Journal of Biological Sciences 8 (2): 478-481, 2008
ISSN 1727-3048


# Epidemic Assessment of Bacterial Agents in Osteomyelitis and Their Antibiotic Resistance Pattern Determination

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**Abstract:** The aim of the present study was to determine the causative agents of osteomyelitis and specifying their antibiotic resistance pattern in patients referred to pediatrics ward of Imam Khomeini Hospital. This study has been performed in Tehran during January to December 2006. In this study, synovial fluid was taken from 90 patients who referred to pediatrics ward of Imam Khomeini. Samples were examined by direct test, culture and biochemical tests. In next step, antibiogram by disk diffusion method (Kirby-Bauer Test) was established on each positive sample and finally, the results were analyzed. Of 90 examined samples, 27 bacterial cases were isolated; *Staphylococcus aureus* with 55.9% was the most epidemic agent and *Klebsiella* sp., coagulase negative *Staphylococci*, *Streptococcus pneumoniae* and *Enterobacter* sp. were the next common agents, respectively. However, it was revealed that more than 40% *Staphylococcus aureus* are sensitivity to Vancomycin, Gentamicin, Sulfamethoxazole and Erythromycin and most of gram negative Rods isolated of were sensitivity to Amikacin, Ampicillin and Cephalothin (50-100%). The most of bacteria (*Staphylococcus aureus*, coagulase negative *Staphylococci*, *Streptococcus pneumoniae* and *Enterobacter* sp.) were sensitive to Vancomycin, gentamicin and Cephalothin. Similar to previous results, it was proved either in our study that *Staphylococcus aureus* is the main agent of osteomyelitis and gram negative bacteria with coagulase negative *Staphylococci* and *Streptococci* are other agents. Also, it was observed that the most isolated bacteria had high antibiotic resistance to common drugs.

**Key words:** Osteomyelitis, bacterial agents, antibiotic resistance pattern, disc-diffusion

## INTRODUCTION

Inflammation of bone and joint is called osteomyelitis and is prevalent in children especially between 3-12 years old. This is common among boys three times more than girls (Prabhakar *et al*., 2000; Ziran, 2007; Brady *et al*., 2006). Various microorganisms can reach to bone through blood and cause inflammation in bone tissue; rarely soft tissue infection may lead to bone damage. Microorganisms reach to the metaphysis of bone through blood flow from skin wounds, upper respiratory tract infections, periodontitis and any other infectious region. Bone metaphysis is a region full of blood vessels and slow bloodstream which can spread the infection. Direct trauma to bone may cause osteomyelitis also (Mader and Calhoun, 2005). Microorganisms' multiplication in metaphysis will cause congestion, edema, exudates, leucocytosis, necrosis and abscess. Clinical symptoms are directly related to infection severity, damaged region and patient condition. Administration of inappropriate antibiotics without considering cause of fever, enough laboratory tests and microbial sensitivity determination, may lead to clinical symptom disappearance and make exact diagnosis very difficult. If diagnosis is made up to 48 h after appearance of clinical symptoms, surgery is not necessary for proper management of osteomyelitis and all attempts are conducted to find the etiologic agent and appropriate treatment will be started after antibiogram. So, culture of synovial fluid and other infectious samples is necessary. Quick and appropriate treatment, health status and weakness of etiologic agent will cause faster and better results in improvement. In the case of inappropriate

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treatment and progress of the disease, surgery and drainage are essential (Prabhakar *et al*., 2000). In situation that inappropriate management or lack of treatment occurs, mortality rate will increase, especially in newborns and children.

Because etiologic factors of osteomyelitis are various, detection of causative agents is essential for appropriate treatment (Mandracchia *et al*., 2004).

In each country, related to antibiotic type and its pattern of consumption, there is a great difference in antibiotic sensitivity and resistance. Since antibiotic consumption in our country is based on other countries treatment program or published books and reports, it may cause unfavorable consequences (Carek *et al*., 2001; Baumert *et al*., 2002).

The goal of this study is to assess etiological agents of osteomyelitis and comparison of different antibiotics effects on microorganisms *in vitro*, in order to utilize its results for quick and appropriate antibiotic prescription before preparation of culture and antibiogram results.

## MATERIALS AND METHODS

This study is Cross sectional. Sampling was performed by easy non-random method. Population was 90 patients who referred to Imam Khomeini Hospital during January to December 2006.

