Case Report

NOSOCOMIAL KLEBSIELLA OXYTOCA INFECTION IN TWO DOGS

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Summary: Despite a large amount of published data on nosocomially-acquired intravenous catheter-related Klebsiella oxytoca infection in human medicine there is a lack of information in veterinary medicine. Intravenous catheter-related Klebsiella oxytoca infection was strongly suspected in two dogs that underwent dental procedures under general anesthesia. Dog 1 was presented with severe osteomyelitis of the shoulder joint from the same leg that was used for intravenous catheter placement during implantation of bilateral direct acrylic inclined plane approximately 3 weeks ago. According to the bacteriology identification that revealed Klebsiella oxytoca infection and susceptibility testing of the synovial fluid from the shoulder joint the dog was treated with amoxicillin+clavulanic acid for one month. In spite of clinical improvement the radiographic examination revealed severe osteoarthritis of the affected joint at the end of the course. Dog 2 died of septic shock associated with disseminated intravascular coagulation 7 hours after the extraction of left mandibular fourth premolar tooth. Klebsiella oxytoca was isolated from several abdominal organs post-mortem. Bacteriology examination of hospital environment and equipment was carried out because of possible common-source epidemic and among other microorganisms, Klebsiella oxytoca was isolated. Additional infection control measures were instituted and so far, eighteen-months after that no nosocomial infection was confirmed.

Key words: cross infection – microbiology; infection control; Klebsiella infections – etiology – microbiology; postoperative complications; dogs

Introduction

Nosocomial infections are infections that may be derived from endogenous flora of the patients or from exogenous microorganisms acquired by patients during their stay in the hospital (1, 2). In the latter case, the infectious agent is often resistant to multiple antibiotics and may be transmitted to or between several patients, thus leading to clusters or outbreaks of nosocomial infections due to the same strain (3). A limited number of small outbreaks is documented in companion animals due to Klebsiella sp. (4), Serratia marcescens (5), Enterobacter cloace (6), Escherichia coli (7, 8), Enterococcus faecium (2), Clostridium difficile (9, 10), Clostridium perfringens (11), Acinetobacter baumannii (2, 12) and Pseudomonas aeruginosa (13). In human medicine, there are several documented outbreaks of catheter or contaminated disinfectant-associated infections with Klebsiella oxytoca (14, 15, 16, 17, 18, 19). The data on catheter associated Klebsiella oxytoca infection in veterinary medicine are sparse (4, 20).

Bacteria from genus Klebsiella are non-motile, rod-shaped, Gram-negative aerobic bacteria that possess a prominent polysaccharide capsule. Some of these bacteria produce an extracellular toxic complex that has been shown to be lethal for and produce extensive lung pathology in mice. It is composed of capsular polysaccharide, lipopolysaccharide, and protein; when introduced to experimental animals in sublethal doses, the animals built up immunization due to antibody production (21). Klebsiella oxytoca and Klebsiella pneumoniae are both opportunistic pathogens found in the environment and in mammalian mucosal surfaces; they are usually passed by the hands of hospital personnel.
**siella oxytoca** and **Klebsiella pneumoniae** are common isolates in clinical microbiology and important producers of extended spectre beta-lactamases (ESBL). Enterobacteriaceae with beta-lactam resistance due to the production of ESBL were discovered in the eighties and since that time became epidemic and endemic in hospitals worldwide (22).

The purpose of this report is to describe nosocomial **Klebsiella oxytoca** infection in two dogs that underwent dental procedures at Clinic for Small Animal Medicine and Surgery, Veterinary Faculty, University of Ljubljana (CSAMS-VFLJ).

**Case 1**

Dog 1, a 7-months-old intact male Airedale terrier, considered healthy after physical examination, weighing 19.5 kg was presented to CSAMS-VFLJ for orthodontic movement of right mandibular canine tooth. Bilateral direct acrylic inclined plane was implanted under general anaesthesia. The dog was premedicated with medetomidine (Domitor, Pfizer, Karlsruhe, Germany) 0.018 mg/kg i/m. Intravenous catheter was placed into cephalic vein 15 minutes later after standard aseptic procedure including clipping the hair with the clipper and disinfection of the skin with a mixture of 2-propanol and benzalkonium chloride (Cutasept, Beiersdorf GsmbH, Wien, Austria). The dog was induced to general anaesthesia with propofol (Propofol 1%, Fresenius Kabi, Graz, Austria) 1.5 mg/kg i/v, endotracheally intubated and maintained with isoflurane (Forane, Abbott, Queenborough, UK) in 100% oxygen for 20 minutes. Atipamezole (Antisedan, Pfizer, Karlsruhe, Germany) 0.038 mg/kg i/m was administered at the end of the procedure to antagonize the effects of medetomidine. During general anaesthesia, Lactated Ringer's solution (B Braun, Melsungen, Germany) was administered at 10 ml/kg/h. One hour and half after atipamezole administration, metoclopramide (Reglan, Alkaloid, Skopje, Macedonia) 0.4 mg/kg s/c was given to treat postoperative nausea. The dog was discharged to a home care few hours later.

The dog was brought to local veterinary practitioner because of lameness of right front leg that was used for intravenous catheter placement the next day. Intense pain was observed at palpation of the skin with a mixture of 2-propanol and benzalkonium chloride (Cutasept, Beiersdorf GsmbH, Wien, Austria). The dog was induced to general anaesthesia with propofol (Propofol 1%, Fresenius Kabi, Graz, Austria) 1.5 mg/kg i/v, endotracheally intubated and maintained with isoflurane (Forane, Abbott, Queenborough, UK) in 100% oxygen for 20 minutes. Atipamezole (Antisedan, Pfizer, Karlsruhe, Germany) 0.038 mg/kg i/m was administered at the end of the procedure to antagonize the effects of medetomidine. During general anaesthesia, Lactated Ringer’s solution (B Braun, Melsungen, Germany) was administered at 10 ml/kg/h. One hour and half after atipamezole administration, metoclopramide (Reglan, Alkaloid, Skopje, Macedonia) 0.4 mg/kg s/c was given to treat postoperative nausea. The dog was discharged to a home care few hours later.

The day after, the dog was presented again with intense pain of right shoulder joint, painful hind part of the body and stiffed gait. The dog was reluctant to walk. According to veterinary practitioner, radiographic examinations of right shoulder joint and hip joints were normal as well as neurological examination. Routine haematology profile was within reference range. The dog was tested for dirofilariosis, boleiosis and ehrlichiosis with commercially available ELISA kit (SNAP 3Dx, Canine Heartworm Antigen/Borrelia Burgdorferi/Ehrlichia Canis Antibody Test Kit, IDEXX Laboratories, Westbrook, Maine, USA), and the results were negative. Despite negative results, the dog was prescribed doxycycline (Clinofug D, Dr. August WOLFF GmbH&Co, Bielefeld, Germany) 10 mg/kg p/o q24h and carprofen 2 mg/kg p/o q12h. The dogs’ condition worsened in the course of therapy, and a week later the dog was referred to CSAMS-VFLJ.

The dog was presented to CSAMS-VFLJ with intense pain of the whole right front leg. Radiographic examination revealed osteomyelitis of the right humeral head. Definitive pattern of lysis and solid pattern of periosteal new bone were seen on the humeral head (Figure 1). Synovial fluid from right shoulder joint was taken and sent to bacteriology identification and susceptibility testing to commonly used antimicrobial agents. Bacteriology examinations were performed in Columbia agar supplemented with 5% of ovine blood. **Klebsiella** microorganisms were identified on the basis of colony morphology, microscopic Gram stain characteristics, indole and oxidase activity. The final confirmation was done with API-20E® enteric identification system (bioMerieux, Marcy l’Etoile, France). The antibiotic susceptibility of isolated strains was determined by disc-diffusion method according to the NCCLS (National Committee on Clinical Laboratory Standards) guidelines (Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Second Edition, M31-A2, Vol. 22 No.6).
The following antimicrobials were assayed: azithromycin, amikacin, amoxicillin, amoxicillin/clavulanic acid, cephalaxin, cephalexin, ciprofloxacin, enrofloxacin, ceftriaxone, gentamicin, neomycin, piperacillin, metronidazole, trimethoprim/sulfamethoxazole and oxytetracycline. Initial beta-lactamase activity was detected by the hydrolysis of nitrocefin (cefinase). Screening tests for extended-spectrum-beta-lactamase activity were evaluated regarding their susceptibility to cefpodoxime, ceftazidime, cefotaxime and cefuroxime (BBL-Difco). Confirmation tests for ESBL were done by E-test (E-test, AB-Biodisk, Dalvägen, Solna, Sweden) for ceftaxime/cefotaxime+clavulanic acid (CT/CTL) and ceftazidime/ceftazidime+clavulanic acid (TZ/TZL).

Bacteriology identification revealed infection by *Klebsiella oxytoca*, beta-lactamase positive by nitrocefin testing, but ESBL negative (Table 1). According to the susceptibility testing, the dog was prescribed amoxicillin+clavulanic acid 20 mg/kg p/o q12h for one month and carprofen 2 mg/kg p/o q12h, the dose of latter to be gradually decreased by the owner according to the intensity of pain.

**Table 1:** Antimicrobial susceptibility profile from microorganisms isolated from dog 1 and dog 2

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<th>Dog 1</th>
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<tr>
<td></td>
<td>osteomyelitis</td>
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<td><em>Klebsiella oxytoca</em></td>
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<td>Susceptibility testing</td>
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<td>azithromycin</td>
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<td>amikacin</td>
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<td>amoxicillin</td>
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<td>cefuroxime</td>
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<td>beta-lactamase activity by nitrocefin testing</td>
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S, sensitive; I, intermediate sensitive; R, resistant; E-test ESBL CT/CTL, extended specter beta-lactamase, ceftaxime/cefotaxime+clavulanic acid; E-test ESBL TZ/TZL, extended specter beta-lactamase, ceftazidime/ceftazidime+clavulanic acid; +, positive; -, negative
Two weeks after the commencement of the therapy, the dog was much less in pain and able to use right front leg, although it was still lame. The dog was brought for a control check 6 weeks after the end of amoxicillin+clavulanic acid therapy. No lameness or pain was observed at the clinical examination and according to the owners it was doing well.

Case 2

Dog 2, a 2 years-old intact working male German shepherd weighing 30.6 kg, considered healthy after physical examination including pulse and respiratory rate and heart and lung auscultation, was brought to CSAMS-VFLJ for extraction of left mandibular fourth premolar tooth three weeks after the dog 1. Body temperature was not measured because of the dog’s uncooperativeness. The extraction was performed under general anaesthesia with isoflurane in 100 % oxygen after premedication with medetomidine 0.025 mg/kg i/m and butorphanol (Torbugesic, Fort Dodge Animal Health, Iowa) 0.1 mg/kg i/m and induction to anaesthesia with propofol at a dose of 2 mg/kg i/v. Intravenous catheter placement procedure was the same as in the dog 1. The dog was given perioperatively carprofen 4 mg/kg i/v and Lactated Ringer’s solution at 10 ml/kg/h. At the end of the procedure, medetomidine was reversed with atipamezole 0.05 mg/kg i/m. Single dose of methadone (Heptanon, Pliva, Zagreb, Croatia) 0.4 mg/kg s/c was administered for postoperative analgesia. Panting that was observed soon thereafter was thought to be a consequence of methadone administration or relatively warm recovery room (23 to 24°C). The dog’s body temperature was not measured because of its aggressive temper. Two hours later, the dog was discharged to a home care with carprofen 2 mg/kg p/o q12h to be given following three days. At that time, it was alert but still mildly ataxic and weak.

Later in the afternoon, the dog’s caretaker noticed that the dog is apathetic and sleepy, and it refused water and food. Its pulse was thready. The dog was given additional dose of atipamezole (dose not known) by staff veterinarian and sent back to CSAMS-VFLJ. On the admission (7 hours after the end of procedure) the dog was in cardiopulmonary arrest. Cardiopulmonary resuscitation (CPR) was performed with no success. During CPR, considerable amount of smelly dark reddish intestinal contents were released.

Post-mortem examination carried out at Institute for Pathology, Forensic and Administrative Veterinary Medicine, Veterinary Faculty, University of Ljubljana revealed severely congested kidneys, liver and small intestinal wall. The small intestine was filled with hemorrhagic contents, while large intestine was empty. The spleen and lungs were congested as well. The subcutaneous blood vessels were congested with non-coagulated blood and the muscles were pale. Non-coagulated blood was found in right ventricle.

Figure 1: Lateral radiograph of the right humeral head 3 weeks after the intravenous catheter-related infection with Klebsiella oxytoca. Osteomyelitis with definitive pattern of lysis and solid pattern of periostal new bone is seen.

Figure 2: Lateral radiograph of the right humeral head 6 weeks after the end of amoxicillin+clavulanic acid therapy (20 m/kg p/o q 12h for one month) shows resolution of the lytic process. The shape of the humeral head is irregular and severe osteoarthritis of the shoulder joint is visible.

