Nigerian Veterinary Journal Vol. 24 (1) 1-7

FREQUENCY AND ANTIMICROBIAL RESISTANCE OF AEROBIC BACTERIA ISOLATED FROM SURGICAL SITES IN HUMANS AND ANIMALS IN NSUKKA, SOUTHEAST NIGERIA

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SUMMARY

This study was carried out to evaluate the frequency of occurrence and antimicrobial resistance of aerobic bacteria isolated from surgical sites in human and animal patients in Nsukka, southeast Nigeria. Wound swabs from 132 patients (96 humans and 36 animals) were cultured for bacterial isolation. Antimicrobial resistance profile of the isolates was evaluated by the agar disc diffusion method. A total of 134 bacterial isolates were obtained from 114 samples that yielded growth. *Staphylococcus aureus* (26.1%) was the most prevalent agent isolated from humans, followed by *E. coli* (17.4%), *Proteus* spp (17.4%), *Enterococcus* spp (13.0%) and coagulase negative *Staphylococcus* [CNS] (10.9%). In animals, the most common agents isolated were *E. coli* (19.0%) and *Proteus* spp (19.0%), followed by *S. aureus* (14.3%), *Enterococcus* spp (14.3%) and CNS (9.5%). A high proportion of Gram-negative isolates were resistant to cephalexin (80%), cotrimoxazole (80%), ampicillin (73.3%), gentamicin (70%) and nalidixic acid (70%) while majority of the Gram-positive bacteria were resistant to streptomycin (40.5%) and erythromycin (40.5%). Isolates demonstrated low resistance rate to ciprofloxacin. This study has provided information that may be needed in designing empirical treatment regimens for post-operative infections in Nsukka area.

KEY WORDS: Bacteria, isolation, surgical site, antimicrobial, resistance.

INTRODUCTION

The skin serves as an important barrier to the invasion of infectious agents. When the skin integrity is physically broken intentionally as during surgery, or accidentally, it becomes contaminated with varying numbers and types of microbial agents leading to local infection. The infectious agents can reach contiguous structures (soft tissue) or be disseminated via the blood stream to distant organs (Roger, 1966). In performing a successful surgical operation, that requires incision of the skin, subcutaneous and muscular tissues, the surgeon must be familiar with the principles and practice of aseptic surgery; which comprise preparation of the patient, the operator and the surgical instrument as well as the theatre (Frank, 1964). However, even the cleanest and most technically correct surgical incision is subject to some level of microbial contamination. Organisms responsible for surgical wound infections appear to be commonly acquired from the patient's endogenous flora, particularly from the gastrointestinal tract (Nochiri, 1973). Exogenous sources of contamination include the healthcare personnel and/or formites (Saini *et al.*, 1992; Fiser and Lanukova, 1997; Lubber and Henston, 1997).

A wide range of microorganisms have been isolated from infected surgical sites in both human and animal patients across the globe. Bacterial agents frequently associated with surgical site infections include *Staphylococcus aureus*, coagulase negative *Staphylococcus* (CNS), Group A *Streptococcus*, *Enterococcus*, *Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp* and *Proteus spp* (Gyang, 1986; Brachman *et al.*, 1980; Gyles, 1997; Huycke *et al.*, 1998; Vogel, 1999; Gales *et al.*, 2000). In addition to microbial factors, complex interactions between the host and the environment have also been reported to result in surgical site infections. Other factors suggested to determine whether a surgical site gets infected or not include the clinical condition of the patient and competence of the surgeon (Holzheimer *et al.*, 1997), type of surgery and the hospital environment (Frank, 1964; Page, 1993; Fiser and Lanukova, 1997), duration of operation and host susceptibility to the microbial contaminants (CDC, 1985; Lane and Cooper, 1999).

