Microbiological analyses of canine infected wounds

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SUMMARY

A total of 57 canine swab samples and wound discharge/aspirates collected during one year from complicated, post operative and infected wounds of traumatic origin have been microbiologically examined. *Staphylococcus intermedius* isolates were the most prevalent Gram positive bacterium evidenced (28.1%) in infected wounds, followed by *Enterobacteria* (26.3%) and especially *E. coli* (21.0%) and *Pasteurella multocida* (21.0%) among Gram negative bacteria. Obligate anaerobes, only represented by *Prevotella melaninogenica* were found in 14.0% of isolates. The other minor isolated germs were β-haemolytic *Streptococci* and actinomycetes among Gram positive ones and *Klebsiella pneumoniae, Proteus mirabilis, Citrobacter spp*. and *Pseudomonas aeruginosa* strains. For *Staphylococci*, the highest resistance percentage (15.0%) was observed against doxicycline and 10% of *Streptococci* were resistant to amoxicillin. Enterobacteria were frequently resistant to amoxicillin (48.2%) and to doxicycline (34.5%) and more scarcely to chloramphenicol (24.1%), whereas *Pasteurella* were sensitive to all tested antimicrobial drugs. All anaerobic isolates were sensitive to amoxicillin, tetracycline and metronidazole. These results emphasize the therapeutic interest of antibiotic combinations according to the bacteria variety of the wound infections in dogs.

Keywords: Dog, wound infections, *Staphylococcus, Streptococcus*, Enterobacteria, *Pasteurella multocida*, antibio-resistance.

RÉSUMÉ

Analyse microbiologique des plaies infectées chez le chien

Au total, 57 frottis et/ou écoulements provenant de plaies compliquées, post-opératoires et infectées d'origine traumatique chez le chien ont été analysés microbiologiquement. *Staphylococcus aureus* a été le germe Gram positif le plus fréquemment isolé (28,1 %) à partir des plaies infectées, suivi par les entérobactéries (26,3 %) et plus particulièrement *E. coli* (21,0 %) et par *Pasteurella multocida* (21,0 %) parmi les germes Gram négatif. Les germes strictement anaérobies, uniquement représentés par *Prevotella melaninogenica*, ont été isolés dans 14,0 % des cas. Les autres germes plus rarement isolés ont été des streptocoques β-hémolytiques et des actinomycètes parmi les Gram positifs ainsi que des souches Gram négatives de *Klebsiella pneumoniae, Proteus mirabilis, Citrobacter spp*. et *Pseudomonas aeruginosa*. Le pourcentage le plus élevé de résistance des staphylocoques a été obtenu envers la doxicycline (15,0 %) et 10 % des streptocoques étaient résistants à l'amoxicilline. Les entérobactéries ont fréquemment résisté à l'action de l'amoxicilline (48,2 %) et de la doxicycline (34,5 %) et plus rarement à celle du chloramphénicol (24,1 %) alors que les pasteurelles ont été sensibles à tous les antibiotiques testés. Tous les anaérobies ont également été sensibles à l'action de l'amoxicilline, de la tétracycline et du métronidazole. Ces résultats soulignent l'intérêt thérapeutique des combinaisons d'antibactériens en fonction de la variété des bactéries impliquées dans les plaies infectées chez le chien.

Mots clés : Chien, plaies infectées, *Staphylococcus, Streptococcus*, Entérobactéries, *Pasteurella multocida*, antibio-résistance.

Introduction

Specific skin infections could be discussed from the point of view of the aetiological agent, the extent of affected tissues (superficial or soft tissues) and their clinical expression [13]. On the other hand, they could be classified according to the epidemiologic status, pathogenesis and prognosis [3].

Complicated secondary skin infections in both humans and animals include acute wound infections, traumatic, bite wound and post operative infections [25]. A substantial part of skin wounds are colonized by aerobe and anaerobe microorganisms originating mainly from mucous coats, the buccal cavity and the gut. The composition and the variety of microbial agents involved in wound infection aetiology are influenced by numerous factors, such as localisation, depth of affected tissues, tissue perfusion quality and intensity and the strength of host antibacterial immune response. The bacterial microflora is better represented in cases of traumatic wounds with foreign bodies and necrotic tissues except in cases when its development is prevented by timely antibacterial therapy [26]. The risk of infection is mainly dependent on the sensitivity of surgical wounds to microbial contamination [23]. Surgical wound infections exhibit polymicrobial aetiology, involving both anaerobic and aerobic microflora [1, 8, 20, 27]. Acute soft tissues infections are commonly associated with *Staphylococcus aureus* [16]. Traumatic infections, especially those with necrotic tissues, are also assumed to be of polymicrobial (aerobic and anaerobic) origin [5]. Bite wound infections in animals, which are also of polymicrobial aetiology, also involve non spore-forming obligate anaerobes as *Peptostreptococcus spp., Bacteroides spp*. [10] apart the facultative anaerobe *S. aureus* and *P. multocida* agents.

Aetiotropic therapy frequently uses cephalosporins, macrolides and semi-synthetic penicillins by reason of the participation of *Staphylococcus aureus* in the aetiology of problematic traumatic wounds [9]. The determination of sensitivity of wound bacterial isolates to antimicrobial drugs should take into consideration the fact that laboratory data are not always corresponding to therapeutic outcome because of the effects of some environmental factors such as environmental pH and total microbial counts [28].

This study aimed to document the complex microbial population, and antimicrobial susceptibility as found in canine infected wounds.

Material and Methods

SAMPLES

Over one-year period (2009-2010), 57 canine swab samples and wound discharge/aspirates collected from complicated, post operative and infected wounds of traumatic origin have been examined in the Clinical Bacteriology Laboratory at the Faculty of Veterinary Medicine, Trakia University, Stara Zagora. Swabs were obtained by using maximum 2 sterile cotton swabs.

MICROBIOLOGICAL INVESTIGATIONS

Specimens were inoculated on trypticase-soy agar with 5-7% sheep blood (TSA II, BBL, USA), MacConkey agar (Difco, UK), Columbia agar (Difco, UK) supplemented with 5% sheep blood with vitamin K (5 mg/L, Sigma Chemicals, Bulgaria), thioglycolate broth (NCPID, Sofia), trypticase-soy broth (NCPID, Sofia).

Antibiograms were performed on Muller-Hinton agar (NCPID, Sofia). For the determination of MICs (Minimal Inhibitory Concentrations) of tested chemotherapeutics against anaerobe isolates, Brucella agar (Oxoid, USA) supplemented with 5 mg/L haemin, 1 mg/L vitamin K and 5% sheep blood was used per NCCLS [19]. For determination of MICs of antimicrobial drugs, bacterial culture inoculates with optical density 1.0 Mc Farland in Brain-Heart Infusion Broth (Oxoid, USA) were prepared as recommended per NCCLS [19].

Cultures were incubated at 37oC for 24-72 hours. The identification of bacteria was performed by conventional methods [22] and on a semi-automated identification system BBL Crystal (Becton Dickinson, USA). The sensitivity of isolates to chemotherapeutics was done by the disk diffusion method and results were interpreted as per CLSI [6] and NCCLS [19]. The intermediate isolates were included in the group of resistant ones (R+I). The following chemotherapeutic disks were used for antibiograms: Amoxicillin-25 μg (BULBIO, Sofia), Gentamicin-10 μg (BULBIO, Sofia), Ceftiofur-30 μg (Oxoid, UK), Erythromycin-15 μg (BULBIO, Sofia), Lincomycin-15 μg (BULBIO, Sofia), Chloramphenicol-30μg (BULBIO, Sofia), Doxicycline-30 μg (Oxoid, UK) and Enrofloxacin-5 μg (Bayer, Germany). MICs of amoxicillin, tetracycline and metronidazole (Sigma-Aldrich, Bulgaria) for anaerobes were determined by agar dilution method [19].

Results

Table I presents the species affiliation of isolates from canine infected wounds. Out of the 57 isolates, 20 were Gram-positive, 29 – Gram-negative and 8 – anaerobes. Among Gram-positive isolates, *Staphylococcus intermedius* isolates exhibited the highest prevalence (28.1%). Each of beta-haemolytic streptococci and actinomycetes had an incidence of 3.5%. Gram negative isolates from the *Enterobacteriaceae* family were 26.3%, 12 of them belonging to *E. coli*, and one to each *Klebsiella pneumoniae, Proteus mirabilis, Citrobacter* species. In addition, *Pasteurella multocida* was found in 12 (21.0%) isolates and *Pseudomonas aeruginosa* in 3.5% of samples. Obligate anaerobic pathogenic microflora was only represented by the species *Prevotella melaninogenica*.

As summarized in Table II, among gram positive bacteria, the highest resistance (15.0%) was exhibited by *Staphylococci* against doxicycline and 10.0% of *Streptococci* were resistant to amoxicillin. Whereas all *Staphylococci* isolated from infected canine wounds were sensible to gentamicin, erythromycin and lincomycin, 5% of them were resistant to amoxicillin, chloramphenicol or enrofloxacin. Resistance of *Streptococci* to gentamicin, erythromycin, lincomycin or doxicycline was also encountered in 5% of cases. Enterobacteria were frequently resistant to amoxicillin (48.2%), doxicycline (34.5%) and chloramphenicol (24.1%) while no resistance against any of tested antimicrobial drugs was evidenced in *Pasteurella multocida* and occasional resistance (6.8%) to amoxicillin and chloramphenicol was found in *Pseudomonas aeruginosa*. All anaerobic isolates were sensitive to amoxicillin, tetracycline and metronidazole.

	Number (frequency)	Species
Gram +	20 (35.1%)	*Staphylococcus intermedius*: 16 (28.1%) β-haemolytic *Streptococci*: 2 (3.5%) Actinomycetes: 2 (3.5%)
Gram -	29 (50.9%)	*Enterobacteriaceae*: 15 (26.3%) *Escherichia coli*: 12 (21.1%) *Klebsiella pneumoniae*: 1 (1.7%) *Proteus mirabilis*: 1 (1.7%) *Citrobacter* spp.: 1 (1.7%) *Pasteurella multocida*: 12 (21.0%) *Pseudomonas aeruginosa*: 2 (3.5%)
Obligate anaerobe	8 (14.0%)	*Prevotella melaninogenica*: 8 (14.0%)

TABLE I: Prevalence of bacteria species from canine infected wounds (n = 57).

The respective MICs are presented in Table III. For *P. melaninogenica* isolates, the MIC of amoxicillin, tetracycline and metronidazole varied from 0.06 µg/mL to 1.0 µg/mL, 0.250 µg/mL to 2 µg/mL, and from 0.03 µg/mL to 0.5 µg/mL, respectively.

Discussion

In the present study, the most prevalent bacteria isolated from canine wounds were *Staphylococcus intermedius, Enterobacteriaceae* and particularly *E. coli*, and *Pasteurella multocida* in 28.1%, 26.3% and 21.0% of cases, respectively. In agreement with that, DINUBILE *et al*. [7] have also reported Gram-positive cocci as the most prevalent agents in the aetiology of complicated skin infections and MEYERS *et al*. [15] have reported that *S. intermedius* was the most prevalent Gram-positive species (12%) isolated from infected and colonized bite wounds in dogs, followed by Gram-negative glucose non fermenting bacteria (16%) and *Pasteurella multocida* (15%). However, Enterobacteria isolates were more numerous (26.3%) in the present study compared to the incidence of 7% reported by MEYERS *et al*. [15]. Among Enterobacteria, these authors have identified *E. coli, Citrobacter* spp., *Proteus* spp., *Klebsiella* spp. and *Y. enterocolitica*. In wound discharges examined here, the composition of Enterobacteria was similar with a highest incidence for *E. coli* (12 strains), followed by *Klebsiella pneumoniae, Citrobacter* spp. and *Proteus mirabilis* and no Yersinia was evidenced. From obligate anaerobes, only *Prevotella melaninogenica* strains were isolated in the current study, whereas MEYERS *et al*. [15] observed also clostridia strains in wound discharges.

In a study on canine bite wounds, MOURO *et al*. [18] observed dominance of Gram-positive pathogens, *S. intermedius* (14.4%) as well as of *Streptococcus* spp. (16%) in wound infection aetiology. Among the Gram-negative microflora, the highest reported prevalence was that of *P. multocida* isolates (16.8%). The *E. coli* involved in the aetiology of canine wound infections (7.2%) taken separately from that of Enterobacteria isolates (9.6%) was also important. From the obligate pathogenic microflora, the authors found out a prevalence of 5.6%, with predominance of *Clostridium perfringens* (2.4%) and only 0.8% incidence of *Prevotella* spp. In the present investigation, the involvement of anaerobe isolates represented only by *Prevotella melaninogenica* was higher (14%).

Studying animal septic wounds, RAHMAN *et al.* [24] also demonstrated the dominating aetiological role of *S. aureus* (33%), followed by *E. coli* (18.9%), *Klebsiella pneumoniae* (16.7%), *Proteus vulgaris* (11.4), *Pseudomonas. aeruginosa* (9.6%) and *Streptococcus* spp. (9.2%). However, the lack of *Pasteurella* spp. and obligate anaerobes is interesting. The reported incidence of aforementioned microbial agents is, in general, similar to what was observed in the present study. First, this is the predominance of coagulase-positive *Staphylococci*, followed by the high percentage of colibacteria, isolated from animal wounds.

Considering the sensitivity of isolates to antimicrobial drugs, MEYERS *et al*. [15] demonstrated as most efficient potentiated sulphonamides (89%), followed by amoxicillin/clavulanic acid (85.4%). The highest resistance was established towards lincomycin (3.6% sensitive isolates), and gentamicin (43.6% sensitive isolates). Such results were observed in this

	A	G	E	L	D	C	Enr
Staphylococcus spp.	5%	0%	0%	0%	15%	5%	5%
Streptococcus spp.	10%	5%	5%	5%	5%	0%	0%
Enterobacteriaceae	48.2%	0%	-	-	34.5%	24.1%	0%
P. multocida	0%	0%	-	-	0%	0%	0%
P. aeruginosa	6.8%	0%	-	-	0%	6.8%	0%

A: amoxicillin; G: gentamicin; E: erythromycin; L: lincomycin; D: doxicycline; C: chloramphenicol; Enr: enrofloxacin.

TABLE II: Frequency of resistance (in %) against tested antimicrobial drugs (amoxicillin, gentamicin, erythromycin, lincomycin, doxicycline, chloramphenicol and enrofloxacin) among Gram positive (*Staphylococci* and *Streptococci*) and Gram negative (*Enterobacteria, Pasteurella multocida* and *Pseudomonas aeruginosa*) germs isolated from canine wounds.

Isolates with *Prevotella melaninogenica*	**Amoxicillin MIC**	**Tetracycline MIC**	**Metronidazole MIC**
1	0.50	0.25	0.03
2	0.50	0.50	0.03
3	1.00	0.50	0.03
4	0.25	0.50	0.06
5	0.12	0.25	0.50
6	0.12	2.00	0.10
7	0.06	0.50	0.06
8	0.12	0.50	0.03
Mean	0.33	0.63	0.11

TABLE III: Amoxicillin, tetracycline and metronidazole MICs (Minimal Inhibitory Concentrations) for *P. melaninogenica* isolated from 8 canine wounds.

