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Nosocomial infections in post-operative wounds due to *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Benue State Nigeria

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Three hundred (300) post-operative wound swab specimens were aseptically collected from four hospitals and investigated. The four hospitals were Federal Medical Centre, Makurdi (FMCM), General Hospital, Gboko (GHG), General Hospital, Otukpo (GHO) and General Hospital North Bank, Makurdi (GHNBM). The swabs were cultured and organisms identified according to standard procedures. A prevalence rate of bacterial isolates (56.7%) was obtained from the post-operative wound sites investigated. *Pseudomonas aeruginosa* was the most encountered pathogen with 20.3% prevalence rate followed by *Staphylococcus aureus* (13.0%), while 8.3% accounted for co-infection of both organisms. Other organisms encountered included *Klebsiella* spp. (4.0%), *Escherichia coli* (3.3%), atypical coliform (2.7%), and *Proteus* spp. (2.3%). *Enterococcus faecalis* and *Streptococcus pyogenes* had the least prevalent rate of 1.3% each. Statistically, Chi square analysis showed that there was no significant difference in the number of isolates from FMCM, GHQ, GHO and GHNBM and in the occurrence of both organisms in relation to sex (p>0.05). The incidence of *P. aeruginosa* was highest (38.4%) at Federal Medical Centre, Makurdi, compared with other collection points investigated while that of *Staphylococcus aureus* was highest (37.5%) at FMCM compared with all other collection points' investigated. Antibiogram studies revealed that *P. aeruginosa* was most susceptible to levoxin to the magnitude of 98.4%. While *P. aeruginosa* was resistant to ampicillin, tetracycline and streptomycin, *S. aureus* was only resistant to tetracycline. The findings have revealed that nosocomial wound infections remain a menace in medical management of wounds.

Key words: Nosocomial, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, antibiotics, post-operative wounds, prevalence.

INTRODUCTION

Nosocomial infection is an infection acquired in a hospital by a patient who was admitted for a reason other than that infection (Wenzel, 2011). These infections are acquired in the hospital but appear after discharge or still when on admission. The organisms that cause most hospital acquired infections are common in the general population and are relatively harmless. The most common are bacteria (*Staphylococcus aureus*, coagulase -negative S*taphylococci*, *Enterococci,* and *Enterobacteria*) including commensal bacteria, which are part of the normal flora, and pathogenic bacteria, which come from an exogenous source. Viruses including Hepatitis B and C, Respiratory Syncytial Virus, rotaviruses, and enteroviruses may also be transmitted nosocomially. During times of prolonged antibiotic treatment and severe immunosuppression, fungi such as *Candida albicans, Aspergillus* spp*., Cryptococcus neoformans* and *Cryptosporidium* including other opportunistic organisms can cause infections (Shittu et al., 2012). The organisms can be transferred from one patient to another (cross-infection). They can be part of a patient's own flora (endogenous infection), or they can be transferred from an inanimate object or from a substance recently contaminated by another human source (environmental transfer). Factors that increase a patient's susceptibility to nosocomial infections include age (e.g. the elderly), decreased immunity, underlying disease, therapeutic and diagnostic interventions (Mangram et al., 2011).

A number of studies in Nigeria have shown that nosocomial infections in post- operative wounds are endemic in parts of the country (Shittu et al*.,* 2012; Kolmos et al., 2013). Akinjogula et al*.* (2010) reported that *S. aureus* was the leading etiologic agent of post-operative wound infection in Calabar and Uyo cities of Nigeria.

In a similar study in Benin City Nigeria, it showed that *Proteus* species were the leading etiologic agents in post-operative wound infections and *P. aeruginosa* was the prevalent agent in parts of South Eastern Nigeria (Shittu et al, 2012). Haghi et al*.* (2010) reported that *S. aureus* was the leading etiologic agent of post-operative wound infections in India, Thailand and Japan. They also found out that *P. aeruginosa* was more prevalent among microorganisms isolated from post-operative wounds in some parts of Jordan.

Data collected from this work will be used to establish the sanitary condition of hospitals where surgical operations are carried out. It will also establish the prevalent microorganisms involved in nosocomial infections. Antibiotic susceptibility test carried out will determine the drug of choice in the treatment of post-operative wound infections. In addition, the knowledge of these infections will help physicians to give adequate treatment when such infections occur and also advise on its prevention.

This study has become necessary to ascertain bacteria implicated in wound infections which delay the normal healing process.

