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BACTERIOLOGICAL EXAMINATION OF CHRONIC OSTEOMYELITIS CASES IN ILE-IFE, SOUTHWESTERN NIGERIA

[1]Ako-Nai, A.K., [2]Ikem, I.C., [1]Aziba, A., [1]Ajayi, A. A., [3]Onipede, O.A.

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[2]Department of Orthopaedics & Traumatology, [3]Department of Medical Microbiology & Parasitology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

Correspondence to: Professor A. K. Ako-Nai, (E-mail: aakonai@oauife.edu.ng)

The bacteriological examination of chronic osteomyelitis cases in Ile-Ife, revealed *Proteus mirabilis* as the predominant isolate encountered (23.1%). Staphylococci constituted 30.7% of the total bacterial isolates with *Staphylococcus aureus* accounting for only 10.2%. Other Gram-positive cocci cultured include, *Staphylococcus spp* (20.5%), coagulase negative staphylococci (CONS) (12.8%) and *Streptococcus spp* (1.2%). Gram-negative rods constituted 55.1% of the total isolates with *Pseudomonas aeruginosa* being 8.5%, *Eschericia coli* 5.1% *Citrobacter freundii, Salmonella spp* 2.5% each. Antibiotic sensitivity test revealed all isolates to be multi-resistant to traditional antimicrobials, which is of epidemiologcal importance in treating cases of chronic osteomyelitis in this environment. The study suggests institution of aggressive therapeutic interventions to avert possible sequalae.

INTRODUCTION

Osteomyelitis is an inflammation of the bone caused mainly by microorganisms and can be categorized into acute and chronic forms. According to Ofiaeli (1), chronic osteomyelitis is perhaps one of the commonest orthopaedic diseases in the tropics among children and adolescents under the age of twenty years. Multiple factors predispose individuals to chronic osteomyelitis among which are mismanagement of acute osteomyelitis (2), haemoglobinopathies such as sickle cell diseases (3,4,5). Haematogenous bone infections, which persist and defy antibiotic therapy, environmental and personal hygiene (6). Prompt bacteriological investigations and aggressive management of patients with chronic ostomyelitis are essential for effective resolution and prognosis of this condition. While treatment modalities of chronic osteomyelitis involve various surgical interventions (7), there is no consensus as to the best surgical option although appropriate, effective and prompt intervention of antimicrobials is desirable when the microbial agents are cultured from patients. Reports from the literature indicate paucity of information on the incidence of chronic osteomyelitis cases in Ile-Ife and its environs except for a study undertaken by Ogunjumo in 1981(6). The present prospective study reports on the current status

of this condition in Ile-Ife and its environs with regard to the patients profile, predisposing factors and the treatment modalities in an effort to assist clinicians in the effective management of this condition to reduce cost and period of hospitalization of these patients.

MATERIALS AND METHOD

Study population

Criteria for inclusion in study were; establishment of osteomyelitis by radiography and only patients who presented at the hospital and were willing to submit themselves for treatment were included. All patients described above in all age groups were considered. 82 consecutive in-patients who satisfied these criteria were admitted into the study. Their ages ranged from 6 months to 90 years, of which 58 were males and 24 were females. The study was conducted between April 1995 to April 1997 at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife, Osun State, SouthWestern Nigeria. The complex is a referral centre for more than half a million people in Ile-Ife and its environs.

Collection of samples

Samples were collected by clinicians from each patient either during routine visit to the hospitals or at the operating table with sterile cotton-tipped applicators or sterile needles and syringes. At least 2-5 ml of venous blood was similarly obtained from each patient. All samples were collected with care to prevent any contamination during transit. Some of these specimens were introduced into transport medium containing Brain Heart Infusion (BHI) broth and Thioglycolate broth (TB) (Difco, Detriot, Michigan, USA).

Processing of samples

Each sample was collected with sterile cotton-tipped applicators and applied unto freshly prepared Mannitol Salt Agar (MSA), Blood Agar (BA) plate and Eosin Methylene Blue (EMB) agar. Each sample was also introduced into Cooked Meat medium and Thioglycolate broth (TB). All inoculated plates were thereafter incubated at 37°C for growth. Approximately 2 ml of venous blood was obtained from each subject and introduced into Thioglycolate broth (TB), which was subsequently incubated at 37°C for growth. The bacterial colonies that grew on the plates were picked and classified into different groups based on their Gram reaction. Those colonies resembling staphylococci were inoculated onto Mannitol Salt Agar and those isolates that fermented mannitol were picked. These were tested for coagulase production using both the slide and tube agglutination test employing pooled

human plasma. Gram-negative isolates that grew on EMB agar were further tested on Triple Sugar Iron (TSI agar), Koser's Citrate and Sulphide Indole Motility (SIM) agar and further characterized on other conventional media.