study, but at lower percentages regarding the resistance of streptococci isolates to gentamicin, lincomycin and erythromycin (5%), and to amoxicillin (10%). In a comparative study on bacteria isolates from dogs and cats during two different time periods (1990-1992 and 2002-2003) AUTHEIR *et al*. [2] established 95% sensitivity of coagulase-positive staphylococci to enrofloxacin, 78% to tetracycline and erythromycin, and 33% to ampicillin. In the present research, the observed resistance to tetracycline was 15% and to amoxicillin – 5%. ERON *et al*. [9] and PERITI *et al.* [21] have discussed about the efficiency of antibacterial therapy in the cases of acute and chronic traumatic wounds in the context of the dominating role of *S. aureus*, and have recommended the use of cephalosporins, macrolides, clindamycin and semi-synthetic penicillins. With regard to the antimicrobial behaviour, the highest sensitivity observed by MOURO *et al.* [18] was against the combination amoxicillin/clavulanic acid (92.6%), followed by sulfonamide/trimethoprim (94.7%). Nevertheless, as many authors [4, 5, 8, 14, 16, 21] have emphasized the participation of anaerobes in aetiology of wound infections, GOLDSTEIN [11] underlined the need to combine broad-spectrum beta-lactams with metronidazole or clindamycin to treat non-surgical infections with anaerobic microbial aetiology. One of the problems encountered in the therapy of infections caused by *Prevotella* spp. is the resistance to amoxicillin, due to production of beta-lactamases, as well as the resistance to macrolides. In human medicine, this problem was mostly discussed with regard to periodontal infections. MOSKA *et al*. [17] reported a resistance of 2.73% to amoxicillin in Prevotella spp. isolates, which is related to high MIC_{90} values ≥ 64 µg/ml. IWAHARA *et al*. [12] communicated a higher resistance (31%) to beta-lactams in periodontal *Prevotella* spp. isolates.

In the study of RAHMAN *et al.* [24], the reported sensitivity of *Staphylococci* to amoxicillin was 40.2% and that of colibacteria – 41.8% whereas in the present study the resistance to this drug was quite lower for *Staphylococci* (5%) and higher for Enterobacteria (48.2%).These authors also reported a sensitivity of 41.5% to tetracycline for *Staphylococci*, and 39.5% for colibacteria which were largely lower than sensitivity obtained here (85% and 65.5%, respectively). In the same way, *Streptococci* resistance to amoxicillin reported by RAHMAN *et al*. [24] was higher (19.1%) than that observed here (10%) but the resistance to gentamicin was quite similar in the 2 studies (4.8% and 5%). By contrast, the reported resistance to chloramphenicol was 2.6% for *Staphylococci* and 7% for colibacteria, whereas in the present work the respective percentages were higher (5% for *Staphylococci* and 24.1% for colibacteria isolates).

The aetiotropic treatment of wound infections in dogs is the most appropriate therapeutic approach, but it is still not introduced as a clinical standard. That is why such investigations could be useful in providing information with regard to the adequate treatment of canine infections with efficient use of chemotherapeutics.