MATERIALS AND METHODS

Study population and area

Patients with post-operative wounds infections were targeted for this study. Three hundred (300) post-operative wounds swabs were collected from this population which comprised ninety-nine (99) from FMCM, seventy from GHG, sixty eight (68) from GHO, and sixty three (63) from GHNBM. Approval was obtained from ethical clearance committees and the Chief Medical Director of each hospital for all the samples used for the study. Confidentiality was maintained in accordance with standard medical practice.

Sample collection and processing

Sterile swab sticks were used to collect pus from the surgical sites of subjects under aseptic conditions. The samples were properly labeled and immediately conveyed to the laboratory for processing. Standard microbiological procedures for handling and transporting of specimens as enunciated by Cheesbrough (2002) were followed.

Cultivation, isolation and identification

All the swabs collected for bacteriological investigations were treated according to the methods of Isenberg et al. (2011). MacConkey, blood and chocolate agars (Oxoid, England) were prepared following the manufacturer's instructions and allowed to solidify. The samples were inoculated onto the agar plates and incubated at 37°C for 24 h. Incubation period was extended to 48 h if there was no bacterial growth within 24 h. Investigations such as characteristics, Gram stain and biochemical reactions of the organisms were carried out in line with standard operating procedures. Identification and biochemical testing of isolates were carried out following standard procedures (Cheesbrough, 2012).

Antimicrobial susceptibility test

Kirby-Bauer disc diffusion susceptibility technique as documented by Isenberg et al. (2011) was adopted for the susceptibility assay. Only *P. aeruginosa* and *S. aureus* isolates obtained were used for this assay. In this technique, a well dried agar plate was seeded with appropriate inoculum. Filter paper discs impregnated with various antibiotics were placed at specific locations on the seeded agar plate. The plates were incubated at 37°C for 18 hrafter which susceptibility to antimicrobial agents was measured in millimeter as zones of inhibition, around the antibiotic discs.

RESULTS

With respect to all the hospitals where samples were processed, 170 (56.7%) bacteria were isolated, with *P. aeruginosa* having the highest 61 (20.3%) followed by *S. aureus* 39(13.0%), *E. faecalis* and *S. pyogenes* the least

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Table 1. Prevalence of bacterial isolates in 300 post-operative wounds examined in Benue State.

Organism	Total number isolated	%
Pseudomonas aeruginosa	61	20.3
Staphylococcus aureus	39	13.0
Klebsiella species	12	4.0
Escherichia coli	10	3.3
Atypical *coliform*	8	2.7
Proteus species	7	2.3
Enterococcus faecalis	4	1.3
Streptococcus pyogenes	4	1.3
Co-infection (*S. aureus* and *P. aeruginosa*)	25	8.3
Total	170	56.7

Table 2. Prevalence of *pseudomonas aeruginosa* and *staphylococcus aureus* in wounds according to type of surgical operation.

Types of operation	Number examined	Number (%)	
		P. aeruginosa	***S. aureus***
Appendicetomy	122	36 (12.0)	24 (8.0)
Caesarean section(c/s)	69	19 (6.3)	15 (5.0)
Herniotomy	63	17 (5.7)	12 (4.0)
Amputation	14	6 (2.0)	5 (1.7)
Cystostomy	11	4 (1.3)	2 (0.7)
Leparatomy	7	2 (0.7)	3 (1.0)
Mastectomy	6	1 (0.3)	2 (0.7)
Prostatectomy	3	1 (0.3)	1 (0.3)
Osteotomy	2	0 (0)	0 (0)
Colostomy	1	0 (0)	0 (0)
Gastrectomy	1	0 (0)	0 (0)
Thyroidectomy	1	0 (0)	0 (0)
Total	300	86 (28.7)	64 (21.3)

4(1.3%) respectively while co-infection of *P. aeruginosa* and *S. aureus* was 25 (8.3%) (Table 1).

Table 2 reveals that appendicetomy has 12and 8% of *P. aeruginosa* and *S. aureus* respectively according to type of surgery while Prostatectomy the least with prevalence of 0.3% for both *pseudomonas* aeruginosa and *S. aureus*. Coinfection was highest in appendicetomy (3.7%) and lowest in amputation (0.3%), mastectomy (0.3%) and prostatectomy (0.3%) (Table 3).

Among the health facilities investigated, FMC has the highest incidence of *P. aeruginosa* (11.0%) with GHO the least (4.7%) (Table 4). Similarly, *S. aureus* has 8.0% prevalence in FMCM while both GHO and GHNBM were lowest (4.0%). The rate of co infection in the various hospitals was more at FMCM (3.0%) with General hospitals Otukpo coming last (1.3%) (Table 5).

