Antimicrobial resistance profiles of bacteria associated with lower respiratory tract infections in cats and dogs in England

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Abstract
Background: Bacterial lower respiratory tract infections (bLRTIs) are common and potentially life threatening in cats and dogs. Antibiotic treatment is often initiated before the diagnosis of bLRTI; therefore improved knowledge of the aetiology and antibiotic susceptibility patterns of these infections is essential to inform empiric antibiotic choices.

Methods: A retrospective study of microbiological, cytological results and their drug susceptibilities from lower respiratory samples (n = 1989) processed in a UK commercial laboratory between 2002 and 2012 was carried out.

Results: Thirty-nine per cent of feline samples and 50% of canine samples were positive for bacterial growth with most yielding a single organism (72 % and 69%, respectively). *Bordetella bronchiseptica* (20.2% from dogs and 2.3% from cats), *Pasteurella* spp. (23.2%, 31.8%), *E. coli* (16.2%, 13.6%) and *Pseudomonas* spp. (11.1%, 11.4%) were most frequently isolated from cytologically positive samples which contained intracellular bacteria (10%, 14%). Amoxycillin-clavulanate, cephalothin, cefovecin, oxytetracycline and trimethoprim/sulfamethoxazole showed modest in vitro activity against *E. coli* from dogs (approximately 70% susceptibility). *Pseudomonas* spp. were resistant to enrofloxacin (50%), ticarcillin (25%) and marbofloxacin (13%) but showed lower or zero resistance to aminoglycosides (approximately 7%) and ciprofloxacin (0%). Multi drug resistance (acquired resistance to three or more antimicrobial drug classes) was particularly common among *E. coli* isolates, with 23% from feline samples and 43% from canine samples.

Conclusion: Resistance to certain first-choice antibiotics was detected in bLRTIs highlighting the need for continued monitoring and sound evidence to inform decision-making in the management of these infections.

1 INTRODUCTION

The correct identification of patients with lower respiratory tract (LRT) disease who will benefit from antibiotics and the early administration of adequate treatment are crucial to ensure successful clinical outcomes and minimise the development of antibiotic resistance. This is complicated by the fact that no combination of clinical, radiographic or gross bronchoscopic findings can reliably confirm the diagnosis of LRT infection or predict its aetiology. A definitive diagnosis is assumed when intracellular bacteria are detected upon cytological examination of a lower respiratory sample and bacterial culture yields a positive result. However, bacteria are only demonstrable in a fraction of cytologic specimens from animals with bacterial lower respiratory tract infections (bLRTIs), and their absence does not rule out bacterial infection. Furthermore, the interpretation of bacterial culture results is hampered by difficulties in differentiating true infection from colonisation as the carina and large airways are not sterile. The resident microflora includes opportunistic organisms that are often responsible for bLRTIs in cats and dogs. Increased numbers of bacteria can be found in patients with reduced airway...
clearance, a complication often associated with conditions such as bronchitis, bronchiectasis and ciliary dyskinesia.11,12 Antimicrobial susceptibility testing should always be recommended to the client, although several factors can affect or delay this investigation. Certain patients may be considered unstable and at greater risk of obtaining a culture sample due to their respiratory disease, financial limitations may hinder the diagnostic procedures required or there may be clinical improvement on empirical treatment and therefore continuation with the chosen antibiotics without any diagnostic procedures.5,11,3 Furthermore, the prolonged turnaround time for bacterial identification and antibiotic susceptibility testing means that microbiology results are not usually available to help guide initial clinical decision-making regarding the management of these patients. As a result, antibiotic treatment is often initiated on an empirical basis in animals with clinical evidence supportive of LRT disease.