In spite of the great improvement in surgical techniques and the use of prophylactic antibacterial agents, sepsis attributable to surgical site infection still remains an important cause of post-surgical death (Roy and Perl, 1997; van de Plasche, 1998). Complications of infected surgical wound include wound dehiscence, delayed healing, scar tissue formation and fibrinous peritonitis (Archibald and Blacky, 1974; Smith *et al.*, 1972; Eze *et al.*, 2002). A successful treatment of surgical site infections require rational selection of appropriate antimicrobial agent (Vaughan *et al.*, 1979), based on knowledge of the infecting agents and their drug-susceptibility patterns.

Thus, this study was carried out to evaluate the frequency of occurrence and antimicrobial resistance profile of aerobic bacteria isolated from surgical sites in human and animal patients in Nsukka, Southeast Nigeria

MATERIALS AND METHODS

Study area/subjects and sample collection

The study was conducted in Nsukka area of Enugu State, situated Latitude $6^{o}45^{1}$ and 7^{o}N and Longitude $7^{o}12.5^{1}$ and $7^{o}36^{1}$ E (Oformata, 1975). Five hospitals (4 Human and 1 Veterinary), were involved in the study. These were hospitals with high admission rates and where various surgical exercises were performed. The consent of human patients and animal owners was sought before the sample was collected. A total of 132 patients (96 humans and 36 animals) were sampled.

The area around the surgical site was thoroughly cleaned with cotton wool soaked in 70% ethanol. Thereafter, a sterile swab (Evepon sterile swab stick®, Nkwelle Ezunaka, Nigeria) was used to swab the wound surface. All samples were processed within 1 hour of collection.

Isolation and identification of bacteria from surgical wounds

The protocol for isolation and identification of the bacterial isolates are according to Cheesbrough (2000). Briefly, each sample was streaked on blood and MacConkey agar and incubated aerobically at 37°C for 24 hours. Representative colonies were Gram-stained and examined microscopically to determine cell morphology and staining reaction. Isolates were further characterized and identified using standard bacteriological procedures (Cheesbrough, 2000).

Antimicrobial resistance studies

Antibiogram of the organisms isolated was determined using the disc diffusion method. Inhibition zone diameters (in millimetres) were measured and recorded to the nearest whole number. The zones were interpreted as resistant or sensitive following the interpretative chart of the Kirby-Bauer Sensitivity Test method (Cheesbrough, 2000). Commercial antimicrobial agents (Oxoid, Basingstoke, UK) used in the study included: ampicillin (25µg), amoxycillin-clavulanic acid (30µg), gentamicin (10µg), streptomycin (30µg), cephalexin (10µg), ciprofloxacin (5µg), norfloxacin (10µg), perfloxacin (5µg), chloramphenicol (10µg), erythromycin (30µg) and cotrimoxazole (50µg). The resistance frequency of the human and animal isolates to each of the antimicrobial agent was computed and compared using the Chi-square statistic.

RESULTS

Out of the 96 human samples cultured, 82 (85.4%) yielded bacterial growth while 32 (88.9%) of the 36 samples from animals contained bacterial agents. A total of 92 bacterial isolates were obtained from the 82 positive human samples and 42 isolates from the animal samples. The rank order of bacteria isolated from the surgical sites is presented in Table I. *Staphylococcus aureus, Escherichia coli, Proteus spp. Enterococcus* spp. and coagulase-negative staphylococcus (CNS) were the five most frequently isolated organisms. Gram-positive bacteria accounted for 55.2% of the total number of isolates while the remaining 44.8% were Gram-negative.

Antimicrobial resistance frequencies of the Gram negative and Gram-positive isolates are shown in Table II. A high proportion of Gram-negative isolates were resistant to cephalexin, cotrimoxazole, ampicillin, and gentamicin while majority of the Gram-positive bacteria were resistant to streptomycin, erythromycin and gentamicin. Isolates demonstrated low resistance rate to ciprofloxacin. Eighty-three percent of Gram-negative isolates were resistant to 2 or more antimicrobials as against 54.1% of the Gram-positive isolates.

