* species was found to be the most common with wounds from Obstetrics and Gynaecological units while *Pseudomonas aeruginosa* was the most frequent in the orthopaedic wounds (Table II). No anaerobic organism was isolated in all the samples examined in this study.

Table II

THE BACTERIAL ISOLATES FROM DIFFERENT UNITS OF SURGICAL WARDS

WARD	GRAM NEGATIVE				GRAM POSITIVE			TOTAL
TYPE OF ISOLATES	*Klebsiella spp*	*Pseudomonas aeruginosa*	*Proteus spp*	*Escherichia coli*	*Staph. aureus*	*Staph. epidermidis*	*Ent. faecalis*	
Obstetrics	21(32.8)	14(21.8)	9(14.1)	5(7.8)	9(14.1)	3(4.7)	3(4.7)	64(34.4)
Gynaecology	9(22.5)	2(5.0)	7(17.5)	4(10.0)	6(15.0)	7(17.5)	5(12.5)	0(21.5
Orthopaedics	17(20.7)	21(25.6)	9(11.0)	8(9.8)	17(20.7)	8(9.8)	2(2.4)	82(44.1)
Total	47(25.3)	37(19.9)	25(13.4)	17(9.1)	32(17.2)	18(9.7)	10(5.4)	186(100)

The sensitivity profile of the isolates to the different antibiotics showed a low susceptibility of the isolates to Ampicillin, Tetracylines, Penicillin and Cotrimoxazole (Table III). However, Gentamicin, Colistin and Tobramycin were more effective against gram negative organism while Chloramphenicol was also more effective against the gram positive organisms. Similarly, all the isolates showed a remarkably high in-vitro susceptibility to the Fluoroquinolones tested (Table III). The analysis of the proforma showed that

Antibiotics	GRAM NEGATIVE ORGANISMS					GRAM POSITIVE ORGANISMS				
	Klebsiella Spp	Ps. aeruginosa	Proteus Spp	E. Coli	***Total***	Staph. aureus	Staph. epidermidis	Ent. faecalis	***Total***	**GRAND TOTAL**
*Ampicillin 25 µg	3(6.38)	1(2.70)	10(40.0)	3(17.6)	***17(13.5)***	4(12.5)	1(5.56)	2(20.0)	***7(11.7)***	**24(12.9)**
*Tetracycline 25 µg	25(532)	13(35.1)	9(36.0)	4(23.5)	***51(40.5)***	16(50.0)	5(27.8)	5(50.0)	***26(43.3)***	**77(41.4)**
*Streptomycin 25 µg	30(63.8)	19(51.4)	14(56.0)	2(11.8)	***65(51.6)***	15(46.9)	3(16.7)	4(40.0)	***22(36.7)***	**87(46.8)**
Cotrimoxazole 25 µg	14(29.8)	3(8.11)	5(20.0)	1(5.88)	***23(18.3)***	11(34.4)	3(16.7)	6(60.0)	***20(33.3)***	**43(23.1)**
Getamicin 10 µg	29(51.7)	25(67.6)	16(64.0)	10(58.8)	***80(63.5)***	22(68.8)	9(50.0)	4(40.0)	***35(58.3)***	**115(61.8)**
Colistin 25 ug	40(85.1)	26(70.3)	14(56.0)	15(88.2)	***95(75.4)***	NT	NT	NT	***NT***	**95(75.4)**
Tobramycin 25 µg	34(72.3)	27(73.0)	16(64.0)	15(88.2)	***92(73.0)***	NT	NT	NT	***NT***	**92(73.0)**
Nalidixic acid 30µg	38(80.9)	11(29.7)	17(68.0)	11(64.7)	***77(61.1)***	NT	NT	NT	***NT***	**77(61.1)**
Nitofurantoin 200 µg	40(85.1)	9(24.3)	15(60.0)	14(82.4)	***78(61.9)***	NT	NT	NT	***NT***	**78(61.9)**
Penicillin 1 i.u.	NT	NT	NT	NT	***NT***	1(3.13)	0(0.00)	0(0.00)	***7(1.67)***	**7(1.67)**
Erythronmycin 5 µg	NT	NT	NT	NT	***NT***	22(68.8)	9(50.0)	6(60.0)	***37(61.7)***	**37(61.7)**
Chloramphenicol10 µg	NT	NT	NT	NT	***NT***	30(93.8)	11(61.1)	7(70.0)	***48(80.0)***	**48(80.0)**
Cloxacillin 5 µg	NT	NT	NT	NT	***NT***	11(34.4)	4(22.2)	1(10.0)	***16(26.7)***	**16(26.7)**
Pefloxacin 5 µg	41(87.2)	31(83.8)	23(92.0)	12(70.6)	***107(84.9)***	27(84.4)	14(77.8)	6(60.0)	***47(78.3)***	**154(82.8)**
Ciprofloxacin 5 µg	43(91.5)	34(91.9)	24(96.0)	12(70.6)	***113(89.7)***	29(90.6)	16(88.9)	8(80.0)	***53(88.3)***	**166(89.2)**
Norfloxacin 10 µg	41(87.2)	32(86.5)	23(92.0)	14(82.4)	***110(87.3)***	29(90.6)	13(72.2)	10(100)	***52(86.7)***	**162(87.1)**