References

1. - ALDRIDGE K.E.: Anaerobes in polymicrobial surgical infections: incidence, pathogenicity and antimicrobial resistance. *Eur. J. Surg. Suppl.*, 1994, **573**, 31-37.
2. - AUTHIER S., PAQUETTE D., LABRECQUE O., MESSIER S.: Comparison of susceptibility to antimicrobials of bacterial isolates from companion animals in a veterinary diagnostic laboratory in Canada between 2 time points 10 years apart. *Can. Vet. J*., 2006, **47**, 774-778.
3. - BISNO A.L.: Cutaneous infections: microbiologic and epidemiologic considerations. *Am. J. Med.*, 1984, **76**, 172-179.
4. - BOWLER P.G., DUERDEN B.I., ARMSTRONG D.G.: Wound microbiology and associated approaches to wound management. *Clin. Microbiol. Rev.*, 2001, **14**, 244-269.
5. - BROOK I., FRAZIER E.H.: Aerobic and anaerobic microbiology of infection after trauma. *Am. J. Emerg. Med.*, 1998, **16**, 585- 591.
6. - CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI): Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard, 3rd edition, National Committee for Clinical Laboratory Standards, Wayne, PA, 2006, M31-A3.
7. - DINUBILE M.J., LIPSKY B.A.: Complicated infections of skin and skin structures: when the infection is more than skin deep. *J. Antimicrob. Chemother*., 2004, **53** (Suppl. S2), 37-50.
8. - DI ROSA R., DI ROSA E., PANICHI G.: Anaerobic bacteria in post-surgical infections: isolation rate and antimicrobial susceptibility. *J. Chemother.*, 1996, **8**, 91-95.
9. - ERON L.J.: Targeting lurking pathogens in acute traumatic and chronic wounds. *J. Emerg. Med*., 1999, **17**, 189-195.
10. - FLEISCHER G.R.: The management of bite wounds. *New. Engl. J. Med.*, 1999, **340**, 138-140.
11. - GOLDSTEIN E.J.C.: Selected non surgical anaerobic infections: therapeutic choices and the effective armamentarium. *Clin. Infect. Dis*., 1994, **18**, 273-279.
12. - IWAHARA K., KURIYAMA T., SHIMURA S., WILLIAMS D.W., YANAGISAWA M., NAKAGAWA K., KARASAWA T.: Detection of cfxA2, the β-lacatamase genes of Prevotella spp., in clinical samples from dentoalveolar infection by Real-time PCR. J. *Clin. Microbiol.*, 2006, **44**, 172-176.
13. - LEWIS R.T.: Soft tissue infections. *World J. Surg*., 1988, **22**, 146-151.
14. - MATHIEU D., NEVIERE R., LEFEBVRE-LEBLEU N., WATTEL F.: Anaerobic infections in soft tissues. *Ann. Chir.*, 1997, **51**, 272-287.
15. - MAYERS B., SCHOEMAN J.P., GODDARD A., PICARD J.: The bacteriology and antimicrobial susceptibility of infected and non- infected dog bite wounds: Fifty cases. *Vet. Microbiol*., 2008, **127**, 360-368.
16. - MEISLIN H.W., LERNER S.A., GRAVES M.H., MC GEHEE M.D., KOCKA F.E., MORELLO J.A., ROSEN P.: Cutaneous abscesses. Anaerobic and aerobic bacteriology and outpatient management. *Ann. Inter. Med*., 1977, **87**, 145-149.
17. - MOSCA A., MIRAGLIOTTA L., IODICE M., ABBINANTE A., MIRAGLIOTTA G.: Antimicrobial profiles of *Prevotella* spp., and *Fusobacterium nucleatum* isolated from periodontal infections in a selected area of southern Italy. *Int. J. Antimicrob. Agents*, 2007, **30**, 521-524.
18. - MOURO S., VILELA C.L., NIZA M.M.R.E.: Clinical and bacteriological assessment of dog-to-dog bite wounds. *Vet. Microbiol.*, 2010, **144**, 127-132.
19. - NATIONAL COMMITTEE FOR CLINICAL AND LABORATORY STANDARDS (NCCLS): National committee for clinical and laboratory standards and methods for antimicrobial susceptibility testing of anaerobic bacteria, Approved standard, 6th edition, NCCLS, Wayne, PA, 2004, Document M11-A6.
20. - NICHOLS R.L., SMITH J.W.: Anaerobes from surgical perspective. *Clin. Infect. Dis*., 1994, **18**, 280-286.
21. - PERITI P., TONELLI F., MINI E.: Selecting antibacterial agents for the control of surgical infection. *J. Chemother*., 1998, **10**, 83-90.
22. - QUINN P.J., CARTER M.E., MARKEY B.K., CARTER G.R.: Section: 2 Bacteriology. *In*: Clinical Veterinary Microbiology, Harcourt Publishers Limited, 1999, pp.: 118-254.
23. - RAAHAVE D., FRIIS-MOLLER A., BJERRE-JESPEN K., THIIS-KNUDSEN J., RASMUSSEN L.B.: The infective dose of aerobic and anaerobic bacteria in postoperative wound sepsis. *Arch. Surg.*, 1986, **121**, 924-929.
24. - RAHMAN M.M., BISWAS D.B., ISLAM M.M., ISLAM M.A.: Cultural sensitivity of septic wound in animals. *Pak. J. Biol. Sci*., 2003, **6**, 741-744.

25. - REVATHI G., PURI J., JAIN B.K.: Bacteriology of burns. *Burns*, 1998, **24**, 347-349.

26. - ROBSON M.C.: Wound infection, a failure of wound healing caused by an imbalance of bacteria. *Surg. Clin. North Am.*, 1997, **77**, 637-650.

27. - SANDERSON P.J., WREN M.W.D., BALDWIN A.W.F.: Anaerobic organisms in postoperative wounds. *J. Clin. Pathol.*, 1979, **32**, 143-147.

28. - WASHINGTON J.A.: The role of the microbiology laboratory in antimicrobial susceptibility testing. *Infect. Med*., 199, **16**, 531-532.