Retrospective Evaluation of Canine Dermatitis Secondary to *Corynebacterium* spp.

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ABSTRACT

Corynebacterium **species are considered nonpathogenic in canine dermatitis; however, potential clinical significance has been demonstrated in canine otitis externa and from a dog bite wound in a human. Objectives of this study were to identify the predominant *Corynebacterium* species present in lesions of canine dermatitis, assess pathogenic role, determine antimicrobial susceptibility, and evaluate clinical response. Of 37 isolates identified as *Corynebacterium,* 31 were *Corynebacterium auriscanis*. Most *Corynebacterium* isolates were susceptible to chloramphenicol (97%), tetracyclines (92%), and amikacin (89%); isolate susceptibilities to β-lactams, trimethoprim-sulfonamides, and fluoroquinolones were <50%. Most cultures grew mixed populations of bacteria; *C. auriscanis* was the only organism isolated in three patients. At recheck, 2–8 wk after initial presentation, pleomorphic rods were absent or significantly decreased in all patients. Two of three *C. auriscanis* isolates were obtained in pure culture and were evaluable, meaning patient had an initial exam and recheck examination. Both patients were already on antimicrobials to which *C. auriscanis* was resistant in vitro. Both improved after doxycycline administration. *C. auriscanis* may act as an opportunistic pathogen in canine dermatitis and may not respond to antimicrobial therapy based on susceptibilities for other organisms in mixed infections. Occasionally, *Corynebacterium* isolated alone may be pathogenic.** (*J Am Anim Hosp Assoc* 2015; 51:372–379. DOI 10.5326/JAAHA-MS-6243)

Introduction

Coryneform is a broad taxonomic term used to describe a variety of Gram-positive pleomorphic rods, including *Corynebacterium, Actinomyces, Archanobacterium, Erysipelothrix, Nocardia,* and *Mycobacterium*.[1] The development of molecular genetic techniques, such as partial 16s rRNA gene sequencing, has allowed more precise taxonomic classification of coryneform bacteria.[2] Bacteria in the genus *Corynebacterium* are Gram-positive rods, facultative anaerobes, and catalase positive; they exhibit cell wall chemotype IV. The rods appear straight to slightly curved with tapered ends, some of which may exhibit a clubbed shape.[1,3] Historically, *Corynebacterium* species have not been considered pathogenic in dogs and cats.[1,3–5] The most significant *Corynebacterium* in human medicine is *Corynebacterium diphtheriae*, with occasional reports of biotypes present in domestic animals.[6] *Corynebacterium* spp. of veterinary significance, such as *Corynebacterium pseudotuberculosis*, have been associated with caseous lymphadenitis, mastitis, urinary tract infections, and endocarditis.[1,7–10]

With the advent of modern molecular techniques, several new species of *Corynebacterium* have been identified.[3–4,11] *Corynebacterium auriscanis*, first described by Collins et al., was reported primarily from dogs with otitis externa; other samples were obtained from deep pyoderma, interdigital cyst, and a vaginal swab.[4] Phenotypic characterization was determined using the API Coryne System[a] and phylogenetic characterization was determined by performing partial 16S rRNA gene sequence analysis. *C. auriscanis* was described as a Gram-positive rod that is catalase positive, aerobic, and nonlipophilic. The rods are club-shaped and can appear singly, in pairs, or in clusters.[4]

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CNA, Columbia Colistin and Nalidixic Acid; TSA, Trypticase Soy Agar

The zoonotic potential of *C. auriscanis* was demonstrated when an otherwise healthy 24 yr old woman developed an abscess subsequent to a dog bite. No organisms were recovered from a culture of the wound obtained 5 days after the bite. The lesion failed to improve after a 7 day course of amoxicillin-clavulanic acid; a heavy growth of a Gram-positive rod with characteristics similar to coryneform organisms was recovered from a culture of purulent exudate. The abscess persisted despite therapy with multiple antimicrobials. A culture obtained from the abscess 6 wk after the bite yielded a heavy growth of Gram-positive rod, identified as *C. auriscanis* by partial 16S rRNA gene sequencing. The abscess resolved and did not recur after treatment with oxytetracycline.[12]

The significance of *C. auriscanis* in canine otitis has been described in two recent studies.[5,11] *C. auriscanis* is typically a component of mixed bacterial infections when causing bacterial otitis externa, and resolves with control of other causative agents. In otitis, *C. auriscanis* acts as an opportunist in mixed infections, although the organism may have pathologic significance when occurring alone.[5] To the authors' knowledge, the significance and role of *C. auriscanis* in bacterial dermatitis have not been described.

The objectives of this retrospective study were to identify the predominant *Corynebacterium* species present in lesions of canine dermatitis, determine antimicrobial susceptibility, evaluate the clinical response to antimicrobials prescribed based on susceptibility data, and assess their role as pathogens in canine dermatitis.