TABLE I: Aerobic bacteria isolated from human and animal surgical sites in Nsukka

Rank order	*Organisms*	Number of isolates (%) Human	Animal	Total
1	*S. aureus*	24 (26.1)	6 (14.3)	30 (22.4)
2	*E. coli*	16 (17.4)	8 (19.0)	24 (17.9)
3	*Proteus spp*	16 (17.4)	8 (19.0)	24 (17.9)
4	*Enterococcus spp*	12 (13.0)	6 (14.3)	18 (13.4)
5	*Coagulase-negative Staphylococcus*	10 (10.9)	4 (9.5)	14 (10.4)
6	*Bacillus spp*	4 (4.3)	2 (4.8)	6 (3.0)
7	*Klebsiella spp*	2 (2.2)	2 (4.8)	4 (3.0)
8	*Streptococcus spp*	0 (0)	4 (9.5)	4 (3.0)
9	*Shigella spp*	2 (2.2)	0 (0)	2 (1.5)
10	*Salmonella spp*	2 (2.2)	0 (0)	2 (1.5)
11	*Citrobacter spp*	2 (2.2)	0 (0)	2 (1.5)
12	*Pseudomonas spp*	2 (2.2)	0 (0)	2 (1.5)
13	*Corynebacterium spp*	0 (0)	2 (4.8)	2 (1.5)
Total		**92**	**42**	**134**

Figures in parenthesis represent percentage of column total

TABLE II: Antimicrobial resistance of Gram-negative and Gram-positive surgical wound isolates

Antimicrobial agents	Number of resistant isolates (%)	
	Gram-negative (n = 60)	*Gram-positive (n = 74)*
Cephalexin (10µg)	48 (80.0)	NT
Cotrimoxazole (50µg)	48 (80.0)	NT
Ampicillin (25µg)	44 (73.3)	NT
Gentamicin (10µg)	42 (70.0)	28 (37.8)
Amoxicillin-clavulanic acid (30µg)	40 (66.7)	NT
Streptomycin (10µg)	38 (63.3)	30 (40.5)
Perfloxacin (5µg)	32 (53.3)	10 (17.2)
Erythromycin (10µg)	NT	30 (40.5)
Chloramphenicol (10µg)	NT	18 (24.3)
Norfloxacin (10µg)	NT	18 (24.3)
Ciprofloxacin (5µg)	14 (23.3)	4 (5.4)

NT = Not tested

Figures in parenthesis represent percentage of column total

Gram-negative isolates from humans were significantly (P < .05) more resistant to each of the test antimicrobial agents (except for gentamicin, streptomycin and perfloxacin) than those from animals (Fig. 1). Gram-positive human isolates were also significantly (P < .05) more resistant to the test antimicrobial agents than those from animal sources (Fig. 2).

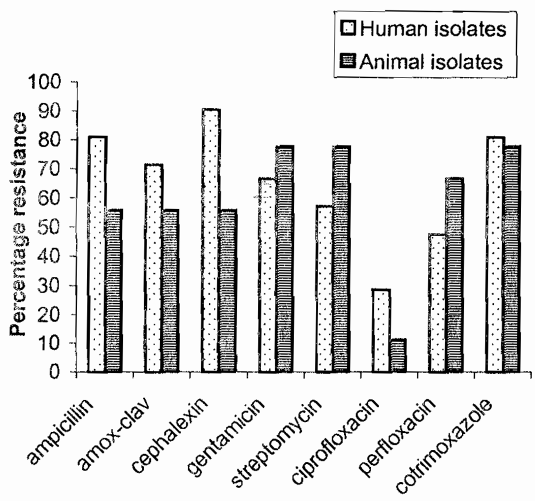

Fig. 1: Antimicrobial resistance profile of Gram-negative bacteria from human and animal surgical wounds in Nsukka, Nigeria