Key: NT = Not Tested

* 10 µg of these antibiotics were used for the Gram Positive. The concentration of others were similar.

( ) Percentage Sensitive

Table III

Sensitivity Profile of Organisms from Post-Operative and non-Operative Wound Infections

the prophylactic antibiotics used pre-operatively varied from a single regime to a triple regime. The prophylactic antibiotics used included Ampicillin, Flagyl, Gentamicin, Zinacef®, Ampiclox, Rocephin®, Floxapen®, and Profloxacin. It was evident that majority of the isolated in this study were resistant to most of the antibiotics used prophylatically.

DISCUSSION

Wound infection is an important determinant of the success or failure of surgery. The total wound infection rate of 7.78% obtained for all the units studied is within the range of 4.8-17% reported earlier from other countries.[9] However this rate is much lower than the 23.3% reported from Tanzania[2]. The difference between these two reports may be related to the relatively lower number of patients per wards in OSUTH, in view of the high number of beds allocated to the surgical wards, because of the hospital's strategic location at the T-junction of two express roads. Reports have shown that overcrowding of patients in a ward, may contribute significantly to the high rate of cross infections in an hospital setting[2]. A statistically significant to the ($P<0.05$) is observed between the non-operative and post – operative wound sepsis. The observed low prevalence of post-operative wound infection is not surprising because pre-operative prophylatic antibiotic are used in majority of the studied cases, which corroborates the previous reports that pre-operative prophylatic antibiotic may significantly reduce the prevalence of wound infections.[10]

Klebsiella species, *Ps. aeruginosa and Staphylococcus aureus* are the most common organisms associated with wound infections in this study, an this is similar to the previous report[4,5]. It is however, interesting to note that while Klebsiella species is more associated with the Obstetrics and Gynaecological wounds, Ps. Aeruginosa is the commonest pathogen in the Orthopaedics wounds. The prevalence of Ps. Aeruginosa in Orthopaedics wounds may probably be attributed to the contamination of the wounds with soil and other environmental microbes, as majority of the orthopaedics cases are traumatic wounds from road traffic accidents. Lowbury, et. Al[11]. have suggested that infections with *Pseudomonas* species is usually acquired from poor environmental sources.

The non-isolation of anaerobic organisms in this study is surprising because of the measures taken to recover these types of organisms from the septic wounds. Such measures include the inoculation of Cooked Meat Medium, a medium known to enhance the growth of the anaerobes, the subculturing onto selective medium as well as the incubation of the plates under anaerobic condition. The inability to isolate such organisms could however, be due to the use of dry swabs for the collection of the wound samples and probably a delay in the inoculation of these swabs into the cooked meat medium. It has been reported that wound specimens collected on swabs are usually subjected to drying. This study is shown that there is a need to improve the methods of collecting clinical samples especially when anaerobes could be among the implicating organisms.