Materials and Methods

This study was a retrospective evaluation of prevalence, antimicrobial susceptibility, and clinical response of canine dermatitis from which *Corynebacterium* spp. was isolated by the dermatology department at a tertiary referral hospital in San Diego, California. Isolates were collected between November 2008 and July 2012. All samples were obtained from patients referred to dermatology service by general practitioners or by other departments within the hospital.

Samples for cytological evaluation were obtained from lesions using direct impression and/or acetate tape preparations, and then stained using a modified Wright-Giemsa stain[b]. Samples were evaluated at high magnification (40x) to find areas of interest, and then examined using oil immersion (100x). A minimum of 15–20 oil immersion fields were examined for each cytological sample. Samples were evaluated for the presence of cocci, rod-shaped bacteria, and *Malassezia*. Skin scrapings were performed to rule out *Demodex* mites when indicated. Bacterial numbers were evaluated on a scale of 0 to 4+, where 0 corresponds to no organisms per oil immersion field, 1+ corresponds to an average of 1–10 per oil immersion field, 2+ corresponds to an average of 10–20 per oil immersion field, 3+ corresponds to an average of 20–30 per oil immersion field, and 4+ corresponds to organisms that were too numerous to count in multiple fields.

Samples for bacterial culture were selected based on cytological evaluation of skin underlying crusts or scale. Cultures were obtained using methods described by White et al.[13] Most lesions were sampled using a direct swab technique; when present, crusts were removed using a sterile scalpel blade, and the tissue under the crust was sampled for culture. Lesions that yielded *Corynebacterium* spp. as a single isolate typically appeared as areas of hypotrichosis or alopecia with crusted scaly dermatitis and were usually multifocal. Lesions were typically dry and not actively erosive under the crusts. Two culture swabs containing Amies transport medium[c] were used for collection and submitted to a local veterinary reference microbiology laboratory for aerobic culture and susceptibility. One swab was used to inoculate MacConkey agar[d], Trypticase Soy Agar (TSA)[e] with 5% sheep blood, and Columbia Colistin and Nalidixic Acid (CNA)[f] agar; the second swab was used to inoculate thioglycolate broth. Cultures were incubated at 37 °C for 72 hr. If no growth occurred on plates, but bacteria were present in the thioglycolate broth, the broth sample was then subcultured to TSA blood agar and incubated as above. Gram stain, catalase test, and wet mount were performed on all morphologically distinct bacterial colonies. Gram-positive rods grown in pure culture on Columbia CNA agar were used to prepare direct suspension inocula for TSA with 5% sheep blood for antimicrobial susceptibilities by Kirby-Bauer disc diffusion. Break points were based on Clinical Laboratory Standards Institute Guidelines for infrequently isolated or fastidious bacteria.[14] Antimicrobials tested were based on standard laboratory panels, which varied somewhat during the course of the study; for example, tetracycline disks were used to determine susceptibility to the tetracycline class of antimicrobials, doxycycline was added to the standard panel late in the course of the study. Gram-positive pleomorphic, non-hemolytic, catalase-positive rods with characteristic short, bent rods on wet mount were subcultured to TSA 5% sheep blood agar and submitted to the Veterinary STAT microbiology laboratory to be frozen at −80 °C and held for further analysis and sequencing.

Forty-five samples were submitted for sequencing. The MicroSEQ[g] microbial identification system was used to sequence a 500 base-pair fragment of the 16S rRNA gene. Results were then compared to MicroSEQ genetic library and GenBank library. A minimum match of 99% identity over the sequence queried was recommended for species differentiation and a minimum of 97% identity over the sequence queried was recommended by the

sequencing laboratory to distinguish between genera. Data from isolates determined to be within the *Corynebacterium* genus are included. Multiple isolates from a single culture were included if they represented different populations of *Corynebacterium* based on antibiograms or separate species of *Corynebacterium* based on sequencing.

The bacterial cultures in which *Corynebacterium* spp. was sequenced were evaluated retrospectively to determine antimicrobial susceptibilities and the presence of other co-cultured bacteria was noted. When available, medical records were reviewed for signalment (age, breed, and sex), physical examination, concurrent conditions, and cytological interpretation of lesions. The results of physical examination and cytological evaluation were used to assess response to treatment. A case was considered evaluable when physical examination and cytological evaluation were performed on the day the sample for culture was obtained and at the time of the recheck examination. Recheck examinations were performed between 2 and 8 wk after the initial visit.

Clinical response was evaluated by referring to the physical examination notes in the medical records, when available, for the initial examination (i.e., when culture was performed) and for the recheck examination. Patient response was divided into four groups based on evaluation of lesion extent and severity at the recheck examination: (1) resolution, no lesions present; (2) significant improvement, reduction in lesion extent and severity by 50–99%; (3) improvement, reduction in lesion extent and severity by 1–49%; and (4) unchanged, no reduction in lesion extent and severity.

Results

A total of 45 isolates from 38 dogs were included in this analysis. Of these, *C. auriscanis* was isolated from one patient on two separate occasions. Two distinct isolates of *C. auriscanis*, based on antibiogram, were obtained from a single culture from one patient. Cultures were repeated for six patients; different species of *Corynebacterium* or *C. auriscanis* with different antibiograms were sequenced in each case. Of the 45 isolates sequenced, 37 isolates from 32 patients were identified as *Corynebacterium* spp.; 31 of those were identified as *C. auriscanis*. Sequence identities for 30/31 *C. auriscanis* isolates were 99 or 100%; one isolate had 95% sequence identity. Other *Corynebacterium* spp. identified were *Corynebacterium amycolatum, Corynebacterium resistens,* and *Corynebacterium freneyi*; species could not be identified in three, which were then grouped as *Corynebacterium* spp. Sequence identity was 99–100% over the sequence queried for all but one, which had a sequence identity of 98%. Eight of the isolates belonged to separate genera, and were identified as *Cellosimicrobium* spp., *Actinomyces, Microbacterium phyllosphaerae, Neisseria, Arthrobacter nicotinovorans,* and *Microbacterium hydrocarbonoxydans*.

Signalment, cytology, and antimicrobial susceptibility data were analyzed for the 32 dogs from which *Corynebacterium* spp. were isolated. The median age at time of culture was 7.9 yr, with a range from 1–15 yr. Neutered males comprised 62.5% of the patients, whereas 3.1% were intact males, 34.4% were spayed females, and none were intact females. Breeds varied widely; mixed-breed dogs were the most common (21.9%), followed by Labrador retrievers and German shepherd dogs (12.5%), English bulldogs and cocker spaniels (9.4%), soft-coated wheaten terriers (6.3%), and American bulldogs (3.1%). Several breeds, Belgian Malinois, bichon frise, Doberman pinscher, field spaniel, golden retriever, Great Dane, and West Highland white terrier, were represented by a single patient.

C. auriscanis was the only organism isolated in cultures from three dogs; the remaining 34 samples from which *C. auriscanis* was isolated were mixed with various other bacteria. Refer to **Table 1**. The percentage of these other bacteria was determined by dividing the number of non-*Corynebacterium* isolates by the number of cultures from which a mixed bacterial population was obtained (n = 34). *Staphylococcus* spp. were identified most frequently (67.6%), and included seven methicillin-susceptible *Staphylococcus aureus*, five methicillin-susceptible *Staphylococcus pseudintermedius*, four methicillin-resistant *Staphylococcus pseudintermedius,* three methicillin-resistant *Staphylococcus aureus,* two methicillin-resistant coagulase-negative *Staphylococcus* spp., and one methicillin-susceptible coagulase-negative *Staphylococcus* spp. Beta-hemolytic *Streptococcus* spp. was identified in 26.5% of cultures; *Pseudomonas aeruginosa* in 17.6%; Group D *Enterococcus*, *Escherichia coli,* and *Proteus mirabilis* each in 14.7% of samples; *Arcanobacterium*-like organisms in 5.9% of cultures; and *Serratia marcescens, Acinetobacter,* and *Pasteurella multocida* each in 2.9% of the samples.

Antimicrobial susceptibilities for all *Corynebacterium* isolates are listed in Table 1. Thirty-four cultures obtained from 32 dogs yielded 37 isolates of *Corynebacterium* spp.; the susceptibility data are reported based on number of isolates, rather than on the number of cultures. **Table 2** lists susceptibility results grouped by antimicrobial, along with percentage susceptibilities. Most isolates were susceptible to chloramphenicol (36/37), followed by tetracyclines (34/37), amikacin (32/36), clarithromycin (28/35), azithromycin (27/35), clindamycin (21/35), imipenem (15/37), ticarcillin clavulanate (15/37), cefazolin (14/37), amoxicillin clavulanate (14/37), ceftriaxone (12/35), enrofloxacin (12/37), trimethoprim sulfa (12/37), ampicillin (11/37), marbofloxacin (9/35), and cefpodoxime (5/37).

TABLE 1

***Corynebacterium* spp. and Co-cultured Bacteria With Antimicrobial Culture and Sensitivity Results for *Corynebacterium* spp.**