Antibiotic susceptibility test

Antibiotic susceptibility of the isolated organisms was determined by the standard agar dilution method of Ericsson and Sherris (8). The antibiotic discs employed included Ampicillin (Amp) 10 µg, Chloramphenicol (Chl) 10 µg, Cloxacillin (Cxc) 5 µg, Erythromycin (Ery) 5 µg, Nalidixic acid (Nal) 10 µg, Gentamicin (Gen) 10 µg, Penicillin (Pen), 1 unit, Ofloxacin (Ofx) 10 µg, Streptomycin (Str) 10 µg and Tetracycline (Tet) 10 µg, all obtained from Abtek Biological Ltd, Liverpool, U.K. Lot No. 5B02/P. The test medium was Mueller-Hinton agar (Oxoid, UK). *S aureus* ATCC 25923 and *Enterobacter aerogenes* ATCC 13042 were run as control organisms.

RESULTS

A total of 82 patients were seen. 58(70.7%) were males and 24(29.2%) were females. The incidence of chronic osteomyelitis was lowest among subjects aged fifteen years and below with 11(13.4%) patients, of whom 8(72.7 %) were males. Overall the incidence of the condition was more than double in males as against the females (See Table 1).

Table 2 shows the types of bone commonly involved in cases of chronic osteomyelitis during the study. Forty-six (56.1%) of these involved the tibia followed by femur (13.4%), humerus (8.5%), ulna (6.1%) and radius (4.8%).

The type of surgical interventions carried out on the patients is shown in Table 3. Wound debridement constituted a large proportion of surgery occurring in 32 cases followed by sequestrectomy, saucerization and curettage in 22 cases. Local muscle flaps accounted for 10; bone grafting, skin grafting and antibiotic impregnation constituted 2 cases each, while plating was done for non-union in only one case. The sources of infections in the chronic osteomyelitis cases varied. Post-traumatic sources were seen in 50 cases and accounted for a considerable proportion of infections. Decubitus ulcers contributed 5 cases. This was followed by diabetic foot with 4 cases. Leg ulcer, pin-tract infection, and crush injury accounted for 3 cases each. Gunshot injuries occurred in 2 cases and acute osteomyelitis occurred in only one case.

Bacterial isolates cultured from chronic osteomyelitis specimens

Seventy-eight bacterial isolates were cultured from 82 cases of chronic osteomyelitis averaging 1.05 microbe per subject. Gram-positive cocci constituted 44.9% of the entire isolates. Staphylococci accounted for 97.1% of these isolates. Among the staphylococci, *Staphylococcus spp* accounted for 47.1%, coagulase negative staphylococci (CONS) 29.4% and *Staphylococcus aureus* 23.5%. Gram-negative rods accounted for 55.1% of bacterial isolates with *Proteus mirabilis* predominating (41.9%) followed by *Pseudomonas aeruginosa* (25.9%), *Escherichia coli* (13.9%), *Klebsiella aerogenes* (9.3%) and *Citrobacter freundii* and *Salmonella spp* constituting 4.6% each of the Gram-negative short rods. None of the blood culture showed any growth.

Antibiotic susceptibility profile of some of the bacterial isolates

The antibiotic susceptibility pattern of the bacterial isolates is shown in Table 6. The staphylococci were all virtually resistant to Benzylpenicillin and Ampicillin. All the *Staphylococcus aureus* and CONS isolates were resistant to these two penicillins but sensitive to Cloxacillin and Ofloxacin both of which are beta-lactamase resistant drugs. *Pseudomonas aeruginosa* isolates were resistant to all drugs used except Gentamicin which 45% of *P. aeruginosa* isolates were sensitive. A similar trend of resistance was seen with *Proteus mirabilis* and *E. coli* isolates (Table 6).

