ABSTRACT... Objectives: To determine the causative organism of long bone chronic osteomyelitis through culture of the sequestrum. Study design: Descriptive case series. Setting and duration: Orthopaedic Surgery Unit, Mardan Medical Complex Teaching hospital, Bacha Khan Medical College Mardan, KPK, Pakistan from September 2011 to April 2012. Methodology: Twenty five patients with radiologically proven chronic osteomyelitis of long bones who had been free of antibiotic therapy for at least 48 hours, excluding those with diabetic foot, decubitus ulcers, and infected implants. At least one specimen of sequestrum was taken from each individual and subjected to complete microbiologic analysis. Results: Staphylococcus aureus was the most frequently found organism (n=11, 44%), followed by Enterobacteriaceae (n=5, 20%), coagulase-negative staphylococci (n=3, 12%), Escherichia coli (n=2, 8%), P aeruginosa (n=1, 4%), Streptococcus species (n=1, 4%) and no growth (n=2, 8%). More than one microorganism was isolated in two (8%) patients. Conclusions: Staphylococcus aureus was the most common organism isolated. Sequestrum culture provides accurate identification of causative bacteria.

INTRODUCTION
The infection of bone that contains bone marrow, called osteomyelitis, is as old as humankind and continues to be an important problem for modern medicine owing to its high morbidity and sequelae.

Osteomyelitis develops within two weeks after disease onset, subacute osteomyelitis within one to several months and chronic osteomyelitis after a few months. Osteomyelitis may be caused from hematogenous spread, direct inoculation of microorganisms into bone, or from a contiguous focus of infection. Pathologic features of chronic Osteomyelitis are the presence of necrotic bone (Sequestrum), the formation of new bone (involucrum), and the exudation of polymorphonuclear leukocytes joined by large numbers of lymphocytes, histiocytes, and, occasionally, plasma cells. The involucrum is irregular and is often perforated by openings through which purulence may track into the surrounding soft tissues and eventually drain to the skin surfaces, forming a chronic sinus. Diagnosis of long bone Osteomyelitis rests on isolation of the pathogen from bone lesion or blood culture. The most important point in relation to chronic bone infections is the difficulty to correctly establish the etiologic agent and the proper treatment to cure the patient. Antibiotic treatment of osteomyelitis should be based on sensitivity studies in meticulously performed cultures of bone taken at the time of debridement or deep bone biopsies. In infants, the pathogens most frequently isolated from blood or bones are Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli. However, in children more than 1 year of age, Staphylococcus aureus, Streptococcus pyogenes, and Haemophilus influenzae are most commonly isolated.

After age 4, the incidence of Haemophilus influenzae infection decreases, and the overall incidence of H. influenzae as a cause of osteomyelitis is dramatically decreasing with the ever-increasing use of an improved H. influenzae vaccine. Perhaps the most significant epidemiologic change regarding long bone osteomyelitis, and all osteoarticular infections, is the ongoing rise of methicillin resistant Staphylococcus aureus (MRSA) and other multidrug-resistant organisms (MDROs). Most recently, MDROs in extremity wounds suffered by

soldiers returning from the wars in Iraq and Afghanistan have become a source of concern in the west\textsuperscript{10,11}. The aim of our study was to determine the causative organism of long bone chronic osteomyelitis by culturing the sequestra in patients presented to our orthopaedic unit.

SUBJECTS AND METHODS
In this study twenty five patients of radiologically proved long bone chronic osteomyelitis of all ages and gender were selected from Out-Patient Department(OPD) of Orthopaedic Unit Mardan Medical Complex. Patients with diabetic foot, decubitus ulcers, infected implant and small bones osteomyelitis were excluded from the study. Informed written consent was obtained from all patients participating in this study. The rationale was explained in accordance with the principles laid down by the Ethics Committee Mardan Medical Complex Teaching hospital. Antibiotic therapy was interrupted at least 48 hours before specimen collection. Sequestra were taken from all patients during open surgery and subjected to culture and sensitivity. Routine and standard laboratory techniques for transportation and culture were followed. The data was analyzed using SPSS version11. Mean, Mode, Median, Percentages, Frequencies and ratios were calculated. Data was presented in tables and graphs where necessary. No statistical test was applied because the study design was descriptive.