Synovial fluid was taken by a specialist and special needles; then samples were sent to the laboratory to determine etiologic agents and pattern of antibiotic resistance (Jacobson and Sieling, 1987; Mackowiak *et al*., 1978). Samples were cultured on chocolate agar, blood agar, EMB agar, MacConkey agar and nutrient agar; in addition after 24-48 h incubation, bacteria were detected by tests such as catalase, oxidase, biochemical characteristics determination (sugar and amino acid fermentation) and complementary tests such as coagulase, culture on special media, e.g. SS agar and manitol salt agar (Carek *et al*., 2001). After determination of etiologic agents, they were used to perform antibiogram by disk diffusion method (Kirby-Bauer Test) (National Committee for Clinical Laboratory Standards, 2000). Pattern of antibiotic resistance for each isolated agent was determined by different antibiotics (Ampicillin, Cephalothin, Amikacin, Cefoxitin, Ciprofloxacin etc.) (National Committee for Clinical Laboratory Standards, 2001). Data were analyzed by means of statistical software. We used descriptive analysis to estimate the frequency.

## RESULTS

Of 90 samples sent to the laboratory, in direct examination and culture, 27 bacterial cases were isolated. Generally, the most prevalent was *Staphylococcus aureus* (55.9%) and *Klebsiella* sp. (14.8%), *Enterobacter* sp., Coagulase negative Staphylococci and *Streptococcus pneumoniae* (each one about 7.4%) and *Acinetobacter* sp. and *Nonhemolytic streptococci* (each one 3.7%), were next agents respectively (Table 1).

Antibiogram was performed on culture-positive samples. Resistance and sensitivity of mentioned bacteria were determined to different antibiotics. Sensitivity of each sample is shown in Table 2. As the Table 2 shows *S. aureus* the most prevalent causative agent of osteomyelitis has the most sensitivity to the Cephalothin, gentamicin, Clavulanic acid, Cefoxitin, Vancomycin, etc.; but its sensitivity is not 100% to these antibiotics, whereas only 13.3% of the cases are sensitive to Cotrimoxazole and Ampicillin. The most of gram negative

Table 1: Frequency of osteomyelitis bacterial agents in patients who referred to Imam Khomeini Hospital in Iran during January to December 2006

| Bacteria | *S. aureus* | *Klebsiella* sp. | Coagulase negative *Staphylococci* | *Pneumococcus* | *Enterobacter* sp. | *Acinetobacter* sp. | Non-hemolytic *Streptococci* |
|---|---|---|---|---|---|---|---|
| Frequency (%) | 55.9 | 14.8 | 7.4 | 7.4 | 7.4 | 3.7 | 3.7 |

Table 2: Susceptibility of bacteria isolated from bone sites of infection in patients who referred to Imam Khomeini Hospital in Iran during January to December 2006

| | Antibiotic | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *S. aureus* | 0 | 0 | 0 | 13.3 | 13.3 | 33.3 | 46.7 | 44.7 | 60 | 53.3 | 53.3 | 60 | 66.7 | 73.3 | 60 |
| *Klebsiella* sp. | 0 | 25 | 25 | 0.0 | 0.0 | 0.0 | 25.0 | 0.0 | 0 | 0.0 | 25.0 | 25 | 25.0 | 25.0 | 25 |
| Coagulase negative *Staphylococci* | 50 | 0 | 0 | 100.0 | 0.0 | 100.0 | 100.0 | 100.0 | 100 | 100.0 | 50.0 | 100 | 50.0 | 50.0 | 50 |
| *Pneumococcus* | 0 | 0 | 0 | 100.0 | 0.0 | 100.0 | 100.0 | 100.0 | 100 | 0.0 | 50.0 | 100 | 100.0 | 100.0 | 100 |
| *Enterobacter* | 0 | 100 | 100 | 0.0 | 0.0 | 0.0 | 50.0 | 50.0 | 0 | 0.0 | 0.0 | 100 | 100.0 | 100.0 | 100 |
| non-hemolytic *Streptococci* group D | 0 | 0 | 0 | 100.0 | 0.0 | 0.0 | 100.0 | 100.0 | 0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 100 |
| *Acinetobacter* sp. | 0 | 0 | 100 | 0.0 | 0.0 | 100.0 | 100.0 | 0.0 | 0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 | 0 |