The use of antibiotics with poor or no in vitro activity against the pathogen(s) at the site of infection and delays in the administration of adequate treatment have been associated with higher morbidity and mortality in cases of human counterparts that are severely ill with bRTIs.13 Comprehensive antimicrobial protocols which guide decision-making for empirical antibiotic therapy have been developed for humans based on epidemiological data, and they take into account the aforementioned patient and antimicrobial factors.14 Guides to responsible use of antibacterials have been produced in veterinary medicine,15 but more elaborate protocols based on epidemiological data such as those developed in humans would be very useful given the high rates of empiric prescribing in small animal practice.16,14

The aims of this study were to obtain data on the aetiology and antibiotic resistance of bLRTIs from a large cohort of canine and feline patients in the UK, and to the author’s knowledge, few studies such as this one have investigated bacterial species from samples that include cytological evidence of respiratory disease.

2 MATERIALS AND METHODS

2.1 Data collection

Data from LRT samples from cats and dogs submitted to NationWide Laboratories (NWL; Lancashire, United Kingdom) for microbiological and cytological examination between May 2002 and May 2012 were retrieved. The data included species, age and gender of the patients, cytology reports and results from bacterial culture and antibiotic susceptibility testing. Samples from the same patient that had the same organisms with identical antibiotic susceptibilities and were submitted within 3 months of the original sample were excluded and those that did not have identical antibiotic susceptibilities were included in the analysis. Multidrug resistance (MDR) was defined as acquired resistance to three or more antimicrobial drug classes as proposed by Magiorakos et al.17

Laboratory methods. Aerobic and anaerobic semi-quantitative bacterial cultures of the LRT samples were performed. A 10 μl loop was used to streak each sample onto standard agar in four sectors consecutively. The plates were incubated overnight (18–24 h) at 37°C and examined the following day. The level of bacterial growth was reported as: no growth; scanty (colonies limited to the initial sector); moderate (colonies on sectors 1–3); or profuse (colonies on all 4 sectors). Microorganism identification was done by Gram-staining and standard biochemical procedures. Antimicrobial susceptibility testing was performed by the Kirby-Bauer method in accordance with the Clinical and Laboratory Standards Institute (CLSI) and the results interpreted based on contemporary criteria published by the same organisation.18–20

2.2 Cytology

Direct smears of the fluid or direct and cytocentrifuged preparations of tracheal wash (TW) or bronchoalveolar lavage (BAL) fluids were stained with Wright-Giemsa stain and examined by clinical pathologists at NWL. Cytology reports recorded overall cellularity, cell preservation, cell types, microorganisms (if present) and interpretation of findings. The authors classified the reports as acute neutrophilic, chronic-active (mixed), chronic or eosinophilic inflammation, haemorrhage or neoplasia. These classifications were based on recommendations in veterinary cytological textbooks and literature.8,21–26 The identification of intracellular bacteria was considered diagnostic of bacterial infection. Samples with evidence of oropharyngeal contamination (squamous epithelial cells, Simonsiella spp.) were excluded from the study.

2.3 Data analysis

Results were expressed as mean ± standard deviation (for age) and percentages. The frequency of different bacterial species in LRT samples was calculated by dividing the number of isolates from a certain species by the total number of bacterial isolates obtained from canine and feline samples. The two-sample (unpaired) t-test was used to compare mean ages at diagnosis between genders and animal species. A p-value < 0.05 was considered significant. Statistical analysis was performed using SPSS software (version 21, SPSS Inc., Chicago, USA).
2.4 | ETHICS STATEMENT

This work would be likely to gain approval in Europe under the European Directive 2010/63/EU (on the protection of animals used for scientific purposes) or an appropriate clinical ethics committee.

3 | RESULTS

3.1 | Patient population, age and sex of the patients

A total of 1989 consecutive non-repetitive lower airway samples from cats and dogs were submitted to NWL for bacterial culture and susceptibility testing during the 10-year period considered in our study. Most of the samples were collected using a (TW) technique (n = 1630) and the remaining by BAL (n = 359). The geographic distribution of the veterinary practices from which the samples originated is shown in Figure 1, a large proportion of which were collected from the West Midlands and Northwest UK. No information was provided on the type of practice (first opinion vs. referral), the samples were submitted from or any previous treatments administered to the patients. A considerable proportion of the LRT samples belonged to older patients aged nine or above (40% of cats and 36% of dogs). Only a small percentage of samples belonged to canine patients younger than 12 months (12%) but this age group accounted for roughly 30% of the cytology-positive samples. The mean age of patients with cytologically-confirmed bacterial infection was higher in cats than in dogs (7.8 vs. 5.1 years), with no significant differences between genders (p < 0.05). No considerable differences were observed between genders of both animal species in terms of proportion of samples positive for bacterial growth or cytological identification of intracellular bacteria.

3.2 | Bacterial culture

Thirty-nine per cent (188/485) of feline samples and 50% (744/1504) of canine samples were positive for bacterial growth, most yielded a single organism (72% and 69%, respectively). Gram-negative species predominated with Pasteurella spp. (37% in cats and 20% dogs), Enterobacteriaceae (15%, 22%) and non-fermenting Gram-negative bacilli (NF-GNB) (18%, 33%) accounting for the majority of isolates present in the respiratory samples (Table 1). Bordetella bronchi septica was the third most prevalent bacterial species in samples from canine patients but only three
TABLE 1  Bacterial species isolated from LRT samples of cats and dogs

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total %</td>
<td>TW %</td>
</tr>
<tr>
<td>α-haemolytic Streptococcus spp.</td>
<td>17</td>
<td>1.7</td>
</tr>
<tr>
<td>β-haemolytic Streptococcus spp.</td>
<td>29</td>
<td>2.8</td>
</tr>
<tr>
<td>S. aureus</td>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td>S. pseudintermedius</td>
<td>43</td>
<td>4.2</td>
</tr>
<tr>
<td>Bordetella bronchiseptica</td>
<td>157</td>
<td>15.4</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>164</td>
<td>16.1</td>
</tr>
<tr>
<td>Pasteurella spp</td>
<td>208</td>
<td>20.4</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>136</td>
<td>13.4</td>
</tr>
<tr>
<td>NF-GNB</td>
<td>333</td>
<td>32.7</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>221</td>
<td>21.7</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>12</td>
<td>1.2</td>
</tr>
<tr>
<td>Growth positive samples</td>
<td>49.5</td>
<td>51.5</td>
</tr>
<tr>
<td>Samples 1 isolate</td>
<td>68.7</td>
<td>69.0</td>
</tr>
<tr>
<td>Samples &gt; 2 isolates</td>
<td>31.3</td>
<td>31.0</td>
</tr>
</tbody>
</table>

Frequency of the different bacterial species in tracheal wash (TW), bronchoalveolar lavage (BAL), Cyto+ (cytology-positive) samples. Overall frequencies (independent of the method used to collect the samples and the results from cytological examination) are also shown (Total).

isolates were found in feline samples. Anaerobic bacteria represented 7.7%\(^2\) of the isolates from cats, and 1.2%\(^2\) of the isolates from dogs and were often found in combination with aerobic organisms (58% and 33% of anaerobe-positive samples from cats and dogs). *Bacteroides* spp., *Clostridium* spp. and *Prevotella* spp. were the most frequently isolated anaerobic species.

The most common combinations of organisms in cultures that yielded mixed bacterial growth were *Escherichia coli/Pasteurella* spp. (n = 5 samples in cats; n = 25 samples in dogs), *E. coli/Pseudomonas* spp. (n = 2 in cats; n = 22 in dogs) and *Pasteurella spp./Pseudomonas* spp. (n = 4 in cats; n = 9 in dogs).

