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ORIGINAL REPORT

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Corneal stromal ulcerations in a referral population of dogs and cats in the Netherlands (2012–2019): Bacterial isolates and antibiotic resistance

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Abstract

Objective: To evaluate bacterial isolates from corneal stromal ulcerations in dogs and cats in the Netherlands, review their antibiotic susceptibility, determine whether recent topical treatment affected bacterial culture results, and investigate whether (multi-drug) resistance patterns changed over time.

Animals studied: Client-owned dogs and cats were diagnosed with corneal stromal ulceration at the Utrecht University Clinic for Companion Animals between 2012 and 2019.

Procedures: Retrospective analysis.

Results: In total, 163 samples were collected from 122 dogs (130 samples) and 33 cats. Positive cultures were obtained from 76 canine and 13 feline samples (59% and 39%, respectively) and included *Staphylococcus* (42 in dogs, 8 in cats), *Streptococcus* (22 in dogs, 2 in cats), and Pseudomonas (9 in dogs, 1 in cats) species. Significantly fewer positive cultures were found in dogs and cats previously treated with topical antibiotics ($\chi^2 = 6.52$, $p = .011$ and $\chi^2 = 4.27$, $p = .039$, respectively). Bacterial resistance to chloramphenicol was more common in dogs previously treated with chloramphenicol ($\chi^2 = 5.24$, $p = .022$). The incidence of acquired antibiotic resistance did not increase significantly over time. In dogs, the incidence of multi-drug-resistant isolates increased significantly between 2012–2015 and 2016–2019 (9.4% vs. 38.6%, $p = .0032$).

Conclusions: *Staphylococcus, Streptococcus*, and *Pseudomonas* species were the most common bacteria associated with canine and feline corneal stromal ulcerations. Previous treatment with antibiotics affected bacterial culture results and antibiotic sensitivity. Although the overall incidence of acquired antibiotic resistance did not change over time, the incidence of multi-drug-resistant isolates in dogs increased over an 8-year period.

KEYWORDS

antibiotic susceptibility, bacteria, canine, cornea, feline, ulcer

1 | INTRODUCTION

Corneal stromal ulcerations are commonly associated with microbial infection.[1–6] They require immediate, accurate, and intensive treatment in order to prevent progression and associated complications such as loss of vision or even loss of the globe.[7] The degradation of the corneal stromal extracellular matrix, associated with this type of ulceration, is the result of an imbalance between proteolytic enzymes (proteinases) and proteinase inhibitors in favor of the proteolytic enzymes.[8] Proteinases and proteinase inhibitors are naturally present in the healthy cornea and precorneal tear film as part of normal corneal tissue maintenance. Additionally, proteolytic enzymes can be produced and released by microorganisms.[9] In case of corneal wounding, the proteinase activity is increased and if infection is present, proteinases secreted by infectious organisms further contribute to corneal damage.[10]

Effective treatment includes appropriate topical antibiotic therapy, which is ideally based on bacterial culture results and susceptibility testing. Since corneal degradation may proceed rapidly, antibiotic therapy needs to be initiated before culture results are available.[7] A variety of bacterial isolates can be involved in corneal infection,[4–6,11] and geographical differences in common bacterial isolates have been observed.[1,3] Therefore, awareness of regionally common pathogenic bacteria and their susceptibility to specific antibiotics is an important aid in choosing the initial antibiotic therapy.

Our study aimed to report the incidence of the most common bacterial isolates associated with corneal stromal ulcerations and their antibiotic susceptibility in a referral population of dogs and cats in the Netherlands, to determine whether patient characteristics, concurrent ophthalmic conditions, and recent topical treatment affected bacterial culture results and to investigate whether the incidence of acquired antibiotic resistance and the incidence of multi-drug-resistant isolates changed over time.

2 | MATERIALS AND METHODS

2.1 | Case selection

All canine and feline ophthalmic bacterial culture samples sent to the Veterinary Microbiologic Diagnostic Centre by the University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht University, the Netherlands, between January 2012 and December 2019, were reviewed. Of the 209 ophthalmic samples, only corneal samples (172) were selected, and bacterial culture results from repeat samples of the same patient were only included if taken from a newly formed ulcer. The resulting patient files were screened.

Patients were included if their file contained an adequate description of the case history and of the size, depth, and aspect of the stromal ulcer. Breed, age, and sex of the patient were documented as well as the affected eye, concurrent ophthalmic diseases, use of topical antibiotics, and use of topical and oral corticosteroids in the 4 weeks prior to presentation. The breeds of canine patients were classified as either brachycephalic or nonbrachycephalic based on the typical facial conformation in the breed and on published or open access scientific data regarding their craniofacial ratio (CFR), breeds with a CFR < 0.5 being regarded as brachycephalic.[12,13]

All eyes underwent complete ophthalmic examination by an ECVO diplomate or a supervised veterinary ophthalmology resident, including slit-lamp biomicroscopy (SL-15 or SL-17 slit-lamp, Kowa Ltd), indirect ophthalmoscopy (Video Omega®2C, Heine Optotechnik GmbH & Co. KG), and fluorescein staining (Bio Fluoro, Fluorescein Sodium Strip 1 mg, Biotech Vision Care). Schirmer tear test (Schirmer Tear Test, MSD Animal Health) measurements and tonometry (Tonovet, Icare) were only performed if the clinician judged these procedures would not be detrimental to the eye.

2.2 | Sample collection and processing

Samples for bacterial culture were collected from the corneal ulcer, avoiding contact with the conjunctiva, lid margin, and hairs, using a sterile swab. No topical anesthetic was used immediately prior to sampling. Swab samples were immediately placed in transport medium (agar without charcoal, Transystem™) and stored at 4–7°C until further analysis and submitted to the Veterinary Microbiologic Diagnostic Centre (VMDC; Faculty of Veterinary Medicine, Utrecht University, the Netherlands).

Swabs were first used to inoculate standard growth media and then placed in Schaedler broth. Culturing media were incubated at 37°C under aerobic and anaerobic conditions for 3–5 days. If no growth was observed after this period, the bacterial culture result was considered negative.

Individual bacterial colonies were identified by routine microbiological methods and since 2014 by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The clinical relevance of mixed cultures was determined at the discretion of a veterinary microbiologist. Mixed culture results that were not deemed clinically relevant were not further analyzed nor tested for antibiotic susceptibility.

2.3 | Antibiotic susceptibility testing

Up to August 2016, antimicrobial susceptibility was tested by an agar diffusion method using Neo-Sensitabs (Rosco). From August 2016 onward, minimum inhibitory concentrations (MICs) were determined by broth microdilution using a commercially available automated MICRONAUT system incorporating a custom-made panel for routine diagnostic testing at the VMDC (MERLIN Diagnostika GmbH). Antimicrobial susceptibility testing was performed as recommended by the manufacturer for inoculum preparation, broth composition and incubation conditions. Clinical breakpoints for systemic use in accordance with the then-current Clinical Laboratory Standards Institute guidelines were used for interpretation (https://clsi.org/). *Staphylococcus* species were screened for methicillin resistance by determining the MIC for oxacillin and cefoxitin. If resistance to these antibiotics was observed, a quantitative PCR test was performed to test for the presence of the *mecA* gene.[14]

Only antibiotics that are available in ophthalmic preparations in the Netherlands were included for further analysis: chloramphenicol, fusidic acid, tetracycline, polymyxin, gentamicin, and fluoroquinolones.

An isolate was defined as multi-drug resistant if it had acquired nonsusceptibility to three or more antibiotic classes.[15]

2.4 | Statistical analysis

Patient characteristics for both dogs and cats were summarized by descriptive statistics. In case a bacterial culture result yielded both a mixed culture and one or more pure bacterial isolates, the mixed culture result was excluded from further analysis. For statistical purposes, intermediate susceptible isolates were classified as resistant. Intrinsic resistance of bacterial isolates to specific antibiotics was excluded from further analysis and is marked separately in the tables and figures.

First, resistance patterns of canine and feline *Staphylococcus* spp., β-hemolytic *Streptococcus* spp., and *Pseudomonas aeruginosa* isolates to selected antibiotics were evaluated using descriptive statistics.

Second, the association between previous treatment with topical antibiotics and reported resistance patterns as well as the probability of a positive bacterial culture result after treatment with topical antibiotics were evaluated (by χ^2 analyses and a Mantel-Haenzsel test for trend, respectively). The association between previous treatment with topical antibiotics and resistance patterns was not further explored beyond chloramphenicol (the most commonly empirically used antibiotic in the Netherlands) due to sample size limitations.

Third, for the canine population, associations between other patient characteristics (i.e., other concurrent ophthalmic conditions, recent treatment with topical corticosteroids, and brachycephalic breed), and bacterial culture results were explored by use of a Fisher's exact test. Due to sample size limitations, these analyses were not attempted for the feline population.