The low level of susceptibility of the isolates of Ampicillin, Penicillin, Tetracyclines and Cotrimoxazole is similar to an earlier report in this environment and this have been attributed to the unrestricted use of these agents. However, the remarkable susceptibility of Gram – negative isolates to aminoglycosides and those of the Gram-positive to Chloramphenicol may be due to the lesser use of these antibiotics as a result of their toxic effect. The aminoglycosides have been reported to cause nephrotoxicity and damage to the eighth cranial nerve in human while chloramphenicol have also been reported to cause bone marrow toxicity. Although the fluoroquinolones are very effective against most of the organisms which are resistant to other antibiotics, it is however worrisome to note that most of the isolates that are resistant to the fluoroquinolones are multiresistant to other antibiotics including those used as prophylactic agents. These genes that specify resistance to a number of useful antibiotics have been located on transposons, thus providing possible explanation for the rapid evolution of R-Plasmids that posses a wide variety of antibiotic resistance determinant.

It is evident that most of the organisms isolates in this studies are multiresistant to majority of the common antibiotics including those used as pre-operative prophylatic agents. There is need therefore, to properly monitor the choice of antibiotics to be used as pre-operative prophylatic agents, if they are to serve the purpose of preventing post-operative sepsis.

REFERENCES

1. Archampong, E.Q: Wounds and Wound Healing. In: Badoe, EA: Archampong, E. Q. Jaja, M.O.A. (Eds). Principles and Practice of Surgery including Pathology in the Tropics. Ghana Publishing Company. 1986.
2. Shija, J.K.: The Incidence and Pattern of Sepsis Among General Surgical In-Patients at Muhimbili Hospital, Dar Es Salaam, 1973 – A Preliminary Report. East African Medical Journal (1976) 53(3) 153 – 159.
3. Anyiwo, M.D. Nosocomial Infections Still a Danger in Hospital Practice. Orient Journal of Medicine (1995) 7 (3/4) 28-32.
4. Enweani, U.N. Surgical Wound Sepsis in Clean Orthopaedic Procedures: Bacteriology and Sensitivity Pattern in a regional Specialist Centre Orient Journal of Medicine (1991) 3(1) 1-6.
5. Otokunefo, T.V. Datubo – Brown, D.D. Bacteriology of Wound Infections in the Surgical Wards of A Teaching Hospital. West African Journal of Medicine (1990) 9(4) 285-290.
6. Greenwood, D: Resistance to Antimiciobial Agents – A personal view. J. Med. Microbiol. (1998) 47(9) 751-755.
7. Sule, A.M. Olusanya, O. In-vitro Antibacterial Activities of fluoroquinolones Compared with common Antimicrobial Agents Against Clinical Bacterial Isolates from Parts of South Western Nigeria. Nigerian Quarterly Journal of Hospital Medicine (2000) 10(1).
8. Koneman, E.W; Allen, S.D; Dowell, V.R: Sommers, H.M; Color Atlas and Textbook of Diagnostic Microbiology J.B. Lippincott Company. Philadelpia. 1979, 6 – 346.
9. Adebo, O. A. Osinowo, O. Post – operative Wound Infection in Thoracic Patients: A Preliminary Report Afr. J. Med. Med. Sci. (1988) 17, 145 – 148.
10. Bodoky, A; Neff, U; Heberer, M. Harder, F. Antibiotic Prophylaxis with Two Doses of Cephalosporin in Patients Managed with Internal Fixation for Fracture of the Hip. The Journal of Bone and Joint Surgery (1993) 75A (1), 61-65.
11. Lowbury E.J.I.; Ayiliffe G.A.J; Geddes, A.M; Williams, J.D: Control of Hospital Infections. A practical handbook Chapman and Hall, London (1977) 169 – 207.
12. Yao, J.D. C Moellering, R.C: Antibacterial Agents. In: Balows, A; Hausler, W.J; Herrman, K.L; Isenberg, H.D. and Shadomy, H.T. (Eds): Manual of Clinical Microbiology 5th ASM Washington DC 1991 1070 – 1073.
13. Joklik, W.K. Willett, H.P; Amos, B.D. Wlifert, C.M: Antimicrobial Agents. In: Zinsser Microbiology 19th ed. Appleton and Lange. USA 1988, 129 –160.