RESULTS
A total of twenty five patients, 17(68%) males and 8(32%) females with mean age 23 years, mode 25 and median 27. The minimum age of the patient was 2 years while maximum age was 56 years. Femur\textsuperscript{(n=10,40%)} and Tibia\textsuperscript{(n=7,28%)} were the most frequent foci of chronic osteomyelitis, followed by humerus \textsuperscript{(n=4,16%)}, Ulna \textsuperscript{(n=3,12%)} and Fibula\textsuperscript{(n=1,4%)}. Sequestra cultures allowed agent identification in 92% of cases. Bacterial aetiology of chronic osteomyelitis based on sequestrum culture is shown in graph below. Staphylococcus aureus was isolated in majority of our patients\textsuperscript{(44%)}. The different kind of bacterial isolation show no relation with age and gender.

DISCUSSION
Appropriate diagnosis and therapy of chronic osteomyelitis require microbiologic cultures of the infected bone. Nonbone specimens are not valid for this purpose\textsuperscript{12}. In our study sequestra culture provided accurate identification of causative bacteria in 92\% of patients. Zuluaga and Galvis reported that bone cultures allowed agent identification in 94\% of cases, including anaerobic bacteria in 14\%\textsuperscript{12}. Another study reported that culture of non-bone specimens to identify the causative organisms in chronic osteomyelitis produced 52% false negatives and 36% false positives when compared against bone cultures. This study concluded that diagnosis and therapy of chronic osteomyelitis cannot be guided by cultures of non-bone specimens because their microbiology is substantially different to the microbiology of the bone \textsuperscript{13}. The choice of bone as the ideal specimen for microbiologic diagnosis of osteomyelitis is based on common sense and a classic retrospective study of 40 patients published by Mackowiak\textsuperscript{14}.

In our study Staphylococcus aureus was the most frequently found organism in chronic Osteomyelitis(44\%). Mackowiack found 60\% of chronic osteomyelitis to be due to S aureus, followed by enterobacteriaceae (23\%), pseudomonas (9\%) and streptococcus (9\%) in his study\textsuperscript{14}. Another study found that inta-operative bone culture appears to predict more reliably the complete etiologic organisms than sinus tract culture in chronic osteomyelitis and organisms isolated from bone cultures were Staphylococcus 69\% (29/42), Escherichia coli...
9.5% (4/42), Pseudomonas aeruginosa 9.5% (4/42), Proteus mirabilis 7% (3/42), respectively. White et al. evaluated the utility of sending both histologic and microbiologic biopsy samples and found eight positive cultures in 19 histologically or surgically proven cases of osteomyelitis, for a culture positivity rate of 42%. Similarly, the commonest infecting organism isolated in a local study was Staphylococcus (54%) followed by enterobacteriaceae (23%) that included (proteus spp (12.5%), E.coli (8%), Klebsiella (2.5%) Pseudomonas aeruginosa (18%), anaerobes (2.5%) and miscellaneous (2.5%). Two (2.5%) anaerobic bacteria were isolated, anaerobic bacteria were peptostreptococci and bacteroides either alone or as a mixed infection.

Sheehy SH et al reported that Staphylococcus aureus was most commonly isolated (32%) amongst a wide range of organisms including gram negative bacilli, anaerobes and coagulase negative staphylococci. This study, however reported high polymicrobial infection (29%) and culture negative cases were common (28%) while in our study polymicrobial infection and culture negative cases were only 8% each. Khudair AH documented staphylococcus aureus was the most common bacteria isolated from 12(60%) patients by operative means and 11(55%) in cases of sinus track cultures. The second most common bacteria was Pseudomonas aerogenosa, 4(20%) patients. E. coli and Klebsiela were the least common microorganism, found in only 1(5%) patient only for each.

Despite the strengths of our study, a few limitations deserve mention. Our sample size may not be large enough to detect a significant difference in microbiology of chronic osteomyelitis. Inspect the fact that the direct specimen from the bone give the more accurate result of causative microorganisms but it is difficult to perform because it must carried out under general anesthesia and patient subjected to operation.

CONCLUSIONS
Staphylococcus aureus was the most common organism isolated. Since sequestra cultures allowed agent identification in 92% of cases therefore all sequestra should be sent to the laboratory for culture and antibiotic sensitivity. It is a cost-effective technique for the isolation of the pathogens and proper treatment of osteomyelitis patients. The value of bone culture in the therapy of osteomyelitis must be emphasized; it is the only reliable means of determining the responsible agent, upon which the antibiotic therapy is based.

REFERENCES


Article Citation: