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Posttraumatic Bacterial Infections in Extremities before and after Osteosynthesis in Dogs

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ABSTRACT

***Background*:** Posttraumatic osteomyelitis is one of the most serious complications after fracture treatment. Although haematogenous osteomyelitis is fairly common in man, it is rarely seen in small animal orthopedic surgery. Predisposing factors to osteomyelitis include bacterial contamination in combination with severe trauma, surgical intervention, the presence of dead bone, or metallic implants, especially if bone or implants are unstable. Posttraumatic osteomyelitis is nearly always infectious in etiology, caused by various pathogens. The purpose of this present study is to find out the type of aerobic bacteria, which plays an important role in the posttraumatic infection before and after fracture osteosynthesis.
***Materials, Methods & Results*:** Our study was performed on 15 patients with long bone fractures operated at the Department of Surgery, Small Animal Clinic, University of Adnan Menderes, Aydin. The patients without an antibiotic treatment 24 h prior to surgery were included in the study. Our study was performed on 15 patients who had undergone osteosynthesis in extremities at Department of Surgery, Small Animal Clinic, University of Adnan Menderes, Aydin. The patients without antimicrobial treatment 24 h before surgery were included in our study. The osteosynthesis procedures were performed by two experienced orthopaedists. Theywere returned to our clinic at 4-8 weeks after operation for follow-up orthopaedic and radiographic examinations. The next follow-ups were carried out at 4-6-week intervals, if needed. The implants for internal fracture were removed after clinical and radiological bone healing. All criteria were analyzed by using $\chi 2$ test and Fischer's exact test with $P < 0.05$ and $P < 0.01$ to evaluate the incidence bone infection in each group of patients. A high percentage of the bacterial isolates was coagulase-negative staphylococci resistent to cephalosporins. the highest sensitivity of all isolates (coagulase-negative *Stapylococcus*, *Staphylococcus aureus*, *Bacillus* spp., *Corynebacterium* spp., *Serratia liquefaciens*, *Alcaligenes faecalis* and *Escherichia coli*) was observed to ceftriaxone (100 %), as well as to danofloxacin (80.9%) and amoxicillin/clavulanic acid (57.1%). The highest resistant isolates was determined against to penicillin (66.6%) and ampicillin/sulbactam (57.1%). Although we use cephalosporins prophylactically in all patients undergoing orthopaedic surgery in our clinic, the incidence of postoperative infections was very low.
***Discussion*:** As the surgical site infection etiology is multi-factorial, the sterilization, preoperative patient preparation and operating theatre environments must be considered as the main sources of this bacterial contamination. Antiseptics used in our practice were theoretically supposed to be effective. Since povidone-iodine and chlorhexidine have a broad spectrum of antimicrobial activity against vegetative bacteria, fungi, viruses, protozoa, and yeasts, they are suitable for removing bacteria from the skin surface at preoperative surgical site. Additionally, povidone-iodine surgical scrub is also used extensively in veterinary practice for the pre-operative preparation of patients and surgeons. Therefore, it is obvious that the use of cephalosporins as preoperative prophylaxis is sufficient in most cases. In the patients at high risk of osteomyelitis development (polytraumatized or immunosuppressed patients, patients receiving prosthetic joint or large metallic implants), or in the patients where the infection is already present, it is important to know hospital-specific pathogens to select adequate complementary antibiotics (in this case we use cephalosporines together with quinolones such as danofloxacin). This emphasizes the need of epidemiologic studies, specific for each clinic.

Keywords: bacteria, osteomyelitis, osteosynthesis, dog, nosocomial infection, posttraumatic infections.