Table 6 indicated the number of males and females infected with *P. aeruginosa* as 15.7 and 13.0% respectively. In contrast, the rates of *S. aureus* infection in males were 9.7% and females 11.6%. Table 7 shows the distribution of *P. aeruginosa* and *S. aureus* infections according to age group with both organisms showing higher occurrence of infections in the young people compare to the elderly. Tables 8 and 9 also show the occurrence of co-infections of both organisms in age and sex of patients respectively with female having a higher prevalence of 4.6% and male 3.7%. Table 10 gave the distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in various hospitals in relation to age of patients with FMCM having the highest prevalence of both organisms when compared to the other hospitals.

DISCUSSION

The results of this study show that the prevalence of *P. aeruginosa* (20.3%) and *S. aureus* (13.0%) post-operative wound infections differed. This finding agrees

Table 3. Co-Infections of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in different types of surgical operation.

Types of operation	Number examined	Number (%) of co-infection
Appendicetomy	122	11(3.7)
Caesarean section (c/s)	69	5(1.7)
Herniotomy	63	4(1.3)
Amputation	14	1(0.3)
Cystostomy	11	0(0)
Leparatomy	7	2(0.7)
Mastectomy	6	1(0.3)
Prostatectomy	3	1(0.3)
Osteotomy	2	0(0)
Colostomy	1	0(0)
Gastrectomy	1	0(0)
Thyroidectomy	1	0(0)
Total	300	25(8.3)

Table 4. Prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in relation to health facilities.

Organism	Frequency (%)				
	FMCM	GHG	GHO	GHNBM	Total
*P. aeruginosa**	33(11.0)	24(8.0)	14(4.7)	15(5.0)	86(28.7)
*S. aureus***	24(8.0)	16(5.3)	12(4.0)	12(4.0)	64(21.3)

*χ^2 = 5.028 (p>0.05); **χ^2 = 1.344 (p>0.05).

Table 5. Prevalence OF *Pseudomonas aeruginosa* and *Staphylococcus aureus* CO-infection in relation to health facilities.

Parameter	FMCM	GHG	GHO	GHNBM	Total
Number examined	99	70	68	63	300
Number co-infected (%)	9 (3.0)	7 (2.3)	4 (1.3)	5 (1.7)	25 (8.3)

χ^2= 0.8773 (p>0.05)

Table 6. Prevalence of *pseudomonas aeruginosa* and *Staphylococcus aureus* according to sex of patients.

Sex	Number examined	Number (%)	
		P. aeruginosa*	***S. aureus*****
Male	156	47 (15.7)	29 (9.7)
Female	144	39 (13.0)	35 (11.6)
Total	300	86 (28.7)	64 (21.3)

*χ^2= 0.3395(p>0.05); **χ^2= 1.458(p>0.05).

with those of Dantas et al. (2013) (18.5%) in Karacchi city of Pakistan, Akinjogunla et al. (2010) (19.7%) in Calabar, and Anjum et al. 2010 (14.3%) in Eastern Nigeria. The rates of *Pseudomonas aeruginosa* and *Staphylococcus*

Table 7. Prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections according to age group.

Age group	Number examined	Number %	
		***P. aeruginosa**	***S. aureus***
10-19	74	24(8.0)	14(4.7)
20-29	76	19(6.3)	16(5.3)
30-39	64	12(4.0)	14(4.7)
40-49	27	7(2.3)	7(2.3)
50-59	25	13(4.3)	5(1.7)
≥ 60	34	11(3.7)	8(2.7)
Total	300	86(28.7)	64(21.3)

*X^2 = 0.5040; (p>0.05); **χ^2 =1.110; (p>0.05).

Table 8. Co-infection of *Pseudomonas aeruginosa* and *Staphylococcus aureus* among age group.

Age group	Number examined	Number co-infected (%)
10-19	74	6(2.0)
20-29	76	7(2.3)
30-39	64	3(1.0)
40-49	27	2(0.7)
50-59	25	3(1.0)
≥ 60	34	4(1.3)
Total	300	25(8.3)

χ^2 = 2.865; (p>0.05)

Table 9. Co-infection of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in relation to sex of patients.