3.3  Cytological examination

Cytology reports were available for 343 of 485 and 885 of 1504 samples from cats and dogs, respectively. Intracellular bacteria were detected in 48 (14%) feline samples and in 92 (10%) canine samples. Analysis of culture results revealed that *Pasteurella* spp., *Pseudomonas* spp., *E. coli*, *B. bronchiseptica* (dogs) and *Streptococcus* spp. (cats) were the bacterial species most frequently isolated from cytology-positive samples (Table 1) which is in agreement with the overall culture results described above. Approximately 25% of the samples that had cytological evidence of bacterial infection were culture negative. Intracellular bacteria could not be detected in the majority of cytological specimens from LRT samples that yielded bacterial growth on culture (73% of samples from cats and 82% of samples from dogs). The relative proportions of the different bacterial species isolated from cytology-positive samples seemed to be in accordance with those isolated by BAL in both cats and dogs. Most cytological specimens (with or without intracellular bacteria) were classified as acute neutrophilic (24% of cat samples and 36% of dog samples) or chronic-active inflammation (28% and 28%, respectively).

3.4  Susceptibility to antibiotics

The resistance profiles of *B. bronchiseptica* (157 in dogs), *Pasteurella* spp. (208 in dogs, 92 in cats), *Pseudomonas* spp. (136 in dogs) and *E. coli* (164 in dogs, 30 in cats) isolates (which represented approximately 65% of the isolates obtained in our study) are shown in Tables 2–3.

Dogs: For most antibiotics, the levels of resistance were low (<9% resistance) among *Pasteurella* spp. and *B. bronchiseptica* isolates (except to ampicillin, trimethoprim/sulfamethoxazole and cefovecin in the latter). Amoxicillin-clavulanate, cephalothin, cefovecin, oxetetracycline and trimethoprim/sulfamethoxazole showed modest in vitro activity against *E. coli* and only fluoroquinolones retained good activity against these isolates (>80% susceptible). *Pseudomonas* spp. were resistant to most of the antibiotics tested, except aminoglycosides (approximately 90% susceptibility to gentamycin, tobramycin and amikacin) and ticarcillin (approximately susceptibility > 70%). Fluoroquinolones were the antibiotics that showed better in vitro activity against the isolates tested. The highest levels of resistance to this
### TABLE 2  Antimicrobial resistance patterns of canine isolates. Only isolates with >30 data points have been included

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Total/Type of growth</th>
<th>P</th>
<th>AMP</th>
<th>AMC</th>
<th>CEF</th>
<th>CEX</th>
<th>CFX</th>
<th>CVN</th>
<th>OT</th>
<th>SXT</th>
<th>ENR</th>
<th>MAR</th>
<th>CIP</th>
<th>CN</th>
<th>AK</th>
<th>TOB</th>
<th>TIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia (164)</td>
<td>Total</td>
<td>56</td>
<td>26</td>
<td>26</td>
<td>–</td>
<td>–</td>
<td>26</td>
<td>32</td>
<td>–</td>
<td>26</td>
<td>12</td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas (136)</td>
<td>Total</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>50</td>
<td>13</td>
<td>0</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Bordetella (157)</td>
<td>Total</td>
<td>–</td>
<td>47</td>
<td>1</td>
<td>–</td>
<td>97</td>
<td>1</td>
<td>–</td>
<td>57</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pasteurella (208)</td>
<td>Total</td>
<td>–</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>4</td>
<td>–</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Abbreviations: AK, amikacin; AMP, ampicillin; AMC, amoxicillin-clavulanate; CEE, cephalothin; CEX, cefalexin; CN, gentamicin; CIP, ciprofloxacin, CLI, clindamycin; CVN, cefovecin; ENR, enrofloxacin; MAR, marbofloxacin; OT, oxytetracycline; P, penicillin; SXT, trimethoprim/sulfamethoxazole; TOB, tobramycin; TIC, ticarcillin.*

