

In vitro antimicrobial resistance of staphylococci isolated from canine urinary tract infection

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Abstract – This study determined the diversity of species and antimicrobial resistance of staphylococci isolated from dogs with a presumptive diagnosis of urinary tract infection (UTI). Urine samples from 348 dogs with clinical signs of UTI, according to clinical examination and urinalysis, were processed for isolation of *Staphylococcus*. Colonies in pure culture were identified by biochemical reactions and tested for susceptibility to 15 antimicrobials. Seventy isolates of staphylococci were obtained (20.1%). *Staphylococcus pseudintermedius* was the most frequent species (32.8%), followed by *S. epidermidis* (18.6%), *S. simulans* (15.7%), *S. schleiferi schleiferi* (11.4%), *S. aureus* (11.4%), *S. schleiferi coagulans* (7.2%) and *S. saprophyticus* (2.9%). All the isolates were resistant to at least 1 drug and 77.1% were multiresistant. The study reports the alarming antimicrobial resistance of members of the *Staphylococcus* genus isolated from canine UTI and highlights the importance of coagulase-negative staphylococci in its etiology.

Résumé – **Résistance aux antimicrobiens de staphylocoques isolés dans une infection du tractus urinaire canin.** Cette étude a déterminé la diversité des espèces et la résistance aux antimicrobiens des staphylocoques isolés chez des chiens avec une présomption de diagnostic d'infection du tractus urinaire (ITU). Des prélèvements d'urine provenant de 348 chiens avec des signes cliniques d'ITU, selon l'examen clinique et l'analyse d'urine, ont été analysés pour l'isolement de *Staphylococcus*. Les colonies en culture pure ont été identifiées par des réactions biochimiques et testées pour la susceptibilité à 15 antimicrobiens. Soixante-dix isolats de staphylocoques ont été obtenus (20,1 %). *Staphylococcus pseudintermedius* était l'espèce la plus fréquente (32,8 %), suivie de *S. epidermidis* (18,6 %), de *S. simulans* (15,7 %), de *S. schleiferi schleiferi* (11,4 %), de *S. aureus* (11,4 %), de *S. schleiferi coagulans* (7,2 %) et de *S. saprophyticus* (2,9 %). Tous les isolats étaient résistants à au moins un médicament et 77,1 % étaient multirésistants. L'étude signale l'alarmante résistance aux antimicrobiens des membres du genre *Staphylococcus* isolés de l'ITU canine et souligne l'importance des staphylocoques à coagulase négative dans son étiologie.

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Introduction

Bacterial urinary tract infections (UTI) are a major clinical problem in dogs and are among the most common indications for antimicrobial therapy (1). A presumptive diagnosis of UTI can be made by the association of clinical signals and urinalysis, but urine culture and antimicrobial susceptibility

tests are required for a definitive diagnosis and an indication of the best choice of antimicrobial treatment. The bacteria most frequently involved in canine UTI include the gram-negative rods *Escherichia* sp., *Proteus* sp., *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp., and the gram-positive cocci *Streptococcus* sp. and *Staphylococcus* sp., particularly *S. aureus* and *S. pseudintermedius* (2).

Staphylococci, besides being commensals on mucosal surfaces and skin, are often implicated in a variety of infections, as otitis externa, abscesses, furuncles, pyoderma, pneumonia, and bacteremia (3). *S. pseudintermedius* (previously referred to as *S. intermedius*) is an important agent of canine UTI (4,5). The earlier *S. intermedius* species entity was useful because it separated these staphylococci from *S. aureus*, avoiding an epidemiologically important source of confusion (6). Since the recognition of *S. pseudintermedius*, strains from dogs identified by traditional means as the former *S. intermedius* are to be reported as *S. pseudintermedius*, unless shown by genomic investigation to belong

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to other related species (6). Since members of this genus have a high frequency of conjugation and frequently acquire plasmids that encode antimicrobial resistance (7), constant surveillance for resistance to antimicrobials is required.

The purpose of this study was to evaluate the diversity of species and the antimicrobial resistance of 70 isolates of staphylococci obtained from urine samples of 348 dogs with a presumptive diagnosis of UTI.

Materials and methods

Samples

Urine samples were obtained from 348 unmedicated adult dogs (aged > 1 y) of both sexes with a presumptive diagnosis of UTI from July 2006 to July 2007. Inclusion criteria included clinical signs of UTI, such as hematuria and dysuria; urinalysis results including red blood cell counts > 5 per field and proteinuria; and > 10³ colony-forming units (CFU) of bacteria per mL of urine at the first plating. Urine samples were obtained by catheterization after thorough cleansing of the genital area.

Bacteriological culture

Urine samples were refrigerated for no more than 1 h after collection and were cultured using calibrated loops to deliver 10 µL and 100 µL of samples onto plates with 5% sheep blood agar (Newprov, Curitiba, Paraná, Brazil) and agar count media (Oxoid, São Paulo, SP, Brazil). All plates were incubated at 37°C for 24 to 48 h. Methodology for isolation of anaerobic bacteria was not employed. If growth occurred after 24 or 48 h, colonies with morphology typical for gram-positive cocci were transferred to Tryptic Soy agar and Mannitol Salt agar (Merck, Rio de Janeiro, RJ, Brazil). Only samples that yielded *Staphylococcus* in pure culture at a concentration of > 10³ viable organisms per mL (1) were considered.

Isolates in pure culture were identified based on colony morphology, Gram stain, pigment production, hemolysis on 5% bovine blood agar, and biochemical reactions; namely catalase test, resistance to bacitracin (0.04 U), acid production in Hugh and Leifson's OF base medium, tube coagulase test, acetoin production, urease (Oxoid; Hampshire, United Kingdom), novobiocin resistance (Pimenta Abreu), deoxyribonuclease test (Becton, Dickinson, Sparks, Maryland, USA), ornithine and arginine dehydrogenase tests, and aerobic fermentation of sucrose, d-mannose, d-cellobiose, d-xylose, l-arabinose, raffinose, d-trehalose, maltose and d-mannitol. *Staphylococcus* species were classified according to the reference methods (8–10).

Antimicrobial susceptibility tests

All the isolates that were identified as staphylococci were tested for susceptibility to antimicrobial agents by the agar disc diffusion method on Mueller Hinton Agar (Himedia, Mumbai, India) incubated at 37°C (10). Six drug classes were included in this study, using commercial discs (Oxoid) containing the drugs. These classes represented the most frequently used antibiotics for cases of UTI in our community. Aminoglycosides were represented by gentamicin (10 µg) and amikacin (30 µg), while fluoroquinolones were represented by ciprofloxacin (5 µg),

Table 1. Resistance patterns of multiresistant staphylococci isolated from canine urinary tract infection

Species	Resistance patterns of multi-resistant strains
<i>S. aureus</i>	ami-crx-eno-sut, amo-amp-gen-sut-oxa, amo-amp-clo-crx-lmx-sut, ami-amo-amp-crx-gen-oxa-sut, amc-ami-amo-amp-cip-clo-eno-gen-lmx-nor-oxa
<i>S. pseudintermedius</i>	amo-amp-gen-sut, ami-amp-cip-gen-oxa-sut, amo-amp-cip-eno-gen-sut, amo-amp-crx-gen-lmx-sut, amc-ami-amo-amp-crx-oxa-sut, ami-amo-amp-clo-crx-gen-oxa-sut, ami-amo-amp-clo-crx-eno-gen-lmx, ami-amo-amp-cip-eno-fff-gen-lmx-sut, ami-clo-crx-eno-fff-gen-lmx-nor-sut, ami-amo-amp-cip-eno-gen-lmx-nor-sut, amc-ami-amo-amp-clo-crx-fff-gen-oxa-sut, amc-amo-amp-cip-clo-eno-fff-lmx-nor-sut, ami-amo-amp-cip-clo-eno-gen-lmx-nor-sut, amc-ami-amo-amp-crx-eno-gen-lmx-nor-oxa-sut, ami-amo-amp-cip-clo-crx-eno-fff-gen-lmx-nor-sut, amc-ami-amo-amp-cip-clo-crx-eno-lmx-nor-oxa-sut
<i>S. schleiferi coagulans</i>	ami-clo-crx-gen-lmx-sut, amc-ami-amo-amp-crx-gen-oxa-sut, ami-amo-amp-cip-clo-crx-gen-nor-sut, ami-amo-amp-cip-clo-crx-eno-lmx-nor-sut, ami-amo-amp-clo-crx-eno-fff-gen-lmx-nor-oxa-sut
<i>S. epidermidis</i>	ami-cip-clo-gen-sut, amo-amp-cip-lmx-oxa, amc-ami-amo-amp-crx-gen-oxa, ami-amo-amp-clo-fff-gen-norl, ami-amo-amp-gen-lmx-nor-sut, ami-cip-clo-gen-sut, amo-amp-cip-lmx-oxa, amc-ami-amo-amp-crx-gen-oxa, ami-amo-amp-clo-fff-gen-norl, ami-amo-amp-gen-lmx-nor-sut, ami-clo-fff-gen-lmx-nor-sut, amc-ami-amo-amp-cip-crx-eno-gen-oxa, ami-amo-amp-cip-clo-crx-gen-lmx-sut, amo-amp-cip-crx-eno-gen-lmx-nor-sut, ami-amo-amp-cip-clo-crx-fff-gen-oxa-sut, amo-amp-cip-clo-eno-fff-gen-lmx-nor-sut, ami-amo-amp-cip-crx-eno-gen-lmx-nor-oxa-sut
<i>S. schleiferi schleiferi</i>	amo-cip-eno-gen, ami-amo-amp-clo-gen, ami-amo-amp-clo-sut, ami-cip-crx-eno-gen-lmx-nor-sut, ami-amo-amp-cip-eno-gen-lmx-nor-sut (2)
<i>S. simulans</i>	ami-amp-gen-lmx-nor, amo-amp-crx-gen-sut, ami-cip-clo-gen-nor-sut, ami-amo-amp-clo-fff-gen-lmx-sut, amo-amp-cip-eno-gen-lmx-nor, amo-amp-cip-eno-gen-lmx-nor-sut, ami-amo-amp-clo-crx-gen-lmx-oxa-sut, amc-amo-amp-cip-clo-crx-fff-gen-nor-oxa-sut
<i>S. saprophyticus</i>	amo-amp-cip-eno-lmx-nor-sut

amc — amoxicillin + clavulanic acid; ami — amikacin; amo — amoxicillin; amp — ampicillin; cip — ciprofloxacin; clo — chloramphenicol; crx — cefuroxim; eno — enrofloxacin; flf — florfenicol; gen — gentamicin; lmx — lomefloxacin; nor — norfloxacin; oxa — oxacillin; sut — trimethoprim-sulfamethoxazole.

lomefloxacin (10 µg), norfloxacin (10 µg), and enrofloxacin (5 µg). Penicillins were represented by the amoxicillin-clavulanic acid combination (AMC) (10 µg), amoxicillin (10 µg), ampicillin (10 µg), and oxacillin (1 µg), and cephalosporins were represented by cefuroxim (30 µg). Trimethoprim-sulfamethoxazole (25 µg), florfenicol (30 µg), and chloramphenicol (30 µg) were also tested. After measuring the diameters of the clear zones around the antimicrobial discs and following the procedures of the National Committee for Clinical Laboratory Standards

Table 2. Resistance to 14 antimicrobials among staphylococci isolated from the urine of dogs with urinary tract infection

Species	amc	amo	amp	oxa	crx	cip	lmx	nor	eno	ami	gen	flf	clo	sut
<i>S. pseudintermedius</i> (n = 23)	7	15	16	6	9	11	13	1	12	13	16	5	9	16
<i>S. aureus</i> (n = 8)	4	6	6	3	3	1	2	8	2	4	5	0	2	6
<i>S. schleiferi coagulans</i> (n = 5)	1	4	4	1	5	2	3	3	3	5	4	1	4	5
<i>S. schleiferi schleiferi</i> (n = 8)	0	7	5	0	1	4	3	3	4	5	6	0	2	5
<i>S. epidermidis</i> (n = 13)	6	10	10	6	6	8	8	7	4	9	11	4	6	9
<i>S. simulans</i> (n = 11)	2	7	8	2	3	5	6	6	3	5	10	2	4	5
<i>S. saprophyticus</i> (n = 2)	0	1	2	0	0	2	2	2	2	0	0	0	0	0

amc — amoxicillin + clavulanic acid; ami — amikacin; amo — amoxicillin; amp — ampicillin; cip — ciprofloxacin; clo — chloramphenicol; crx — cefuroxime; eno — enrofloxacin; flf — florfenicol; gen — gentamicin; lmx — lomefloxacin; nor — norfloxacin; oxa — oxacillin; sut — trimethoprim-sulfamethoxazole.

(10), the strains were categorized as sensitive or resistant to the drug. Intermediate susceptibility was infrequent and was regarded as resistant.