Radiographic examination of the right shoulder joint showed resolution of the lytic process. The shape of the humeral head was irregular and severe osteoarthritis of the shoulder joint was visible (Figure 2).
Histopathology showed disintegrated epithelium of small intestine. Small intestinal mucosal venous dilatation was accompanied with lymphocyte and macrophage infiltration in the lamina propria. Inflammatory cells, predominantly lymphocytes, were present in liver vessels. Liver sinusoids were congested and hepatocellular atrophy was observed in centrilobular zone. In both kidneys, hemorrhagic renal cortical necrosis with tubular casts was found. Reactive hyperplasia of spleen involved lymphoid tissue of the white pulp and macrophages of the red pulp. Several small colonies of bacteria were found in blood vessels and in parenchymatous tissue of the spleen. Severe congestion with multiple focal intraalveolar haemorrhages was found in bilateral lungs, and a small number of alveoli contained air. In the heart, prominent multiple epicardial haemorrhages and myocardial hyperaemia were observed. Abdominal organs were sent to bacteriological examinations and *Klebsiella oxytoca* was isolated abundantly from liver, kidneys and spleen. No bacteria were isolated from lungs. *Klebsiella oxytoca* and haemolytic *Escherichia coli* were isolated from small intestine while *Klebsiella oxytoca* and *Clostridium sporogenes* were isolated from the small amount of peritoneal fluid, the latter probably contaminating abdominal cavity post mortem. Initial tests of isolated bacteria regarding beta-lactamase activity by nitrocefin were strongly positive (Table 1). Screening tests for extended-specter-beta-lactamase (ESBL) were negative and confirmation tests with E-test were negative as well. The antibiotic susceptibility of isolated strains of *Klebsiella oxytoca* and *Escherichia coli* was determined as in dog 1 (Table 1).

The anaesthesia staff was concerned about a possible common-source epidemic of *Klebsiella oxytoca* infection after the second case occurred, and environmental surfaces and equipment in pre-/post-operative rooms (PPR), operating theatres and wards (tables, cages, incubator, clippers, muzzles, Doppler pulse detector and pulse oximetry probes, breathing circuits, endotracheal tubes, laryngoscope blades, dentistry equipment) as well as opened vials of propofol, acepromazine and carprofen and intravenous fluids were bacteriologically examined. beta-lactamase negative *Klebsiella oxytoca* (by nitrocefin testing) was isolated from PPR cages and *Staphylococcus intermedius, Enterococcus sp.*, and *Bacillus sp.* from muzzles. beta-lactamase positive *Klebsiella oxytoca* and beta-lactamase positive *Escherichia coli* (by nitrocefin testing) were isolated from tables, cages and clippers in wards.

Additional infection control measures for PPR, operating theatres and wards were instituted, including restrictions on the personnel entrance into PPR and operating theatres, strict disinfection of clippers, tables, cages and other equipment between the patients and regular disinfection of hands before handling another animal or before intravenous, arterial and urinary catheter placement. Particular care was taken at manipulation of catheters and disconnection of infusion lines. The use of single-use gloves was strongly encouraged when manipulating the animals. So far, 24-months after the additional infection control measures were instituted; no nosocomial infection was confirmed at CSAMS-VFLJ.

**Discussion**

90% of nosocomial bloodstream infections in human hospitals are related to intravenous catheter use (23). These infections can range in severity from localized phlebitis to fatal bacteraemia and sepsis, and are caused by contamination of the catheter either at the time of insertion or during use (24). Microorganisms may be introduced on the hands of the person placing or handling the catheter. They may be a part of the health care worker’s own skin flora, may be part of the patient’s skin flora acquired from a site that has not been disinfected, or may be faecal or other types of bacteria that the health care worker has handled (1).

There are few data in the veterinary literature documenting the incidence of catheter-related bloodstream infections in companion animals (2, 4, 5, 6, 7, 9, 10, 11, 12, 13). Most catheter-related infections involve organisms that are ubiquitous to the patient’s skin flora, nasopharynx or intestinal tract and organisms carried on the hands of hospital personnel (25). Surveillances study in a veterinary hospital reported 26% positive jugular catheter bacterial cultures after an average of 2.7 days. More than 50% of the organisms were *Klebsiella spp.* and *Enterobacter spp.* (1).