Finally, the incidence of acquired antibiotic resistance as well as the incidence of multi-drug-resistant isolates in canine and feline patients were compared for the period 2012–2015 and 2016–2019 by use of a Fisher's exact test.

Statistical analyses were performed by statistical software IBM® SPSS® Statistics version 26. Values of $p \leq .05$ were considered significant.

3 | RESULTS

In total, 163 samples were collected from 122 dogs (130 samples) and 33 cats. Bilateral corneal stromal ulcers were present in six dogs and repeated sampling from a newly formed ulcer (one in the ipsilateral eye, one in the contralateral eye) occurred in two dogs.

3.1 | Dogs

Thirty-six breeds (including the category of crossbreed) were included, of which the Shih Tzu (19.7%), French Bulldog (16.3%), and Pug (10.7%) were overrepresented compared with the overall clinic population (Table S1). In total, 65% of the dogs were considered to be of a brachycephalic breed (Table S1). There were 80 males and 49 females with a median age of 7.3 years (range, 6 months–15 years). The right eye was affected in 68 and the left eye in 62 cases.

Concurrent ophthalmic conditions were observed in 87/130 affected eyes (67%) (Table 1). Seventy percent of dogs had received treatment with topical antibiotics prior to referral: 41% of the dogs with one class of topical antibiotics, 29% with two or more classes.

Positive cultures were obtained in 76 canine samples (76/130; 59%). Two cultures contained three pure strains of bacterial isolates, and 9 cultures contained two pure strains of bacterial isolates, making the total number of bacterial isolates found in dogs 89 (Table 2).

Staphylococcus species were the most commonly isolated pathogens ($n = 42$; 47%) in which *Staphylococcus pseudintermedius* was predominant ($n = 32$). *Streptococcus* species were the second most commonly

TABLE 1 Concurrent abnormalities found on ophthalmic examination in, and medication administered within the 4 weeks prior to referral of, 122 dogs and 33 cats with corneal stromal ulcerations presented to the University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht University, the Netherlands between January 2012 and December 2019

	Canine		Feline	
	n	%	*n*	%
Concurrent ophthalmic findings				
Keratoconjunctivitis sicca	53	40.8	9	27.3
Monoculus	3	2.3	1	3.0
Phthisis bulbi contralateral eye	1	0.8	0	0
Corneal ulcer contralateral eye	6	4.9	1	3.0
Corneal fibrosis contralateral eye	20	15.4	2	6.1
Trichiasis - periocular hair	20	15.4	0	0
Trichiasis - nasal fold	16	12.3	0	0
Trichiasis - caruncle	7	5.4	1	3.0
Macroblepharon	7	5.4	0	0
Lagophthalmos	8	6.2	2	6.1
Entropion	20	15.4	7	21.2
Distichiasis	14	10.8	0	0
Ectopic cilia	1	0.8	0	0
Topical antibiotics				
Chloramphenicol	74	51.7	17	51.5
Polymyxin B	33	23.3	11	33.3
Gentamicin	30	21.0	12	36.3
Fusidic acid	10	7.0	5	15.6
Ofloxacin	6	4.2	3	9.1
Trimethoprim	4	2.8	0	0
Tetracyclines	4	2.8	2	6.1
Neomycin	2	1.4	0	0
Oxacillin	1	0.7	0	0
Corticosteroids				
Topical corticosteroids	11	7.7	7	21.2
Oral corticosteroids	8	6.2	2	6.1

isolated pathogens ($n = 22$; 25%), all were beta-hemolytic streptococci. *Pseudomonas* species were the third most isolated pathogens ($n = 9$; 10%). Of the 89 isolates, 25 were considered to be multi-drug-resistant: 19 of these were *Staphylococcus pseudintermedius*, including two methicillin-resistant *Staphylococcus pseudintermedius* (MRSP).

Acquired resistance in *Staphylococcus* spp. was most commonly observed for chloramphenicol, in β-hemolytic-*Streptococcus* spp. for fusidic acid and in *Pseudomonas aeruginosa* for enrofloxacin (Figure 1).

The probability of a positive culture result was significantly lower in dogs previously treated with one or more classes of topical antibiotics ($\chi^2 = 6.52$, $p = .011$, Mantel-Haenzsel test for trend; Figure 2) and was predominantly assigned to previous treatment with chloramphenicol ($\chi^2 = 10.6$, $p = .001$). Resistance of bacterial isolates to chloramphenicol was more common in patients that previously received treatment with chloramphenicol ($\chi^2 = 5.23$, $p = .022$, Figure 3).