Results

Bacteriological culture

From the 348 dogs that met the inclusion criteria, 70 yielded staphylococci in pure culture and in concentrations $> 10^3$ CFU/mL of urine, representing 20.1% of the dogs. Urine from the other 278 dogs yielded mixed cultures of gram-positive cocci with gram-negative rods [146 (42%)] or gram-negative rods in pure culture [132 (38%)]. Coagulase-positive staphylococci (CoPS) were isolated from 51.4% (36/70) of the samples that yielded staphylococci in pure culture, and most of these isolates were *S. pseudintermedius* [23/70 (32.9%)] followed by *S. aureus* [8/70 (11.4%)] and *S. schleiferi coagulans* [5/70 (7.1%)]. Coagulase negative staphylococci (CoNS) were isolated from 34 samples (48.6%), and were distributed among *S. epidermidis* [13/70 (18.6%)], *S. simulans* [11/70 (15.7%)], *S. schleiferi schleiferi* [8/70 (11.4%)] and *S. saprophyticus* [2/70 (2.9%)].

Antimicrobial susceptibility tests

All isolates were resistant to at least 1 drug, and 53 (75.7%) were multiresistant, defined herein as resistance to 3 or more antimicrobial classes. Multiresistance was detected in 26 (72.2%) CoPS and 28 (82.4%) CoNS. Three strains of *S. schleiferi coagulans*, 3 of *S. pseudintermedius*, 2 of *S. epidermidis*, and 2 of *S. simulans* were resistant to all classes of antimicrobial agents. Three strains of *S. pseudintermedius*, 1 of *S. schleiferi coagulans*, and 1 of *S. epidermidis* were resistant to 12 of the 14 antimicrobial drugs. Susceptibility patterns of the multiresistant isolates are listed in Table 1.

Resistance to the penicillins was observed in 53 (75.7%) isolates; ampicillin and amoxicillin were the less effective penicillins, while resistance to AMC and oxacillin was present in 28.6% and 25.7% of resistant isolates, respectively. Resistance to the fluoroquinolones, lomefloxacin, ciprofloxacin, enrofloxacin, and norfloxacin ranged from 42.9% to 52.9%. Resistance to trimethoprim-sulphamethoxazole was also a common finding, being observed in 48 (68.5%) isolates. Resistance to gentamicin occurred in 74.3% and to amikacin in 58.6% of the isolates. The number of resistant strains of each species to all the antimicrobials is listed in Table 2.

Discussion

The 70 isolates of staphylococci represent a rate of 20.1% of the 348 urine samples. Other studies in the literature cite prevalences of no more than 10% of UTI samples being positive for staphylococci (4). In this study, however, rigorous inclusion criteria were adopted in the selection of animals and only samples that yielded staphylococci in pure culture were considered; therefore, *Staphylococcus* sp. appear to be more prevalent in the present population than what is usually reported.

Coagulase positive species were slightly more common (51.4%) than CoNS; this agrees with other studies (11,12). Among the CoPS, the predominance of *S. pseudintermedius* over *S. aureus* also agrees with most of these studies and was not unexpected, since it is well-known that *S. pseudintermedius* is the most common species of *Staphylococcus* in canine infections (1,3,13,14), including UTI (15). Phenotypic differentiation between *S. intermedius* and *S. pseudintermedius* is difficult and commercial kits are not available for their adequate identification (16). Identification was based on the observation that canine *Staphylococcus* strains meeting the phenotypic identification of the former *S. intermedius* should now be considered *S. pseudintermedius* (6).

Coagulase-negative staphylococci constitute a major component of the normal microflora of human beings, dogs and cats (17). They are opportunistic pathogens but their importance in canine UTI is not well established and requires further attention. It is noteworthy that the dogs were not hospitalized, as long-term hospitalization is a predisposing factor to infections by opportunistic microorganisms. Also, urine samples were obtained by catheterization, thus reducing the possibility that these isolates were contaminants of the genital mucosa.

There was a high frequency of resistance, with all isolates being resistant to at least 1 antimicrobial drug and multiresistance being present in 77.1% of the isolates. Older studies (12,15,18–20) reported lower rates of resistance and the present results suggest an increasing tendency towards resistance among staphylococcal isolates of canine origin. Although there are some differences in the population and in methodology, it has been previously noted (15) that rapid development and spread of antimicrobial resistance has occurred in the genus *Staphylococcus*. The samples were provided by clinical practitioners and the possibility that some of those animals may have previously

been treated with antimicrobial agents cannot be ignored, and could contribute to the high resistance rates observed among these isolates.

Resistance to trimethoprim-sulphamethoxazole was detected in 67.2% of the isolates, consistent with data from other countries, such as 74.4% in Canada (15). This drug was widely used to treat canine UTI in the recent past and resistance directed towards this class of drugs has increased rapidly; 25 years ago resistance to this drug was reported as only 2% (21). Due to the widespread and increasing resistance, the use of sulphonamides associated with trimethoprim for UTI cannot be recommended in the absence of antimicrobial susceptibility tests.