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INTRODUCTION

Osteomyelitis is defined as an inflammation of the bone, cortex and periosteum [30,33,41,45]. Although haematogenous osteomyelitis is fairly common in man, it is rarely seen in small animal orthopedic surgery. Predisposing factors to osteomyelitis include bacterial contamination in combination with severe trauma, surgical intervention, the presence of dead bone, or metallic implants, especially if bone or implants are unstable [18]. Posttraumatic osteomyelitis is nearly always infectious in etiology, caused by various pathogens. The typical causative agents of this infection are aerobic bacteria especially staphylococcal species [30,34,42]. Even though staphylococci cause about 50-60% of bone infections in dogs [33], many organisms such as pyogenic streptococci [6], gram-negative aerobic bacteria (eg. *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp.) [21], and coagulase-negative staphylococci [1,36,41] are commonly found. Although aerobic bacteria are commonly responsible for these causative posttraumatic infections, some uncommon microorganisms such as *Brucella canis* [38], *Mycobacterium avium* [9] can be found. Furthermore, anaerobic bacteria such as *Peptostreptococcus anaerobius*, *Propionibacterium* spp., *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Bacteroides* spp., *Clostridium villosum* were isolated. It is also reported that *Wolinela recta*, *Leishmania donovani* [24,25], *Blastomyces dermatitidis* [31], *Scedosporium prolificans* [43] might cause osteomyelitis in humans and domestic animals [23,32] but their incidences are rare.

The purpose of this present study is to find out the type of aerobic bacteria, which plays an important role in the posttraumatic infection before and after fracture osteosynthesis.

MATERIALS AND METHODS

Our study was performed on 15 patients who had undergone osteosynthesis in extremities at Department of Surgery, Small Animal Clinic, University of Adnan Menderes, Aydin. The patients without antibiotic treatment 24 h before surgery wereincluded in our study. The osteosynthesis procedures were performed by two experienced orthopaedists. They were returned to our clinic at 4-8 weeks after operation for follow-up orthopaedic and radiographic examinations.

The next follow-ups were carried out at 4-6-week intervals, if needed. The implants for internal fracture were removed after clinical and radiological bone healing.

All data of the patients, including breed, age, sex, type of fracture, methods of internal fixation, interval between the time of injury and surgery, type of bone healing, type of anaesthesia, radiographic finding were recorded and evaluated. Routine preoperative patient preparation and disinfectants used were theoretically evaluated. Preoperative surgical site preparations were performed starting with hair clipping with an electric clipper, and then 3-5 min povidone-iodine scrubbing, and chlorhexidine spraying were applied to the preoperative operation site. All surgical instruments were sterilized with autoclave (132-135 C; 10-25 min). Povidone-iodineand alcohol were used for hand-scrubs of surgical team.

This study was divided into two parts; bacterial culture was performed before osteosynthesis and when the internal fixations were removed. In the first part, 1 gram of tissue and/or bone fragment at the fracture site was collected. Furthermore, another sample was taken by means of sterile cotton sponge, which was saturated with blood. Both types of sample were collected from 15 patients when the fractured bone was approached to perform internal fixation. Samples were collected in Amies transport medium (CM 425)[1], and or in modified Cary-Blair medium (CM 519)[1]. Toperform bacterial cultivation, samples were inoculated onto Blood agar base (CM 854)[1] and MacConkey's agar (CM 7b)[1], and also into TH broth in order to enrich bacterial cells. The inoculated media were incubated at 37°C for 1-3 days. The bacteria found in each sample were identified and tested for antibiotic sensitivity by means of discs diffusion method according NCCLS.

In the latter part, one gram of tissue and internal fixations were obtained from each of 15 patients when the internal fixations were removed. One gram of tissue at the fracture site was transferred into Amies transport medium $(CM\ 425)_1$, and internal fixation devices were put into Cary-Blair medium (CM 519, Oxoid) as the transport medium. The bacterial cultivation was performed in the same procedure as in the first part.

Since coagulase-negative staphylococci (CoNS) strains were frequently isolated, we separately evaluated the incidence of CoNS infection in each group of patients in order to properly determine the incidence of bacterial bone infection. Ceftriaxone

sodium (20 mg/kg)[2] was intravenously administered to all patients as soon as the sample was collected.

Antimicrobial Susceptibility Testing

The behaviour of isolates to antimicrobial drugs was tested by the disk diffusion method and the results were interpreted according CLSI recommendations (National Committee for Clinical Laboratory Standards, 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. M31-A2. National Commmittee for Clinical and Laboratory Standards, Wayne, PA).

Statistical analysis

All criteria were analyzed by using $\chi 2$ test and Fischer's exact test with $P < 0.05$ and $P < 0.01$ to evaluate the incidence bone infection in each group of patients.