Patient characteristics, including concurrent ophthalmic conditions, recent treatment with topical corticosteroids, and brachycephalic breed could not be related to the outcome of bacterial culture results and were not further explored.

The frequency of previous topical antibiotic therapy did not differ significantly in the canine patient group between the period 2012–2015 and 2016–2019 ($p = .094$). The incidence of acquired antibiotic resistance did not change significantly over time between the period of 2012–2015 and 2016–2019 ($p > .05$), except for gentamicin, for which it declined ($p = .029$). The incidence of multi-drug-resistant isolates increased significantly between 2012–2015 and 2016–2019 (9.4% vs. 38.6%, $p = .0032$).

We decided against reporting the results of possible associations between other patient characteristics (i.e., other

TABLE 2 An overview of the bacterial culture results from canine clinical patients with corneal stromal ulcerations, referred to the Ophthalmology Section at the University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht University, the Netherlands between January 2012 and December 2019

	2012–2015	2016–2019	Total
Cultures, *n*	49	81	130 (100%)
Negative, *n*	15	24	39 (30%)
Mixed, *n*	7	8	15 (11.5%)
Positive, *n*	27	49	76 (58.5%)
Bacterial isolates, *n*[a]	32	57	89 (100%)
Staphylococcus species, *n*	13	29	42 (47.2%)
S. pseudointermedius	11	21	
MRSP	1	1	
Other *Staphylococcus spp.*	1	7	
β-hemolytic *Streptococcus*, *n*	8	14	22 (24.7%)
Pseudomonas aeruginosa, n	5	4	9 (10.1%)
Other species, *n*	6	10	16 (18.0%)
E. coli	1	3	
Fusobacterium spp.	1	1	
Corynebacterium spp.	1	0	
Mycoplasma spp.	1	1	
Pasteurella dagmatis	0	1	
Capnocytophaga spp.	1	0	
Pantoea agglomerans	0	1	
Gram-negative rods	0	2	
Gram-positive rods	0	1	
Gram-positive anaerobic rods	1	0	
Multi-drug-resistant isolates	3	22	25 (28.1%)

[a]Please note that the total amount of canine isolates is higher than the total amount of positive canine bacterial cultures. In 2 dogs 3 bacterial isolates and in 9 dogs 2 bacterial isolates were obtained.

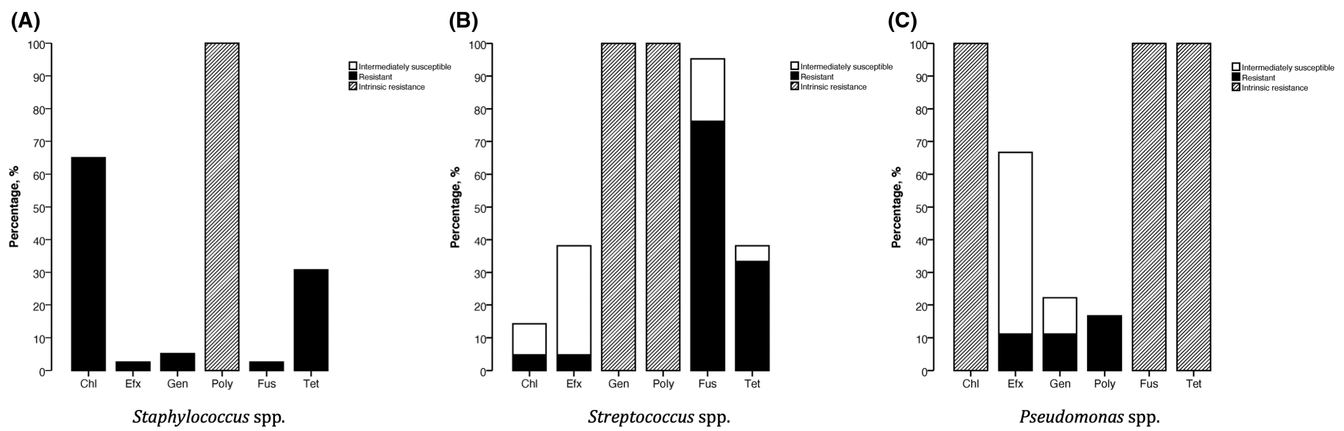

FIGURE 1 Antibiotic resistance in the three most commonly cultured isolates from canine corneal stromal ulcerations between 2012 and 2019. Intrinsic resistance is marked separately. (A) Acquired resistance in *Staphylococcus* spp. was most frequently observed against chloramphenicol. (B) Acquired resistance in *Streptococcus* spp. was most frequently observed against fusidic acid. (C) Acquired resistance in *Pseudomonas* spp. was most frequently observed against enrofloxacin. Chl, chloramphenicol; Efx, enrofloxacin; Fus, fusidic acid; Gen, gentamicin; Poly, polymyxin B; Tet, tetracycline

concurrent ophthalmic conditions, recent treatment with topical corticosteroids, and brachycephalic breed) and bacterial culture results, as none of these comparisons yielded a statistically significant association.