In the present report, the cages and clipper from wards were contaminated with beta-lactamase positive *Klebsiella oxytoca*, which was also isolated from both affected dogs. None of dogs entered the wards; therefore the only possible way of transmission could be by the hands of personnel rotating between PPR and wards or the clipper from the wards although the rotation of clipper between the wards and PPR is not a common practice at CSAMS-VFLJ. However, since the culturing of microorganisms from environmen-
tal surfaces and equipment was carried out 4 days after the dog 2 died, regular cleaning and disinfection procedures in a meantime might have an influence on the microbiology results and other sources of intravenous catheter contamination can not be excluded. While there is the possibility that the infection entered the bloodstream at the oral cavity during dental procedure in dog 2, it is highly unlikely in dog 1 because during the implantation of bilateral direct acrylic plane the integrity of gingival mucosa was not interrupted. However, the lack of catheter cultures makes it hard to be certain of the source of the infection.

Clinical presentation of Klebsiella spp. bacteraemia is indistinguishable from that of bacteraemia caused by other microorganisms. If bacteraemia develops, symptoms include fever or hypothermia, leukocytosis with left shift or neutropenia, and shock. The incidence of septic shock is remarkable; in a five-year study in human hospital 22% of the patients with documented bacteraemia due to Klebsiella spp. developed septic shock (26). In the present report, shock was overlooked in a dog 2, because the dog was discharged soon after the end of procedure due to his aggressive temper and uncooperativeness, and the early signs of impending shock such as panting and change in mental status were unfortunately misinterpreted.

The results of post-mortem examination of dog 2 indicate that the dog died because of septic shock associated with disseminated intravascular coagulation, and β-lactamase positive Klebsiella oxytoca was isolated from several abdominal organs, including small intestine. The authors can not affirm, whether Klebsiella oxytoca was introduced into the dog through intravenous catheter or during dental procedure or the dog was already the reservoir for Klebsiella oxytoca, which spread to extraintestinal sites through mesenteric lymph node complex as a result of bacterial translocation. Bacterial translocation in hosts with an intact intestinal barrier can occur by intracellular route, however the primary mechanism promoting bacterial translocation in these cases is bacterial overgrowth due to recent antimicrobial therapy (27), which was not the case in dog 2. The other mechanism, which promotes bacterial translocation is increased permeability of intestinal mucosal barrier, for example due to hemorrhagic or endotoxic shock, where indigenous bacteria translocate intercellulary (28). The various species of indigenous bacteria do not all translocate at the same rate. Gram-negative, facultative anaerobic Enterobacteriaceae, such as Escherichia coli, Klebsiella spp., and Proteus mirabilis, translocate from gastrointestinal tract at a greater rate than the other bacteria (27). In the present report, the authors doubt that uneventful short-term anaesthesia could provoke bacterial translocation and sepsis by itself in previously healthy dog.

The susceptibility profile of Klebsiella oxytoca isolated from dog 1 and dog 2 showed that the organism was not resistant to a broad spectrum of antibiotics including those commonly used in the hospital (amoxicillin+clavulanic acid, gentamicin, enrofloxacin). Dog 2’s antimicrobial sensitivity profile showed an intermediate resistance to cephalixin, where dog 1’s organism was sensitive. This might represent a developing and broadening resistance pattern as the organism has been presumably in a hospital setting for a longer period of time, but on the other hand cephalixin is not an antibiotic used on a regular basis in a hospital setting.

Amoxicillin+clavulanic acid to which Klebsiella oxytoca was sensitive, is used most often for prophylactic antibiotic treatment of surgical and dentistry patients at CSAMS-VFLJ. The lipopolysaccharide capsule of Klebsiella oxytoca that protects the organism from phagocytosis and aids in adherence (21) might be the cause for the severity and rapid development of the infection in dog 2.

In conclusion, the present report demonstrates the potential for nosocomial infection with Klebsiella oxytoca in small animal hospitals. Strict infection control measures, particularly regular disinfection of hands and use of disposable gloves should be strongly encouraged as the most important factors in preventing nosocomial infections.

References

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BOLNIŠNIČNA OKUŽBA DVEH PSOV Z BAKTERIJO KLEBSIELLA OXYTOCA

A. Seliškar, I. Zdovc, B. Zorko


Ključne besede: bolnišnična okužba – mikrobiologija; infekcija, nadzor; Klebsiella infekcije – etiologija - mikrobiologija; pooperacijske komplikacije; psi