Sex	Number examined	Number co-infected (%)
Male	156	11 (3.7)
Female	144	14 (4.6)
Total	300	25 (8.3)

χ^2= 0.6993; (p>0.05)

aureus wound infections reported in their results fall within the range obtained in this study. The rate of infection obtained in this study were however higher than (10.5%) reported by Joshi et al. (2011) in Benin City and (8.6%) reported by Shittu et al. (2012) in south west Nigeria. The disparity in infection rate could be attributed to differences in geographical location and possible differences in hygienic practices. Other scientists have obtained increasing prevalence of *P. aeruginosa* and *S. aureus* in post-operative wound infections especially in recent years. It is thus clear that the prevalence of *P. aeruginosa* and *S. aureus* obtained in this study is in agreement with what is obtained in other hospitals in Nigeria.

The microbial analysis revealed that *P. aeruginosa* and *S. aureus* were the leading etiologic agents of post-operative infection in this study. Similar results were obtained by et al. (1992) in Bombay town of India, Konno (2011) and Akinjogunla et al. (2010). The virulence of the microorganisms may be responsible for their high infection rates as suggested by Coffin et al. (2011).

The rate of *P. aeruginosa* and *S. aureus* were higher at Federal Medical Centre Makurdi than in other hospitals. In Federal Medical Centre, patients on admission stay long in the overcrowded wards, and are therefore exposed to cross infections

The prevalent rate of *P. aeruginosa* was higher than *S. aureus* in all the hospitals and this finding agrees with

Table 10. Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in relation to age of patients in various hospitals.

Age group	FMCM			GHB			GHO			GHNBM		
	No. exam.	No. (%) *P.aeru.**	No. (%) *S.aur.***	No. exam.	No. (%) *P.aeru**	No. (%) *S.aur***	No. exam.	No. (%) *P.aeru**	No. (%) *S.aur***	No. exam.	No. (%) *P.aeru.**	No. (%) *S.aur.***
10-19	23	8 (2.7)	5 (1.7)	15	6 (2.0)	4 (1.2)	18	5 (1.7)	2 (0.7)	18	5 (1.7)	3 (1.0)
20-29	22	7 (2.3)	5 (1.7)	19	7 (2.3)	5 (1.7)	19	2 (0.7)	3 (1.0)	16	3 (1.0)	3 (1.0)
30-39	18	6 (2.0)	6 (2.0)	15	3 (1.0)	3 (1.0)	17	2 (0.7)	3 (1.0)	14	2 (0.7)	2 (0.7)
40-49	9	1 (0.3)	2 (0.7)	5	1 (0.3)	1 (0.3)	6	2 (0.7)	2 (0.7)	7	2 (0.7)	2 (0.7)
50-59	11	5 (1.7)	2 (0.7)	6	4 (1.2)	1 (0.3)	4	2 (0.7)	1 (0.3)	4	1 (0.3)	1 (0.3)
60 and above	16	6 (2.0)	4 (1.2)	10	3 (1.0)	2 (0.7)	4	1 (0.3)	1 (0.3)	4	1 (0.3)	1 (0.3)
Total	99	33 (11.0)	24 (8.0)	70	24 (8.0)	16 (5.3)	68	14 (4.7)	12 (4.0)	63	15 (5.0)	12 (4.0)

*χ^2=10.525 (p>0.05); **χ^2=4.933 (p>0-05). FMCM = Federal Medical Centre Makurdi; GHG = General Hospital Gboko; GHO = General Hospital Otukpo; GHNBM= General Hospital North Bank Makurdi. *P.aeru =Pseudomonas aeruginosa*; *S.aur = Staphylococcus aureus*; No. = Number; exam. = examined

the reports of Joshi et al. (2011) and Cheadle W (2010). In another study by Prinsloo et al. (2010) and Burke (2012), *P. aeruginosa* was reported to be responsible for most nosocomial infections. This could be as a result of its ability to grow in disinfectants, sinks, water and other materials in the hospitals. It is also possible that patients may have developed immunity to *S. aureus* infection but this assumption contradicts the report of Johnson et al. (2013) where patients were more infected with *S. aureus* than *P. aerginosa* infection.

Incidence of *P. aeruginosa* was higher in males (except in General hospital Gboko) than females who were more infected mostly with *S. aureus* (except in General hospital Otukpo). This result is consistent with the reports of Kolmos et al. (2013) and Dulworth and Pyenson (2012), but contrary to that of Church et al. (2010) in which females were more infected with *P. aeruginosa*. It is possible that there are differences in hygienic practice of both males and females including the hospitals environment.