Resistance to amoxicillin reached 71.5% and to ampicillin, 72.9%. Although extremely high, this was not surprising, since it has been recently demonstrated (14) that 62% of the isolates of *S. pseudintermedius* strains of canine pyoderma origin were β -lactamase producers. The occurrence of β -lactamase producers in this genus has substantially grown compared to a previous study of a similar canine population (3). Lilenbaum et al (3) found resistance frequencies of 41.2% of the CoPS and 37.0% of the CoNS, compared with 69.4% and 79.4% (respectively) in the present study. For the combination of clavulanic acid and amoxicillin only 28.6% of the isolates were resistant. Therefore, it seems that the combination of amoxicillin with clavulanic acid, an inhibitor of β -lactamase, is still a valuable antimicrobial for the treatment of staphylococcal infections.

Resistance to oxacillin, although limited (25.7%), has dramatically increased from 4.6% in a previous study of a similar canine population (3). In a recent retrospective study conducted in the United States (22), resistance to oxacillin from the years 2001 to 2005 had also substantially increased in clinical samples of canine origin. Oxacillin is used in laboratory testing to predict microbial susceptibility to other penicillinase-resistant penicillins such as methicillin or dicloxacillin. Methicillin resistant staphylococci (MRS) have been reported in staphylococci from dogs in various countries (23,24). In the present study, 3 of the 18 MRS isolates were *S. aureus*, and therefore MRSA, represented 37.5% of the isolated *S. aureus*.

Florfenicol is not approved for use in dogs and it is therefore not surprising that resistance to this drug was low. Chloramphenicol, however, has been widely used for many years and resistance to this drug was much more frequent (38.6%); this is much higher than that reported from Canada (15).

The increasing use of fluoroquinolones in companion animal practice over the past 10 y has led to an increasing frequency of resistance (4,12,19). In order to avoid further increases, fluoroquinolones should not be used as a first line choice for staphylococcal UTI, and should be reserved for cases in which the results of culture and sensitivity support their use.

The present study reports the alarming antimicrobial resistance of members of the *Staphylococcus* genus isolated from canine UTI and highlights the importance of CoNS in its etiology. It also emphasizes the need for bacterial culture with species identification and antimicrobial susceptibility tests in order to choose appropriate antimicrobial agents to treat canine UTI.

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Book Review

Compte rendu de livre

Equine Respiratory Medicine and Surgery

McGorum BC, Robinson NE, Schumacher J, Dixon PM, eds. Saunders Elsevier, Philadelphia, Pennsylvania, USA. 2007. 600 pp. Hardcover, ISBN 978-0-7020-2759-8. \$262.96.

Equine Respiratory Medicine and Surgery is edited by 4 well-known editors and has over 50 contributors. It provides the reader with a fully comprehensive, state-of-the-art reference source in the field of equine respiratory medicine and surgery.

The text is of high quality, well-written, and nicely bound. It consists of 7 sections and 46 chapters. The 1st section covers the basic sciences related to the respiratory system of the horse, including anatomy, physiology, immunology, and pharmacology. The 2nd section deals with diagnostic techniques pertaining to equine respiratory diseases. It starts with special examination of the respiratory system and the collection and analysis of respiratory samples. The different imaging techniques (ultrasonography, radiography, scintigraphy) and endoscopy, blood gas analysis, pulmonary function testing, lung biopsy, and postmortem examination follow. The 3rd section discusses viral and bacterial infections of the equine respiratory tract. The 4th and 5th sections covers medical and surgical diseases

and disorders of the equine respiratory system organized anatomically (upper and lower respiratory tract, respectively). This coverage includes medical and/or surgical treatment(s) where applicable. The 6th section is restricted to respiratory diseases of the foal (recognition, diagnosis, and treatment). The book concludes with a section about the disorders of the thoracic wall, pleura, mediastinum, and diaphragm.

The index is accurate, easy to use, and appropriately cross-referenced. One of the main strengths of this book is the high quality radiographic, ultrasonographic, and endoscopic images, CT scans, MRI images, color clinical photographs, microscopic images, electron micrographs, and schematic diagrams which aid in understanding the pathology, diagnosis, and treatment.

This book is highly recommended for veterinary students, practitioners, instructors, and researchers that need a source for an in-depth coverage of the medical and surgical diseases and disorders of the equine respiratory system.

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