3.2 | Cats

The majority of cats (14/33) were domestic short-haired cats, with nine other breeds also represented, including

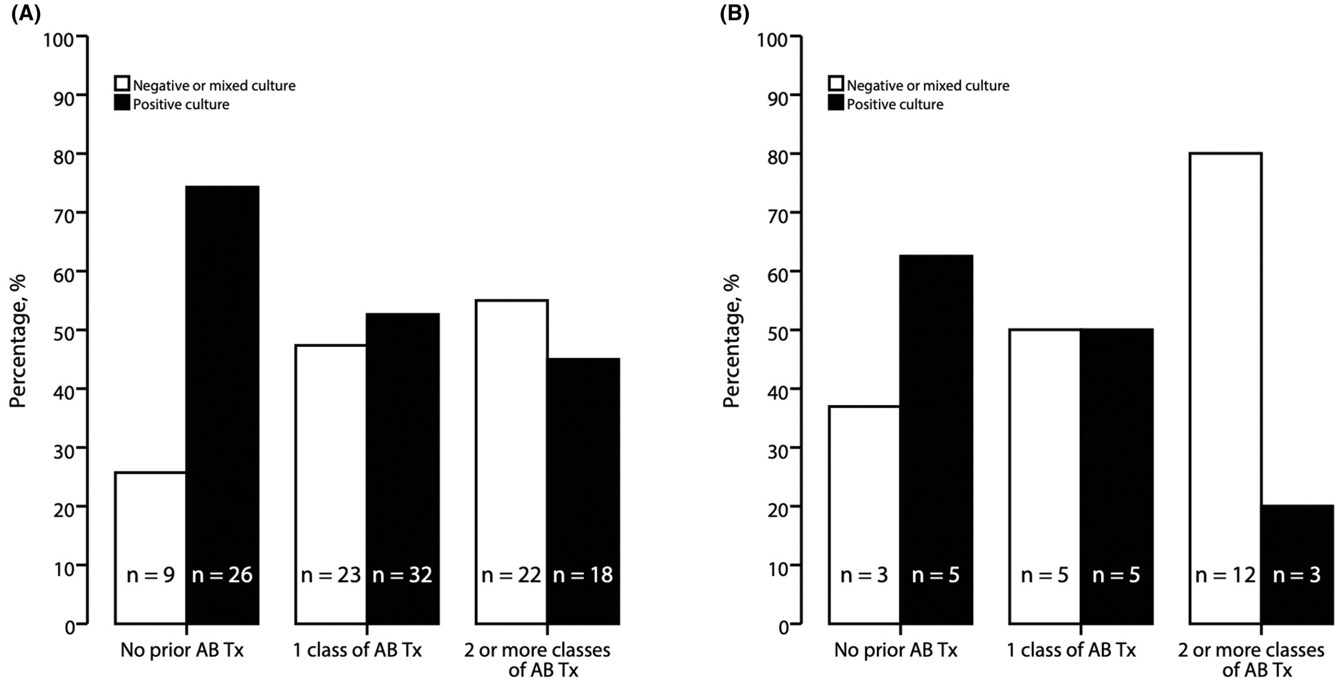

FIGURE 2 Association between previous treatment with topical antibiotics and bacterial culture results in (A) dogs and (B) cats with corneal stromal ulcerations. The probability of positive culture results was significantly lower in patients previously treated with one or more classes of topical antibiotics ($\chi^2 = 6.52$, $p = .011$ and $\chi^2 = 4.27$, $p = .039$, in dogs and cats, respectively; Mantel-Haenzsel test for trend)