Patients within the age groups 10-19 years, 50-59 years and ≥60 years are at the highest risk of infections. Maltezou et al. (2012) in southern Uganda had reported that the age groups 10-19 years and ≥50 years were the most infected. According to Joshi et al. (2011) in south east Nigeria, children less than 13 years old were infected with post-operative wound infections whcich is also common in other parts of the world. Our results also agree with findings of Dantas et al. (2013) that infections were more common among the young and debilitated elderly people. From this study also, the age group 30-39 years had the least rate of infection in most of the hospitals. This may be due to good hygienic practices and avoidance of cross-infections exhibited by these groups of patients.

The rate of co-infection of *P. aeruginosa* and *S. aureus* according to health facility, gender and age differs but was not statistically significant. This result implies that co-infection is not influenced by these factors.

Chi square analysis at 99% confidence limit did not show any significant difference in the number of organisms isolated from the four hospitals.

The susceptibility rate of *P. aeruginosa* and *S. aureus* isolates to the eight antibiotics tested *in vitro* were relatively low compared to the sensitivity pattern to different anti pseudomonal and staphylococcal drugs reported worldwide (Haghi et al., 2010). In this study, *P. aeruginosa* isolates was highly susceptible *to* levoxin (97.7%) followed by ciprocin (81.4%) and norbactin (70.9%) (Table 11), while *S.aureus* isolates was also highly susceptible to levoxin (98.4%), ciprocin (93.8%), and norbactin (81.3%) (Table 12). Other drugs showed very low percentage of susceptibility. The non-hygienic measures in hospitals, the ability of some bacteria to grow in hospital materials or indiscriminate use of antibiotics, fake drugs, and self-prescription among patients are favourable conditions which overtime encourages the development of antibiotic resistant bacteria.

The isolates were completely resistant to three of the antibiotics (ampicillin, tetracycline and streptomycin) tested *in vitro*, which is much higher compared to a Belgian study (Prinsloo et al., 2010) but lower than the Turkish study where one third of the isolates were multidrug resistant. This could be due to misuse of these drugs without running sensitivity tests thereby resulting to development of resistant organisms.

The prevalence and sensitivity of *P. aeruginosa* and *S. aureus* often varies between communities, hospitals in the same community and among different patient populations in the same hospital (Kolmos et al., 2013). Faced with these variations, the physician in clinical practice has the responsibility of making clinical judgments, and should have access to recent data on the prevalence and antimicrobial resistance pattern of commonly encountered pathogens. It is therefore important to institute a system for the surveillance of antimicrobial resistance that will involve the clinical collection of

Table 11. Susceptibility pattern of *Pseudomonas aeruginosa* isolates to common antibiotics.

Antibiotics	No. of isolates sensitive (%)	No. of isolates resistant (%)
Ampicillin	0(0)	86(100)
Gentamycin	34(39.5)	52(60.5)
Colistin	47(54.7)	39(45.3)
Streptomycin	0(0)	86(100)
Tetracycline	0(0)	86(100)
Levoxin	84(97.7)	2(2.3)
Norbactin	61(70.9)	25(29.1)
Ciprocin	71(81.4)	16(18.6)

The concentration of each antibiotic was 10µg. The number of isolates tested against each antibiotic was 86.

Table 12. Susceptibility pattern of *staphylococcus aureus* isolates to common antibiotics.

Antibiotics	No. of isolates sensitive (%)	No. of isolates resistant (%)
Ampicillin	10(15.6)	54(84.4)
Gentamycin	31(48.4)	33(51.6)
Colistin	43(67.2)	21(32.8)
Streptomycin	25(39.1)	39(60.9)
Tetracycline	0(0)	64(100)
Levoxin	63(98.4)	1(1.6)
Norbactin	52(81.3)	12(18.8)
Ciprocin	60(93.8)	4(6.0)

The concentration of each antibiotic was 10 µg. The number of isolates tested against each antibiotic was 64.

microbiological data. Shittu et al. (2012) found that patients were the sources of bacteria in all cases of wound infection and that increase in post-operative infections was due to high penicillin resistant carrier rate in hospital personnel and patients as a result of widespread use of Penicillin. However, Kolmos et al. (2013) reported that cleaners and patients were the major source of wound contamination.

The high incidence of *P. aeruginosa* and *S. aureus* may be related to indiscriminate use of antibiotics without laboratory diagnosis and antibiotic sensitivity report. This single factor could eliminate the normal flora and provide a non-competitive environment for *P. aeruginosa* and *Staphylococcus aureus* to occur. The resistance of the organism to antimicrobial agents, nutritional versatility and the difficulties encountered in maintaining proper hygienic standards especially among personnel involved in wound dressing and general care of patients may have contributed to the high rate of *P. aeruginosa* and *S. aureus* infections.

Conflict of interest

The authors have not declared any conflict of interests.

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