Culture ID	Bacteria	Other Bacteria	AMK	CLA	AZI	CPD	CHL	CLR	CLI	ENR	SXT	TET	MAR	DOX
1	*C. auriscanis*	MRSA	S	R	S	R	S	S	S	R	R	S	R	S
2	*C. amycolatum*	*E. coli*, MRSA	S	S	R	R	S	I	R	R	R	S	R	-
3	*C. auriscanis*	MRSP	S	S	S	S	S	R	R	I	R	S	R	-
4	*C. amycolatum*	*E. coli*, β-Strep, *P. mirabilis*	S	S	R	R	S	R	R	R	R	S	R	S
5	*C. auriscanis*	MSSA	R	R	S	R	S	S	R	R	R	S	R	-
6	*C. auriscanis*	MSSP, *C. resistens*	S	S	S	S	S	S	S	I	S	S	I	-
7	*C. resistens*	MSSP, *C. auriscanis*	S	R	S	R	S	S	S	R	R	S	R	-
8	*C. auriscanis*	β-Strep, MRCN	S	S	R	I	R	R	R	R	S	S	S	-
9	*C. auriscanis*	*Enterococcus*	S	R	R	R	S	R	R	S	R	S	S	-
10	*C. auriscanis*	MRCN, MSSP	S	R	S	R	S	S	S	S	R	S	S	-
11	*C. auriscanis*	MRSA	S	R	R	R	S	S	R	R	R	S	R	-
12	*C. auriscanis*	*E. coli,* MSSP, β-Strep	-	R	S	R	S	S	I	R	R	I	R	-
13	*C. auriscanis*	MSSA, MSCN	S	S	S	R	S	S	S	S	R	S	S	-
14	*C. auriscanis*	β-Strep, MSSA	S	S	S	S	S	S	S	R	S	S	R	-
15	*C. auriscanis*	*P. mirabilis*, MSSA, β-Strep, *Enterococcus*	S	S	R	R	S	R	R	S	S	R	S	-
16	*C.* spp	*Enterococcus*, *C. auriscanis*	S	S	S	R	S	S	S	R	S	S	R	-
17	*C. auriscanis*	*Enterococcus*	S	R	S	R	S	S	R	R	R	S	R	-
18	*C. auriscanis*	*Rhodococcus* like	S	R	S	R	S	S	S	R	R	S	R	-
19	*C. auriscanis*	*E. coli, P. aeruginosa, S. marcescens*, MSCN	S	R	S	R	S	S	S	I	R	S	I	-
20	*C.* spp	*E. coli*, β-Strep, MRSP	S	S	S	R	S	S	S	R	R	S	R	-
21	*C. auriscanis*	*Acinetobacter*, *P. mirabilis, E. coli, P. aeruginosa*, β-Strep, Group D *Enterococcus*, MSSA	S	R	S	R	S	S	S	R	R	S	R	-
22	*C. auriscanis*	Same as above	S	S	S	R	S	S	S	S	S	S	S	-
23	*C. auriscanis*	*Pseudomonas*, β-Strep, *Arcanobacterium* like	S	R	S	R	S	S	S	R	R	S	R	-
24	*C. auriscanis*	*Pseudomonas sp., E. faecalis*	S	R	-	R	S	-	-	S	S	S	*	-
25	*C. auriscanis*		S	R	S	R	S	S	S	R	R	S	R	-
26	*C. auriscanis*	MRSP	R	R	R	R	S	S	I	S	R	I	I	-
27	*C. auriscanis*		S	R	-	R	S	-	-	R	S	S	*	-
28	*C. auriscanis*	*P. aeruginosa, P. mirabilis*, MSSA	I	S	S	R	S	S	R	S	R	S	R	-
29	*C. freneii*	MSSP, MSSA, MSCN	S	S	S	S	S	S	S	S	S	S	S	-
30	*C. auriscanis*	MSSA	S	R	S	R	S	S	S	S	S	S	R	-
31	*C. auriscanis*	MSSP	S	R	S	R	S	S	S	S	R	S	S	-
32	*C. auriscanis*		S	R	S	R	S	S	S	I	S	S	I	S
33	*C. auriscanis*	*P. multocida*, β-Strep	S	S	S	S	S	S	S	S	R	S	S	-
34	*C. auriscanis*	*P. mirabilis, P. fluorescens,* MSSA	S	R	S	R	S	S	R	I	R	S	R	-
35	*C. auriscanis*	MSSP,	S	R	S	R	S	S	I	I	R	S	R	-
36	*C. auriscanis*	β-Strep, *Enterococcus*	R	R	R	R	S	R	S	R	R	S	R	-
37	*C. auriscanis*	MRSP, *E. coli*, Group D, non-hemolytic Strep	S	R	S	R	S	S	S	R	S	S	R	-

Antimicrobials: AMK, amikacin; CLA, clavamox (amoxicillin clavulanic acid); AZI, azithromycin; CPD, cefpodoxime; CHL, chloramphenicol; CLR, clarithromycin; CLI, clindamycin; ENR, enrofloxacin; SXT, trimethoprim sulfamethoxazole; TET, tetracycline; MAR, marbofloxacin; DOX, doxycycline

Bacteria: *C.* spp., *Corynebacterium* species; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MSSP, methicillin-susceptible *S. pseudintermedius*; MRSP, methicillin-resistant *S. pseudintermedius;* MSCN, methicillin-susceptible coagulase-negative *Staphylococcus*; MRCN, methicillin-resistant coagulase-negative *Staphylococcus; Strep*, *Streptococcus*