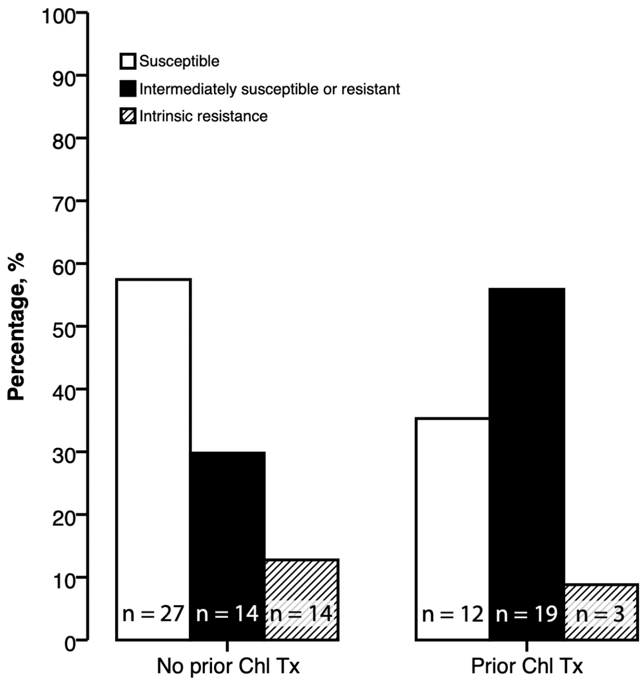

FIGURE 3 Antibiotic susceptibility to chloramphenicol of bacterial pathogens isolated from corneal stromal ulcerations in dogs with or without previous treatment with topical chloramphenicol within 4 weeks of presentation. Previous treatment with chloramphenicol was associated with a higher incidence of acquired resistance to chloramphenicol ($\chi^2 = 5.23$, $p = .022$)

Persian (6/33), British short-haired (3/33), Maine Coon (3/33), Sphynx (2/33), Burmese (1/33), Ragdoll (1/33), Siberian (1/33), Abyssinian (1/33), and Exotic short-haired (1/33) cats. The right eye was affected in 17 and the left eye in 16 cases. There were 24 males and nine females with a median age of 6.8 years (range, 2 months–15.4 years). Concurrent ophthalmic conditions were observed in 21 affected eyes and 29 cats had recently received treatment with topical antibiotics and topical or oral corticosteroids, as shown in Table 1. Previous treatment with topical antibiotics was noted in 76% of the cats: 30% received treatment with one class of topical antibiotics, and 46% with two or more classes.

Positive cultures were obtained from 13 feline samples (13/33; 39%) and all contained a pure strain of one bacterial isolate (Table 3). *Staphylococcus* species were the most commonly isolated pathogen (n = 8; 62%), of which all but two were *Staphylococcus felis*. No methicillin-resistant Staphylococci were found. *Streptococcus* species were the second most commonly isolated pathogen (n = 2; 15%), and both of the isolates were beta-hemolytic streptococci. *Pseudomonas aeruginosa* (n = 1; 8%), *Mycoplasma* species (n = 1; 8%), and Gram-positive anaerobic cocci (n = 1; 8%) were less commonly found. One of the feline isolates was considered to be multi-drug resistant.

Resistance of feline *Staphylococcus* spp., beta-hemolytic-*Streptococcus* spp., and *Pseudomonas aeruginosa* isolates

TABLE 3 An overview of the bacterial culture results from feline clinical patients with corneal stromal ulcerations, referred to the Ophthalmology Section at the University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht University, the Netherlands between January 2012 and December 2019

	2012–2015	2016–2019	Total
Feline cultures, *n*	10	23	33 (100%)
Negative, *n*	5	13	18 (54,5%)
Mixed, *n*	1	1	2 (6.1%)
Positive, *n*	4	9	13 (39.4%)
Feline bacterial isolates, *n*	4	9	13 (100%)
Staphylococcus species, *n*	3	5	8 (61.5%)
Staphylococcus felis	2	4	
Staphylococcus pseudointermedius	1	0	
Staphylococcus aureus	0	1	
Streptococcus canis, *n*	0	2	2 (15.4%)
Other species, *n*	1	2	3 (23.1%)
Pseudomonas aeruginosa	1	0	
Mycoplasma spp.	0	1	
Gram-positive anaerobic cocci	0	1	
Multi-drug resistant isolates	0	1	1 (3.0%)