1 = Penicillin, 2 = Ciprofloxacin, 3 = Carbenicillin, 4 = Ampicillin, 5 = Cotrimoxazole, 6 = Chloramphenicol, 7 = Amikacin, 8 = Sulfamethoxazole, 9 = Cephalothin, 10 = Erythromycin, 11 = Ceftriaxone, 12 = Gentamicin, 13 = Cefoxitin, 14 = Clavulanic acid, 15 = Vancomycin

Rods isolated of were sensitivity to Amikacin, Ampicillin and Cephalothin (50-100%) and the most of bacteria (*Staphylococcus aureus*, coagulase negative *Staphylococci*, *Streptococcus pneumoniae* and *Enterobacter* sp.) isolated of were Sensitive to Vancomycin, gentamicin and Cephalothin.

## DISCUSSION

Bone and joints infection is painful for patient and causes a condition of disappointment for both patient and physician (Prabhakar *et al*., 2000; Ziran, 2007). Owing to the fact that main causative agent of osteomyelitis and their antibiotic sensitivity are highly various in different parts of the world, there is no appropriate antibiotic treatment nowadays for it (Mader *et al*., 1997; Mandracchia *et al*., 2004).

No study about etiologic agents of osteomyelitis has been done in Iran and it seems that this one is the first. In this study *S. aureus* was isolated from 55.9% of patients and *Klebsiella* sp., *Pneumococcus* and *Enterobacter* sp. were the next. Also, in this study one *Acinetobacter* sp. positive-sample was isolated. Previous studies have revealed that *Staphylococci*, *Streptococci*, *Enterobacteriaceae* and anaerobes are main causative agents of osteomyelitis. In one study in 2003, in USA, *S. aureus* was the main causative agent of osteomyelitis and *Klebsiella* sp., *Proteus* sp., *Enterobacter*, *E. coli*, *Streptococci viridans*, *Pseudomonas aeruginosa* and *Haemophilus influenza* were next agents, respectively (Brady *et al*., 2006).

In next study in USA, Sixty-one cases of bacterial vertebral osteomyelitis were analyzed. Gram-negative rods were the predominant bacteria isolated (Patzakis *et al*., 1991).

In the study, determine Salmonellae and *S. aureus* accounted for 37.9 and 62.1%; 64 and 4.9% in isolates from the Middle East and Europe, respectively (Thanni, 2006).

In one thy study, 60% of *S. aureus* was sensitive to Vancomycin, 66.7% to Cephoxitin, 60% to gentamicin and Cephalotin and 73.3% to Clavulanic acid and had a very low sensitivity to Penicillin, Ampicillin and Cotrimoxasol. Also, *Enterobacteriaceae* causing osteomyelitis was often sensitive to Carboxicillin, Amikacin, Cephoxitin and kanamycin and had no sensitivity to Amoxicillin, Cephalotin and Penicillin.

As mentioned, causative agents and their sensitivity to antibiotics are not the same in different parts of the world (Korakaki *et al*., 2007). In one study in USA, 53.1% of *S. aureus* was sensitive to Oxacillin, 52.2% to Ceftriaxon, 55.1% to Cefotaxime, 50.1% to Ciprofloxacin and 100% to Vancomycin. Also, 93.9% of *Streptococci viridans* were sensitive to Ceftriaxon, 94.4% to Cefotaxime, 94.6% to Lomefloxacin and 100% to Linezolid and Vancomycin. Most of *Enterobacteriaceae* (*Klebsiella* sp., *Enterobacter* sp. and *E. coli*) were sensitive to Ceftriaxon and Cefepim (Sahm *et al*., 1999).

## CONCLUSION

As indicated in this study, the causative agents of osteomyelitis are various and consist of gram-positive cocci (*Staphylococci* and *Streptococci*) and gram negative bacilli (*Enterobacteriaceae* and *Acinetobacter*). Besides, their antibiotic resistances are different and varying persistently. Because of definite and effective treatment necessity for osteomyelitis and preventing waste of time and cost, annual studies should be done in each country to update information about causative agents, antibiotic sensitivity and resistance and should be presented to physicians for treatment. With regard to the results of this study, it is recommended that physicians consider the fact that osteomyelitis is a multi-factorial disease, the most important of which are *Staphylococci* and since these bacteria are resistant to the most of antibiotics, treatment process should begin with antibiogram procedure. In addition, to prevent the increment of antibiotic resistance, antibiotic administration should not be done without definite diagnosis and necessity of treatment.

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