TABLE 2

Percentage of Susceptible, Resistant, and Intermediate for *Corynebacterium* spp. Isolated in This Study

Antimicrobial	AMK	AMP	CLA	AZI	CPD	CHL	CLR	CLI	ENR	MAR	SXT	TET/DOX	TIM
Sensitive	32	11	14	27	5	36	28	21	12	9	12	34	15
%	88.89	29.73	37.84	77.14	13.50	97.30	80.00	60.00	32.43	25.71	32.43	92.50	40.54
Resistant	3	26	23	8	31	1	6	11	19	22	25	1	22
%	8.33	70.27	62.16	22.86	83.78	2.70	17.14	31.43	51.35	62.86	67.57	2.50	59.46
Intermediate	1	0	0	0	3	0	1	3	6	4	0	2	0
%	2.78	0	0	0	2.70	0	2.86	8.57	16.22	11.43	0	5.00	0

Antimicrobials: AMK, amikacin; AMP, ampicillin; CLA, clavamox (amoxicillin clavulanic acid); AZI, azithromycin; CPD, cefpodoxime; CHL, chloramphenicol; CLR, clarithromycin; CLI, clindamycin; ENR, enrofloxacin; SXT, trimethoprim sulfamethoxazole; TET/DOX, tetracycline and doxycycline combined; MAR, marbofloxacin; TIM, ticarcillin clavulanic acid

Results of cytological evaluation of skin lesions were analyzed when available from medical records (n = 34). Cytological evaluation was performed at both the initial and recheck examinations for 15/34 patients; these cases were considered evaluable. Samples for cytological evaluation were obtained during the recheck examination unless declined by the patient's owner or from some patients in which lesions were no longer present. Recheck range was between 2 and 8 wk after initial presentation when the culture was obtained. Results were available from the initial examination alone in 16/34 cases. No cytological analyses were available for 3/34 patients. Pleomorphic rods were the only organisms observed in cytological samples obtained from 2/34 patients; in both, *C. auriscanis* was the only organism cultured. Additional organisms were observed in all other samples obtained during the initial examination, cocci in 29/31 of the samples, rods in 15/31, and *Malassezia* in 10/31. Antimicrobials chosen based on susceptibility data included amoxicillin/clavulanic acid, azithromycin, cephalexin, chloramphenicol, doxycycline, enrofloxacin, marbofloxacin, and trimethoprim sulfonamide. Physical examination findings for the 15 evaluable cases showed lesion resolution at the time of the recheck in 3/15 patients, significant improvement in 8/15, improvement in 3/15, and no improvement in one patient. The patient for which no improvement was observed did not receive a complete course of antimicrobial therapy since the patient was resistant to the owner's attempts to administer oral medications. Based on cytological analysis of samples obtained at the recheck examination, rods were eliminated in 87% and decreased in 13% of the patients. Cocci were no longer present in 77% of the samples, decreased in number in 15%, and had not changed in 7%. *Malassezia* were not detected in 50%, decreased in number in 33%, and numbers of *Malassezia* were unchanged in 17%. Refer to **Table 3** for summary of cytology, antimicrobials chosen, and clinical response results.

Two of the three cases in which *C. auriscanis* was the only organism isolated were considered evaluable. The first patient, a 4 yr old, male, neutered cocker spaniel, failed to respond to a 2 wk course of amoxicillin clavulanate. Physical examination revealed multifocal annular areas of hypotrichosis and scale over the dorsal thorax; cytological analysis of samples obtained from these lesions showed pleomorphic rods (abundance = 4+). Culture yielded a single isolate, *C. auriscanis*, which was resistant to amoxicillin clavulanate, but susceptible to doxycycline. The patient was re-evaluated after a 3 wk course of doxycycline (5 mg/kg twice daily). Dermatologic lesions had resolved completely, and no organisms were observed in samples obtained for cytological analysis. The second evaluable case was a 4.5 yr old, intact, male Belgian Malinois that was referred to the dermatology service for evaluation of a mixed bacterial infection that failed to respond to a 4 wk course of enrofloxacin therapy. A culture submitted by the referring veterinarian to a commercial veterinary microbiology laboratory had yielded *Corynebacterium* sp. (abundance = 4+), *Pseudomonas* (*Flavimonas*) *oryzihabitans* (abundance = 1+), and *Pantoea* sp. (abundance = 1+). The *P. oryzihabitans* and *Pantoea* sp. were susceptible to several antimicrobials, including enrofloxacin. Susceptibility testing was not performed for the *Corynebacterium* sp., as the laboratory considered the organism to be nonpathogenic. Upon presentation to the dermatology service, pleomorphic rods (abundance = 1+) were observed in cytological samples obtained from the dorsal trunk and paws. A single isolate of *C. auriscanis* was cultured from that site; the organism was resistant to enrofloxacin but susceptible to doxycycline. Significant improvement was noted at the recheck examination after a 3 wk course of doxycycline. The third case, an 11 yr old, spayed, female boxer mix