RESULTS

The most frequently fractured bones were the femur (60% of long bone fractures), followed by the tibia (26.66%), and the humerus (13.33%). Once each fracture was categorized by localization and type of fracture line, the diaphyseal complex (55.2%), diaphyseal transverse (24.7%), metaphyseal and/or physeal and/or epiphyseal extraarticular (10.7%), and diaphyseal oblique or spiral (10.3%) fractures were found. The positive bacterial findings were frequently detected inthe patients with femoral (64.23%) and tibial (50%) fractures.

Bacterial culture before implantation

The negative and positive results of bacterial culture were found in 8 patients (53.3%) and in 7 patients (46.6%), respectively. Among the patients with the isolation of bacteria, there was mixed bacterial infection in 3 patient and single bacterial infections in 4 patient. In all patients bacteria can be isolated via enrichment in broth media. Bacterial species found in the patients before were CoNS (50%), *Staphylococcus aureus* (10%), *Bacillus* spp. (10%), *Corynebacterium* spp. (10%), *Serratia liquefaciens* (10%) and *Escherichia coli* (10%).

Bacterial culture after metallic implantation

The negative results of bacterial culture were found only in 7 patients (46.6%) whereas the positive results were found in 8 patients (53.3%) after implantation. The incidence of bacterial infection after implantation was higher than that before implantation. Among the patients with culture of bacteria, there was mixed bacterial infectionin 2 patients (25 %) and single bacterial species in 6 patients (75%). The incidences of mixed and single bacterial infection before and after implantation were not different. As a whole, bacteria found in the patients before and after implantations were almost in thesame species or genus and incidences including CoNS (40 %), *Staphylococcus aureus* (40%), *Pseudomonas* spp. (10%) and *Alcaligenes faecalis* (10%).

Antimicrobial susceptibility

As per Table 1, the highest sensitivity of all isolates (CoNS, *Staphylococcus aureus*, *Bacillus* spp., *Corynebacterium* spp., *Serratia liquefaciens*, *Alcaligenes faecalis* and *Escherichia coli*) was observed to ceftriaxone (100 %), as well as to danofloxacin (80.9%) and amoxicillin/clavulanic acid (57.1%). The highest resistant isolates was determined against to penicillin (66.6%) and ampicillin/sulbactam (57.1%).

DISCUSSION

In this study, incidence rate of type and localization of long bone fractures were different as in the previous report [11,40]. It was found the most frequently fractured bones were the femur (60% of long bone fractures), followed by the tibia (26.66%), and the humerus (13.33%) whereas the previous report [11] showed that the radius and ulna were the most commonly fractured. However, our number of patients is lower than that reported in the previous study. In addition, our study excluded the patients with previous antibiotic treatment. Furthermore, we also found similar to the previous reports that the incidence of bone infection is high in tibia fractures. Due to less dense muscle covering the bone at these sites, it was found that infection always occurred in the tibia and the radius [4,5,7,8,15,25].

Considering the surgical site infection etiology as multi-factorial, the sterilization, preoperative patient preparation and operating theatre environments must be considered as the main sources of this bacterial contamination. Antiseptics used in our practice were theoretically supposed to be effective. Since povidone--iodine and chlorhexidine have a broad spectrum of antimicrobial activity against vegetative bacteria, fungi, viruses, protozoa, and yeasts, they are suitable

Table 1. Results of antimicrobial susceptibility obtained from dogs before and after osteosyntesis.

Isolates	Positive Samples	Antimicrobials					
		P	AMC	SAM	DFX	CRO	GN
Staphylococcus aureus	1D	R	R	R	S	I	R
	4A	R	S	R	R	S	S
	4C	I	S	I	I	I	S
	5C	S	S	S	I	I	R
	10D	R	S	R	S	S	S
Coagulase- negative staphylococci	2A	R	S	I	S	S	S
	2B	R	S	S	I	I	S
	2C	R	S	S	S	S	S
	3A	R	R	R	I	S	S
	6B	S	S	I	I	S	S
	6D	I	S	I	S	S	I
	7B	S	S	S	S	S	I
	9D	R	R	R	R	I	S
	10B	R	R	R	I	S	S
	10C	R	R	R	I	S	S
Escherichia coli	4B	R	R	R	R	I	R
Pseudomonas spp.	5D	I	S	S	I	S	I
Corynebacterium spp.	3B	S	S	R	I	I	S
Serratia liquefaciens	5B1	R	R	R	R	I	R
Bacillus spp.	5B2	R	R	R	I	I	S
Alcaligenes faecalis	3D	R	R	R	S	I	S