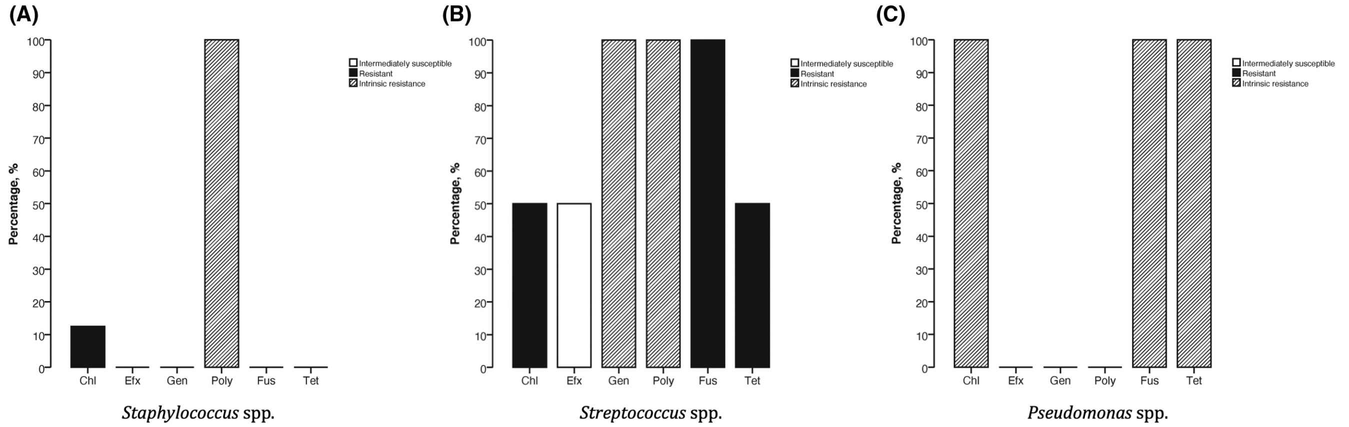

FIGURE 4 Antibiotic resistance in isolates from feline corneal stromal ulcerations between 2012 and 2019. Intrinsic resistance is marked separately. (A) Acquired resistance in *Staphylococcus* spp. was most frequently observed against chloramphenicol. (B) Acquired resistance in *Streptococcus* spp. was most frequently observed against fusidic acid. (C) In this single isolate of *Pseudomonas* spp., acquired resistance was not observed. Chl, chloramphenicol; Efx, enrofloxacin; Fus, fusidic acid; Gen, gentamicin; Poly, polymyxin B; Tet, tetracycline

to selected antibiotics was observed, as shown in Figure 4. Acquired resistance in *Staphylococcus* spp. was observed for chloramphenicol, in beta-hemolytic-*Streptococcus* spp. for chloramphenicol, fusidic acid, and tetracycline. No acquired resistance was seen in the single *Pseudomonas aeruginosa* isolate.

The probability of a positive culture result was significantly lower in cats previously treated with one or more classes of topical antibiotics ($\chi^2 = 4.27$, $p = .039$, Mantel-Haenzsel test for trend; Figure 2). Previous treatment with chloramphenicol was not associated with an increase in resistance patterns ($p = .556$).

Previous topical antibiotic therapy in the feline patient group did not differ significantly between the period 2012–2015 and 2016–2019 ($p = .205$). Neither the incidence of acquired antibiotic resistance nor the incidence of multi-drug-resistant isolates changed significantly over time between the period of 2012–2015 and 2016–2019 (0% vs. 11.1%, $p > .05$).

4 | DISCUSSION

In this study, the most common bacterial isolates cultured from corneal stromal ulcerations were *Staphylococcus*, *Streptococcus*, and *Pseudomonas* species in dogs, and *Staphylococcus* and *Streptococcus* species in cats. In general, this corresponded with those found in previous studies.[4–6,11,16] Our study did not demonstrate an increase in antibiotic resistance over an 8-year time period. A previous study by Tolar et al. evaluated the resistance patterns to a selection of antibiotics in 97 dogs with bacterial keratitis from 1993 to 2003 and also did not observe significant changes in antibiotic resistance.[4] However, in the current study, an increase in the amount of multi-drug-resistant isolates was observed over the years. This might be explained by the clustering of resistance within specific isolates in the second time cohort, instead of a more general spread of resistance among all isolates in the first time cohort. The increasing incidence of multi-drug-resistant isolates fits the known emerging antibiotic resistance in (veterinary) ophthalmology.[2,11,16–18]

The trend toward more antibiotic resistance to commonly prescribed empirical topical antibiotics, which was demonstrated for chloramphenicol in dogs in this study, was also described by several other authors in both veterinary and human ophthalmology.[4,16,19,20]

The results of this study demonstrated that the majority of bacterial isolates were sensitive to enrofloxacin. Therefore, it might be tempting to choose fluoroquinolone as the initial antibiotic until bacterial culture results are available. However, several studies have shown that the common use of fluoroquinolones is associated with a rapid increase in antibiotic resistance among bacteria.[11,21] The World Health Organization has classified fluoroquinolones and polymyxin as reserved, last resort antibiotics,[22] the use of which should thus be avoided.