TABLE 3

Cytological Evaluation, Antimicrobial Selected for Therapy, and Level of Improvement Determined at the Time of Visit 2 for Patients That Had an Initial Visit With Cytology and a Recheck Examination With Cytology

Patient	Recheck	Visit 1	Visit 2	Antimicrobial	Improvement
1	4	C3, M1, R1	C1	SXT & DOX	significant
2	4	C1, M3	no	CLA	significant
3	3	C4, R4	no	CFL	resolution
4	4	C1	no	DOX	same
5	2	C1, M1, R1	1C, 1M, 0R	DOX	significant
6	3	C4	no	Only topical	improvement
7	4	C4	no	CFL	significant
8	3	4C, 4R	no	DOX	significant
9	3	4C, 4R	0C, 2R	CLA,MAR,DOX	improvement
10	5	1R	no	ENR, DOX	improvement
11	6	1C, 1M, 2R	0C, 1M, 0R	CHL	significant
12	3	4R	no	DOX	resolution
13	2	2C	1C	MAR, DOX	resolution
14	8	1C	0C, 1M	DOX, MAR	significant
15	4	1C, 1M	no	CLA, DOX	significant

Recheck examinations are listed in wk after therapy was initiated, antimicrobials are the antimicrobials administered to the patients based on culture results, and abbreviations are as follows: CFL, cephalexin; CHL, chloramphenicol; CLA, amoxicillin clavulanic acid; DOX, doxycycline; ENR, enrofloxacin; MAR, marbofloxacin. Cytology results are expressed on a scale of 0–4+ for visit 1 and visit 2, where 0 corresponds to no organisms per oil immersion field; 1+ corresponds to an average of 1–10 per oil immersion field; 2+ corresponds to an average of 10–20 per oil immersion field; 3+ corresponds to an average of 20–30 per oil immersion field; and 4+ corresponds to organisms that were too numerous to count in multiple fields. Cytology results are represented in this table with the following abbreviations: C, cocci and diplococci; M, *Malassezia*; and R, rods.

with a history of mast cell tumors, was presented to the dermatology service for evaluation of interdigital furunculosis that failed to respond to a 3 wk course of cephalexin. A single isolate of *C. auriscanis* was cultured from the left front paw; the organism was resistant to cephalosporins but susceptible to doxycycline. The patient developed a necrotic mast cell tumor on the proximal aspect of the right pelvic limb that required aggressive surgical and oncological intervention. Although this case was not considered to be evaluable because the patient was not rechecked by the dermatology, no dermatologic abnormalities were observed in the physical examination performed by the oncology service after a 4 wk course of doxycycline.

Concurrent conditions were reported for the dogs from which *Corynebacterium* spp. were cultured. Each patient was counted only once in the evaluation of prevalence of concurrent disease, whether or not multiple cultures were obtained from the patient or multiple isolates of *Corynebacterium* spp. recovered from culture. Clinical signs exhibited in 63% of dogs were consistent with allergy: flea, food, atopy, or a combination. Deep skin scrapings revealed *Demodex canis* in 10% of patients. Endocrine or metabolic diseases were present in 22% of the study population; all of these patients were diagnosed with hypothyroidism and one had concurrent diabetes mellitus. Other disease conditions included pemphigus foliaceus, discoid lupus erythematosus, lupoid onychodystrophy, perianal fistulas, fistulous disease, seasonal flank alopecia, and hepatocutaneous disease.

Discussion

Bacterial flora from canine skin and hair follicles are classified as either resident or transient organisms. Resident bacterial flora colonize surfaces of the skin and are often there for extended periods of time. Transient bacterial flora may colonize areas of the skin briefly, but do not persist over time. *Corynebacterium* spp. are considered transient organisms, along with *Escherichia coli, Proteus mirabilis, Bacillus* spp., *Pseudomonas* spp., and coagulase-negative *Staphylococcus*.[15] Although multiple studies have investigated the role of *Corynebacterium* spp. in canine otitis externa and media, little information has been reported about the involvement/function of these organisms in canine dermatitis.[5,11] Other *Corynebacterium* spp., such as *C. jeikeium, C. urealyticum,* and *C. resistens*, can be significant pathogens in human beings, particularly in immune-compromised patients.[1,17] Understanding the potential pathogenicity of *Corynebacterium* spp., particularly *C. auriscanis* in dermatitis, can help guide diagnostic laboratories when determining which organisms require susceptibility testing and help clinicians determine appropriate antimicrobial therapy.