TABLE 1: PROILE OF CHRONIC OSTEOMYELITIS CASES SEEN AT OAUTHC BETWEEN 1995-1997

Age	Number (%) Male	Number (%) Female
0-15	8(13.8)	3-(12.5)
16-30	20(34.5)	10-(41.7)
31-45	17(29.3)	5-(20.8)
>46	13(22.4)	6 – (25.0)
Total	58 (70.7)	24 (29.2)

TABLE 2: TYPES OF BONE INVOLVED IN CASES OF CHRONIC OSTEOMYELITIS AT OAUTHC, ILE-IFE

BONE TYPE	FREQUENCY (%)
Tibia	46(56.1)
Femur	11(13.4)
Humerus	7(8.5)
Ulna	5(6.1)
Radius	4(4.8)
Phalanges	2(2.4)
Sacral Bone	2(2.4)
Tarsal	1(1.2)
Clavicle	1(1.2)
Knee	1(1.2)
Elbow	1(1.2)
Ribs	1(1.2)

TABLE 3: TYPES OF SURGICAL INTERVENTIONS EMPLOYED AT THE OAUTHC, ILE-IFE

PROCEDURE	FREQUENCY
Sequestrectomy, saucerization and curettage	22
Wound debridement	32
Local Muscle flaps	10
Amputation	7
Drainage of Pus	5
Bone grafting	2
Skin grafting	2
Antibiotic impregnation	2
Plating (after control of infection)	1

TABLE 4: SOURCES OF INFECTION IN CHRONIC OSTEOMYELITIS AT OAUTHC, ILE-IFE

ROUTE OF INFECTION	FREQUENCY
Post traumatic	50
Decubitus ulcers	5
Diabetic foot	4
Leg ulcer	3
Pintract infection	3
Sacral pressure (bed sore)	3
Crush injury	2
Gunshot injury	2
Acute osteomyelitis	1

TABLE 5: BACTERIAL SPECIES ISOLATED FROM CHRONIC OSTEOMYELITIS PATIENTS AT THE OAUTHC, ILE-IFE

BACTERIAL ISOLATES	FREQUENCY (%)
Gram Positive	
Staphylococcus spp.	16(20.5)
Coagulase nagative Staphylococci (CONS)	10(12.8)
Staphylococcus aureus	8(10.0)
Streptococcus spp.	1(1.2)
Gram Negative rods	
Proteus mirabilis	18(23.1)
Pseudomonas aeruginosa	11(14.1)
E.coli	6(8.5)
Klebsiella aerogenes	4(5.1)
Citrobacter freundii	2(2.5)
Salmonella spp.	2(2.5)
Total	78

TABLE 6: ANTIBIOTIC RESISTANT PROFILE OF BACTERIAL ISOLATES

Organism	Total No. Tested	% Resistant								
		PEN	AMP	ERY	CHL	STR	TET	GEN	CXC	OFX
Staphylococcus aureus	8	100	100	450	25	25	100	25	0	0
Staphylococcus spp	16	75	44	13	44	100	100	13	0	0
Coagulase Negative Staphylococci (CONS)	10	100*	100	20	20	20	100	0	0	0
Streptococcus spp	1	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	11	100	100	100	100	100	100	45	0	0
Proteus mirabilis	18	100	100	100	100	100	100	33	0	0
Klebsiella aerogenes	4	100	100	100	100	100	100	25	0	0
Escherichia coli	6	100	100	100	100	100	100	33	0	0
Salmonella spp	2	100	0	0	0	0	0	0	0	0

*** Only major organisms were tested**

DISCUSSION

This work was prompted because of the paucity of information regarding chronic osteomyeliitis in Ile-Ife and its environs. Except for the study carried out by Ogunjumo in 1981 (6), there is no other report of chronic osteomyelistis in this environment. Unlike acute osteomyelitis, a substantial proportion of the patients with chronic osteomyelitis were adults (78%) and a number of these were males (67%) indicating the predominance of this condition amongst males (2.41:1). Our results show that the tibia was the predominant bone affected followed by the femur and humerus. The majority of the chronic osteomyelitis cases and others were as a result of post-traumatic infections due to compound fracture, crush injury and pin tract infection. Although other organisms have been implicated in chronic osteomyelitis, bacterial species are the most common. The bacterial aetiology of chronic osteomyelitis varies but according to most studies (9,16), *Staphylococcus aureus* seems to be the predominant organism encountered. In a study carried out at this Centre by Ogunjumo (5), *S. aureus* was the predominant organism isolated from patients. While staphylococci still remain the significant organisms cultured from chronic osteomylitis patients, our study reveals that *Staphylococcus spp* (20.5%) has become the predominant Gram positive cocci recovered, followed by coagulase negative staphylococci (CONS) (12.8%). *S. aureus* came a distant third (10.3%). This observation underscores the changing pattern of bacterial aetiology of this condition even within the same environment.