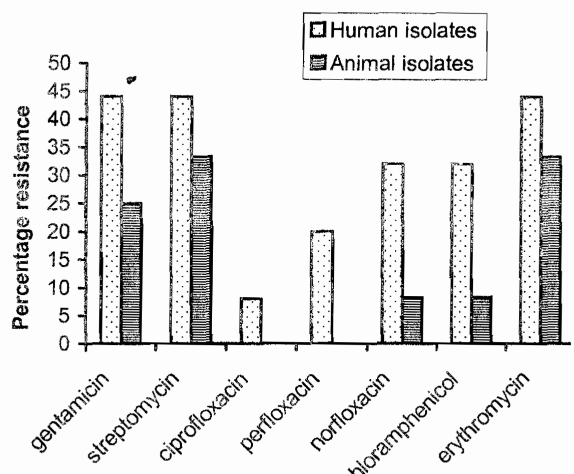

Fig.2: Antimicrobial resistance profile of Gram-positi bacteria from human and animal surgical wounds in Nsukka, Nigeria

DISCUSSION

The results of this study have shown that bacterial infection of surgical wound is common in both human and veterinary hospitals. This finding is in agreement with previous reports (Jarvis and Martone, 1992; Doern *et al.*, 1999; Gales *et al.*, 2000). Microbial infection of surgical wounds may elicit a systemic septic response as well as interfere with wound healing processes. The overall effect of this infection is prolonged hospitalization and high cost of patient care. *Staphylococcus aureus* was apparently the most frequent isolated pathogen in surgical wound infection in this and several other studies (Gyang, 1986; Jarvis and Martone, 1992; Janda *et al.*, 1997; Gyles, 1997; Vogel, 1999; Gales *et al.*, 2000).

High resistance rates were demonstrated by most bacterial isolates, especially the Gram-negatives. No isolate showed susceptibility to all the test antimicrobial agents. High frequency of multi-drug resistance among bacteria has been severally reported (Vogel, 1999; Huykce *et al.*, 1998; WHO, 2000) and the emergence of such strains has been variously blamed on improper use of the drugs both in and out of the hospital. Such selective pressures often promote evolution and dissemination of antimicrobial resistant bacteria. The high antimicrobial resistance rates recorded in this study may be a reflection of improper use of these agents in the studied area.

In order to prevent post-surgical sepsis, antimicrobial administration immediately before surgery, has been suggested especially for operations involving the gastrointestinal tract, operation lasting more than three hours and when contaminated or surgical incision in field animals is envisaged (Page, 1993). Effective surgical prophylaxis generally requires knowledge of the likely pathogen(s) and a careful selection of a reasonably safe and effective antimicrobial agent (Page, 1993; Irvin and Roger, 1994). In the present study, *S. aureus, E. coli, Proteus spp, Enterococcus spp* and coagulase-negative *Staphylococcus* were the most common surgical wound contaminants encountered. Thus, knowledge of the antibiotic resistance pattern provided by this study is important for the purpose of prophylaxis and treatment of post-operative sepsis. The apparently low resistance to ciprofloxacin may be because it is not affordable by many and, therefore, not subject to abuse. Therefore, it seems a drug of choice in the management of post-operative sepsis. On the other hand, combination of antibiotics such as streptomycin and penicillin, penicillin or ampicillin and gentamicin has been conventionally employed in combating post-operative infections in man and animals (Gyang, 1986; Irvin and Roger, 1994).

For optimum use of antibacterial agents in the therapy of bacterial infections, knowledge of the infecting agents and their resistance profiles are of paramount importance. The results of this study have provided information on the likely pathogens associated with surgical wound infection in the study area. Also the local resistance patterns of prevalent pathogens have been identified. The results have therefore provided information that may be required in designing empirical treatment regimens for post-operative infections in the area. The necessity for regular surveillance of surgical wound contaminants and determination of their antibiogram cannot be over-emphasized, given the importance of such knowledge in prophylaxis as well as treatment of post-operative wound sepsis.

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