Results of cytological evaluation of samples obtained from skin lesions in this study were comparable with previously reported observations in which coryneform bacteria were typically associated with other bacteria or *Malassezia*.[5,11,16] Three samples in this study yielded single-isolate cultures of *C. auriscanis*, whereas this organism was recovered as a single isolate in 11/61 samples obtained from otic exudate.[5] Skin lesions from which *Corynebacterium* spp. was cultured were dry, rather than exudative. Concentration of these organisms in dermatitis lesions appears to be less than that in infected ears.

C. auriscanis was the primary *Corynebacterium* species identified by sequencing in canine dermatitis, and was also the predominant species reported in canine otitis.[11] Additional species identified in this study included *C. amycolatum, C. resistens,* and *C. freneyi*; however, partial 16s rRNA sequencing may not accurately differentiate *C. freneyi* and *C. amycolatum* among other *Corynebacterium* spp. [18]

Culture results and antimicrobial susceptibilities were presented for all *Corynebacterium* spp. isolates. The antimicrobial susceptibilities for the *C. auriscanis* isolates for this study as compared to previous canine otitis studies are similar, and are as follows: chloramphenicol (97, 98%) and doxycycline (92, 93%). Antimicrobial susceptibilities in this study for clarithromycin and azithromycin are 80 and 77%, respectively; one canine otitis study found susceptibilities to erythromycin at 74%.[5,12,19] Resistance to erythromycin and azithromycin was reported to be common for *Corynebacterium jeikeium, Corynebacterium urealyticum, Corynebacterium xerosis,* and *Corynebacterium striatum*.[19] Susceptibilities to beta lactam antimicrobials, trimethoprim sulfonamides, and fluoroquinolones for *C. auriscanis* in this study were <50%. Although these results differed from *C. auriscanis* in canine otitis, in which 59% of isolates were susceptible to fluoroquinolones and 67% were susceptible to amoxicillin trihydrate/clavulanate potassium.[5] These results were consistent with those from earlier reports of *Corynebacterium* spp. susceptibilities in which resistance to beta lactam antimicrobials was common and some strains were resistant to ciprofloxacin.[19]

Antimicrobials were selected on the basis of culture and susceptibility for all bacteria present, including *Corynebacterium*. In 24/34 patients with mixed bacterial infections, *C. auriscanis* was susceptible to the antimicrobial selected for the non-coryneform bacteria. In 10/34 patients, *C. auriscanis* was resistant to the antimicrobial required for additional organisms and an additional antimicrobial (doxycycline) was administered concomitantly. Rods were eliminated (87%) or were reduced in number (13%) in all patients by the recheck examination. The three patients from which *C. auriscanis* was the only organism isolated responded well to therapy using doxycycline; each of these patients had failed to respond to empirical therapy using beta-lactam antimicrobials or rational antimicrobial-therapy-based susceptibilities of associated bacteria.

Lesion response in this study may have reflected the high prevalence of concurrent chronic dermatologic conditions. Most patients were diagnosed with allergic dermatitis, atopy, and/or adverse reaction to food. Resolution of those dermatologic lesions caused in part by associated epidermal barrier abnormalities typically requires more than 2–8 wk.

C. auriscanis should not be considered a primary pathogen in canine dermatitis, but is present in some mixed infections. *C. auriscanis* can act as an opportunistic pathogen and has the potential to cause lesions and clinical infections by itself, as demonstrated by the three cases where *C. auriscanis* was the only bacterial species isolated from culture.

C. auriscanis is often resistant to beta lactam antimicrobials, which are common empirical selections in the treatment of canine dermatitis. *C. auriscanis* may not respond to antimicrobial therapy based on susceptibilities of other bacteria cultured along with it. Antimicrobial therapy targeting *C. auriscanis* is recommended when the organism is recovered as a single isolate and may be necessary if dermatologic lesions fail to resolve after antimicrobial therapy based on susceptibilities of other bacteria in mixed infections.

This study was limited by its retrospective nature. Future prospective investigations would benefit from (1) the implementation of a more objective system of lesion scoring, such as the canine atopic dermatitis extent and severity index, which would provide better quantification of data; (2) standardization and enforcement of recheck examinations and sample collection for cytological evaluation; and (3) evaluation of the need to add an additional antimicrobial specifically for *C. auriscanis* in mixed infections.[20–21] Genetic sequencing may not be practical in all cases. Increased use of phenotypic test, such as the API Coryne System and the RapID CB Plus System[h], may provide additional data on the prevalence of *Corynebacterium* spp. in canine dermatitis.