As CLSI and manufacturer breakpoints are not available to define resistance to topically applied antibiotics, systemic breakpoint data were used in our study. The use of systemic breakpoint data for topically applied antibiotics is controversial.[23,24] For most topical agents, the sound pharmacokinetic data that are needed to set proper clinical breakpoints are not available. It is commonly assumed that corneal tissue concentrations of topically applied antibiotics will be equal to or greater than serum concentrations after systemic administration. Consequently, the in vitro resistance of bacteria to an antibiotic may be overcome by the high corneal drug concentrations achieved with topical antibiotic therapy in vivo. Thus, in vitro susceptibility testing for corneal isolates may underestimate the actual percentage of bacteria susceptible to an antibiotic when the patient is treated topically.

There are several limitations to this study. Due to the retrospective nature of this study, we could only test for trends and associations. To determine causal relations prospective experimental studies are warranted. Furthermore, it is possible that, for a number of patients, data on clinical brachycephaly related characteristics such as medial entropion were not complete because they had not been noted as a separate entity in the patient file. For this study, only conditions were scored that were specifically noted in the file. Therefore, incomplete descriptions in patient charts could have caused an underestimation of concurrent ophthalmic conditions.

Isolates graded 'intermediately susceptible' in susceptibility testing were classified 'resistant' to a particular antibiotic for statistical testing in order to be able to pick up on any trend toward decreasing antibiotic susceptibility over time, and fully answer the objectives of this study. However, when translated into a clinical setting, the results of this study design could have led to an overestimation of antibiotic resistance. The breakpoints used are based on systemic dosing and pharmacokinetics/pharmacodynamics. As ophthalmic treatments are usually applied topically, much higher concentrations of antibiotics can be achieved locally. This means that antibiotics classified as resistant based on systemic breakpoints might still be effective when applied topically. The classification used particularly affected enrofloxacin with seven out of 21 Staphylococcus spp. isolates and five out of nine Pseudomonas aeruginosa isolates testing intermediately susceptible.

Measurement of the craniofacial ratio (CFR) of patients was not part of the exam. For the purpose of this study, craniofacial ratios described for specific dog breeds by Packer et al. were used, and dogs were classified as belonging to a brachycephalic breed.[12,13]

During the period of this study, the methods for antibiotic susceptibility testing were changed, which could have had an effect on test results. However, this effect is expected to be limited, as both tests included the specific antibiotics of interest and were interpreted by the CLSI Performance Standards for Antimicrobial Susceptibility Testing.[25] This change in testing methods could have had an effect on determining multi-drug-resistant isolates because more types and more classes of antibiotics were included in the microbroth dilution method (22 types, 13 classes) compared with the disk diffusion method (18 types, 11 classes) and it was decided that the change in method coincided with the start of the second time cohort. Although the chances of finding a multi-drug resistant isolate theoretically increased by adding more antibiotic classes to the antibiotic susceptibility testing method, this effect was expected to be minor. The two antibiotic classes that were added in 2016 were metronidazole and nitrofurantoin, neither of which is topically applied in veterinary ophthalmology. If these two antibiotic classes were excluded from the analysis of multi-drug-resistant isolates, the number of multi-drug-resistant isolates remained unchanged.

All data were collected at a referral hospital. As most patients had already been seen by a referring veterinarian, most animals had received previous treatment with at least one type of topical antibiotic. Previous treatment with topical antibiotics could have had an effect on the type of bacterial pathogens found and their corresponding antibiotic susceptibility. Therefore, the incidence of bacterial pathogens and resistance to specific antibiotics found in this study could differ from the incidence at first presentation in primary veterinary practice.

5 | CONCLUSION

This study found *Staphylococcus*, *Streptococcus*, and *Pseudomonas* species as the most common pure isolates in dogs and *Staphylococcus* and *Streptococcus* species as the most common pure isolates in cats with corneal stromal ulcerations in a referral population in the Netherlands.

Although the incidence of acquired antibiotic resistance in isolates of dogs and cats did not change significantly over the 8-year time period, the incidence of multi-drug-resistant isolates in dogs increased significantly over time. In addition, the resistance of bacterial isolates to chloramphenicol was more common in dogs that received previous treatment with chloramphenicol. These results underline that prudent antibiotic use is important, both in the first opinion and in referral practice.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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