P-(Penicillin); AMC-(Amoxicillin/clavulanic acid); SAM-(Ampicillin/Sulbactam); DFX-(Danofloxacin); CRO-(Ceftriaxone), GN-(Gentamicin). S: Sensitive; I: Intermediate; R: Resistant. A-before osteosyntesis (cotton sponge); B-before osteosyntesis (1 gr tissue of fracture); C-after osteosyntesis (cotton sponge); D-after pin extirpation.

for removing bacteria from the skin surface at preoperative surgical site [27]. Additionally, povidone-iodine surgical scrub is also used extensively in veterinary practice for the pre-operative preparation of patients and surgeons [2].

The microbiological cultivation methods used in our present study were supposed to be effective in identifying aerobic bacteria [13,35]. Amies and Carry Blair transport mediums were used as transport mediums in our study. Both are acceptable as effective transport mediums that can prolong the survival of both aerobic and anaerobic bacteria such as *Staphylococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Enterococcus* spp., *Peptostreptococcus* spp., *Prevotella* spp., *Streptococcus* spp., *Haemophilus* spp., *Neisseria* spp., *Fusobacterium* spp. etc. [13,35]. Bacterial cultivation was performed by inoculation samples onto the blood agar medium and MacConkey's agar (solid media). The enrichment in broth media was used to detect only few bacterial cells in the sample. The types of bacterial species found in our study were in agreement with previous reports [4-6,8-10,13,14,17,20,28,39]. Since CoNS were frequently found in our study, we assumed that it was the main nosocomialpathogen in our operating theatre. Furthermore, this species was also classified as the main nosocomial pathogen in humans [29,41,44]. The mixed bacteria identified from the samples before and after implantation werein the same incidence. However, in the present study, multimicrobial bone infections were commonly found in the patients with open fractures and always contained mixtures of staphylococci as well as gram-negative aerobic rods such as *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp. [21].

Furthermore, according to our investigation, CoNS were frequently found in the sample of the patients with closed fractures before and after implantation. Thus, it was concluded that CoNS might be the main nosocomial pathogen in our operating theatre.

Other factors, which can promote cryptic infection of the bone, are the degree of soft tissue damage, type of internal fixation, and interval from the time of injury to the time oftreatment, avascular bone fragment, and duration of surgery [34]. Soft tissue

trauma and fracture fixation using metallic implants may produce structural and functional damage to the local host tissue causing devascularization, malperfusion, disturbance of endothelial permeability, hypoxia, acidosis, haematoma, edema, and increased intracompartmental pressure [19,47]. This may result in an impaired humoral and cellular immune competence [22]. On a local level it may decrease resistance to the pathogenic microbiological load with subsequent manifestation of infection in the traumatized tissue [3,22,34]. Nevertheless, numerous clinical investigations have shown that operative treatment of closed fractures with severe soft tissue injury is associated with a higher risk of infection than that without severe soft tissue injury [26,37]. These investigations indicate that even without major bacterial contamination, soft tissue damage and its pathophysiological consequences act as a catalyst for infection since they reduce resistance to infection.

Given that the overall infection rate for clean, elective surgical procedures in the present study was in line with values reported for human patients [42], it is unlikely that an abnormally high bacterial burden was a problem in our hospital. Operating room protocols and surgical sterility appeared to be adequate. However, compared with previous reports, the high percentage of CoNS isolates was an unusual finding. Therefore, a change may be warranted in the choice of antimicrobials used in our hospital in patients in which infections are suspected but results of bacterial culture and susceptibility testing are not yet known. Knowledge of the commonly isolated organisms and their antimicrobial susceptibility patterns within a given hospital assists in the selection of appropriate antimicrobial treatment.

According to our results, it is recommended to administer the first generation of cephalosporins during preoperative operation in every patient [12,30,46]. In patients with a high risk of infection, such as polytraumatized or immunosuppressed individuals, the patients who have undergone prosthetic joint or large metallic implants, the quinolone group such as danofloxacin should be added and postoperative care should be performed intensively to prevent posttraumatic osteomyelitis [16].

SOURCES AND MANUFACTURERS

[1]Oxoid, Hemakim, Turkey.

[2]Novazef, 1 g.,Eczacibasi, Turkey.

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