However, Gram negative short rods constituted 55.1% of the total bacterial isolates cultured from various specimens with *Proteus mirabilis* accounting for 41.9%) of the Gram negative rods. This was followed by *Pseudomonas aeruginosa* (25.9%). Overall, *Proteus mirabilis* was the predominant bacterial isolates in chronic osteomyelities at this centre. While Ogunjumo (6) previously reported *S. aureus* as the predominant organism in chronic osteomyelitis at this centre, our study however, corroborates Oguachuba (10) who reported *Proteus spp* predominance in Jos, Northern Nigeria. The increasing prominence of *Pseudomonas aeruginosa* in chronic osteomyelitis is of concern because of this organism resistance to various antimicrobial agents. However, none of the subjects from whom pseudomonas were cultured had sickle cell disease (11). An average of two types of organisms

each was cultured from 24 patients that participated in the study. Seven of the clinical specimens cultured did not grow on all the media employed. The occurrence of multiple organisms in some specimens cultured, suggests polymicrobial nature of chronic osteomyelitis which appears to be a feature of this condition compared with acute osteomyelitis cases, where over 50% of clinical specimens cultured contained a single organism (12).

Many factors other than the age affect the development of chronic osteomyelitis (1,5,13). Osteomyelitis resulting from direct extension of a decubitus ulcer, wound infection or open fracture may be difficult to detect clinically before the infection progresses to chronic osteomyelitis. Therefore early recognition of acute osteomyelitis is highly desirable for favourable prognosis. Even when roentgenographic abnormalities of the bone may be helpful in confirming the presence of infection, if roentgenographically diagnostic changes are delayed, the process may often progress to chronic form. In patients with chronic recurrent osteomyelitis, antimicrobial therapy is usually dictated by the microflora present within the infected bone. Even when cultures of sinus tract drainage yield bacteria, which are not present in the bone or fail to yield organisms, such cultures of sinus tract are only useful as guidelines for initial therapy.

The selection of antimicrobial agents for the treatment of chronic osteomyelitis caused by Gram-negative rods may be difficult. The *in vitro* sensitivity testings carried out with these isolates underscores this point. Virtually all the gram-negative rods recovered from the clinical source were multiply resistant to the various antibiotics used at least *in vitro*. Except for four isolates of *Staphylococcus spp*, all the other staphylococcal isolates were resistant to Benzylpenicillin and Ampicillin, suggesting these were beta-lactamase producers. Such observation with *S. aureus* isolates had been reported by Mollan and Piggot (12), in which seventy-four percent of these strains were resistant to Benzylpenicillin. Gentamicin was marginally effective in *vitro* with only five isolates of *Pseudomonas aeruginosa* sensitive. Similarly the *Proteus mirabilis* recovered from the patients were resistant to most antibiotics used in treating Gram-negative infections except to Gentamicin. The ineffectiveness of many of these antibiotics *in vitro* against the majority of our isolates corroborates reports of some investigators of the existence of multiply resistant enteric rods even amongst apparently healthy

individuals in this environment and elsewhere (14,15).

We recommend that clinicians practicing in this environment should be mindful of this phenomenon and therefore employ in their treatment modalities as combination of effective drugs and also follow-up patients' treatment for considerable length of time in order to resolve the condition. According to Jensen and Lassen (17), Fusidic acid given in combination with other antibiotics were effective in preventing emergence of resistant strains of *S.aureus* when administered as first line treatment. It is interesting that the bacterial isolates cultured in our study responded very well to Cloxacillin and Ofloxacin both of which are known to be effective against beta-lactamase producers.

While antimicrobial therapy is desirable in the control of chronic osteomyelitis, surgery remains the therapeutic and diagnostic procedure, which should be carried out early to resolve the condition (1,18). Our study therefore recommends prompt detection of acute osteomyelitis cases so that these do not progress to chronic form. Aggressive chemotherapeutic management of acute osteomyelitis is suggested. Early bacteriological investigations to determine bacterial aetiology are highly desirable so that first line antimicrobials are rigorously administered. Since the majority of chronic osteomyelitis cases are posttraumatic, orthopaedic surgeons should be more conscious of the risk of such injuries progressing to chronic forms and educate patients of prompt and proper management at initial stages to avert subsequent complications. The prevalence of multiply antibiotic resistant organisms in this study should be brought to the notice of Health Administrators in the Ministry of Health who should educate the public and dissuade communities in engaging in self-medication.

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