FOOTNOTES

[a] API Coyne system; bio Mérieux Industry, Marcy l'Étoile, France
[b] Quick III; Astral Diagnostics Incorporated, West Deptford, NJ
[c] Amies transport media; Hardy diagnostics, Santa Maria, CA
[d] MacConkey's agar; Hardy Diagnostics, Santa Maria, CA
[e] TSA (tryptic soy) agar; Hardy Diagnostics, Santa Maria, CA
[f] Columbia CNA (colistin and nalidixic acid) agar; Hardy Diagnostics, Santa Maria, CA
[g] Micro SEQ; Applied Biosystems, Foster City, CA
[h] Rapid ID CB Plus System; Thermo Fisher Scientific, Lenexa, KS

REFERENCES

1. Coyle MB, Lipsky BA. Coryneform bacteria in infectious disease: clinical and laboratory aspects. *Clin Microbiol Rev* 1990;3:227–246.
2. Ruimy R, Riegel P, Boiron P et al. Phylogeny of the genus *Corynebacterium* deduced from analyses of small subunit ribosomal DNA sequences. *Int J Syst Bacteriol* 1995;45:740–746.
3. Funke G, Von Graevenitz A, Clarrigde J, et al. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* 1997;10:125–159.
4. Collins MD, Hoyles L, Lawson P, et al. Phenotypic and phylogenetic characterization of a new *Corynebacterium* species from dogs: description of *Corynebacterium auriscanis*. *J Clin Microbiol* 1999;37:3443–3447.
5. Henneveld K, Rosychuk R, Olea-Popelka FJ. *Corynebacterium* spp. in dog and cats with otitis externa and/or media: a retrospective study. *J Am Anim Hosp Assoc* 2012;48:320–326.
6. Hall AJ, Cassiday PK, Bernard KA, et al. Novel *Corynebacterium diphtheriae* in domestic cats. *Emerg Infect Dis* 2010;16:688–691.
7. Barksdale L, Linder R, Sulea IT, et al. Phospholipase D activity of *Corynebacterium pseudotuberculosis* (*Corynebacterium ovis*) and *Cory-*

nebacterium ulcerans, a distinctive marker within the genus *Corynebacterium*. *J Clin Microbiol* 1981;13:335–343.
8. Fernandez-Garayzabal JF, Collins MD, Hutson, et al. *Corynebacterium mastitidis* sp. nov., isolated from mild of sheep with subclinical mastitits. *Int J Syst Bacteriol* 1997;47:1082–1085.
9. Fernandez-Garayzabal JF, Collins MD, Hustson, Gonzalez I, et al. *Corynebacterium camporealensis* sp. nov., associated with subclinical mastitis in sheep. *Int J Syst Microbol* 1998;48:463–468.
10. Aguado JM, Ponte C, Soriano F. Bacteria with a multiply resistant species of *Corynebacterium* (*Corynebacterium* group D2): an unnoticed cause of urinary tract infections. *J Infect Dis* 1987;156:144–150.
11. Aalbaek B, Bemis DA, Schjaerff M, et al. Coryneform bacteria associated with canine otitis externa. *Vet Microbiol* 2010;145:292–298.
12. Bygott JM, Malnick H, Shah JJ, et al. First clinical case of *Corynebacterium auriscanis* isolated from localized dog bite infection. *J Med Microbiol* 2008;57:899–900.
13. White SD, Brown AE, Chapman PL, et al. Evaluation of aerobic bacteriologic culture of epidermal collarette specimens in dogs with superficial pyoderma. *J Am Vet Med Assoc* 2005; 226:904–908.
14. Clinical and Laboratory Standards Institute. M45–A2 Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria: Approved guidelines. 2nd ed. Wayne (PA): Clinical Laboratory Standards Institute; 2010.
15. Miller WH, Griffin CE, Campbell KL, eds. Bacterial Skin Diseases. In: Muller and Kirk's *Small Animal Dermatology*. 7th Ed. St. Louis: Elsevier Mosby; 2013:184–185.
16. Cole LK, Kwochka, Kowalski JJ, et al. Microbial flora and antimicrobial susceptibility patterns of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media. *J Am Vet Med Assoc* 1998;212:534–8.
17. Otsuka Y, Kawamura Y Koyama T, et al. *Corynebacterium resistens* sp., a new multidrug-resistent coryneform bacterium isolated from human infections. *J Clin Microbiol* 2005;43:3713–3717.
18. Renaud FN, Coustumier AL, Wilhem N, et al. *Corynebacterium hansenii* sp. nov., an alpha-glucosidase-negative bacterium related to *Corynebacterium xerosis*. *Int J Syst Evol Microbiol* 2007;57:1113–1116.
19. Soriano F, Zapardiel J, Nieto E. Antimicrobial susceptibilities of *Corynebacterium* species and other non-spore-forming Gram-positive bacilli to 18 antimicrobial agents. *Antimicrob Agents Chemother* 1995; 39:208–214.
20. Olivry T, Muller R, Nuttall T, et al. Determination of CADESI-03 thresholds for increasing severity levels of canine atopic dermatitis. *Vet Dermatol* 2008;19(3):115–119.
21. Plant JD, Gortel K, Kovalik M, et al. Canine atopic dermatitis extent and severity index development and validation of canine atopic dermatitis lesion index, a scale for the rapid scoring of lesion severity in canine atopic dermatitis. *Vet Dermatol* 2012;23(6):515.