### TABLE 3  Antimicrobial resistance patterns of feline isolates. Only isolates with >30 data points have been included

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Total/Type of growth</th>
<th>P</th>
<th>AMP</th>
<th>AMC</th>
<th>CEF</th>
<th>CEX</th>
<th>CFX</th>
<th>CVN</th>
<th>OT</th>
<th>SXT</th>
<th>ENR</th>
<th>MAR</th>
<th>CIP</th>
<th>CN</th>
<th>AK</th>
<th>TOB</th>
<th>TIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia (30)</td>
<td>Total</td>
<td>47</td>
<td>23</td>
<td>23</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pasteurella (92)</td>
<td>Total</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Abbreviations: AK, amikacin; AMP, ampicillin; AMC, amoxicillin-clavulanate; CEE, cephalothin; CEX, cefalexin; CN, gentamicin; CIP, ciprofloxacin, CLI, clindamycin; CVN, cefovecin; ENR, enrofloxacin; MAR, marbofloxacin; OT, oxytetracycline; P, penicillin; SXT, trimethoprim/sulfamethoxazole; TOB, tobramycin; TIC, ticarcillin.*

Antibiotic class were observed in *Pseudomonas* spp. The in vitro susceptibility of *Pseudomonas* spp. to ciprofloxacin was 100%, while it was 87% to marbofloxacin and 50% to enrofloxacin. MDR was particularly common among *E. coli* isolates (43%).

Cats (isolates > 30 samples): For most antibiotics, the level of resistance was low among *Pasteurella* spp. (<9% resistance). Resistance to ampicillin was considered high against *E. coli* isolates (approximately 50%) but cefovecin and doxycycline showed good in vitro activity against these isolates (approximately 10% resistance each). Amoxicillin-clavulanate and cefalothin retained good activity against *Pasteurella* spp (100% susceptible) and moderate activity against *E. coli* isolates (approximate susceptibility > 70%). 23% of *E. coli* isolates were MDR.

### DISCUSSION

Cats and dogs suspected of bLRTI often receive antibiotic treatment before the diagnosis is confirmed. Overuse or inappropriate use of antibiotics, particularly in a veterinary hospital environment in the USA, can lead to increased numbers of resistant bacteria being identified. In human medicine, initial antibiotic choices can have a significant impact on patient outcomes as inappropriate treatment, defined as the use of antibiotic(s) to which the documented pathogen is resistant, is associated with higher morbidity and mortality. Another study found that dogs suffering from pneumonia were often infected with bacteria resistant to antibiotics administered for 4 weeks on an empirical basis; although this should be interpreted with caution as confounding factors such as acquired bacterial resistance and changing bacterial populations should also be considered. An up-to-date knowledge of the aetiology and antibiotic resistance of bLRTIs is needed to improve the likelihood of adequate empiric coverage.

The data reported here are largely in agreement with previous reports on the aetiology of bLRTIs in cats and dogs although with differences in the frequency of the bacteria detected. Variations in these frequencies between the different studies may reflect differences in study design. For example the number of cases tested, difficulty differentiating between primary infectious disease and non-infectious respiratory disease, disparities in geographical location, timeframes of sampling and the fact that some studies lacked information on previously administered antimicrobial treatment before sampling. Furthermore, as our study was performed over a 10-year period, it is possible that results from parts of this period as well as results performed after the completion of this study may vary.

In our study, the majority of samples that yielded bacterial growth isolated a single pathogen (71.8% from cats and 68.7% from dogs). *Pasteurella* spp., *E. coli*, *Pseudomonas* spp. and *B. bronchiseptica* (the latter only in dogs) were the bacterial species most commonly implicated, accounting for 65% of isolates. A recent study in the USA has highlighted the potential of dysbiosis as associated with canine pneumonia.
while acknowledging the importance of overgrowth by a single dominant phylotype.32

Cytology positive samples were found to be very low in our study (14% in cats and 10% in dogs) compared to culture positive samples (39% and 50%, respectively), and intracellular bacteria were not detected in the majority of cytological specimens that were culture positive (73% and 82%, respectively). A recent study examining TW cytology results from dogs with respiratory disease revealed similar results of discordancy with a high number of cytology negative and culture positive results (55.4%).31 Pre-treatment with antibiotics may affect cytology results and as no information was provided on the administration of medication to patients before sample collection in our study, this influential factor cannot be excluded.2,33 In addition, culture of commensal organisms in animals with non-infectious respiratory disease may have also affected these results or even bacteria that are difficult to culture but still detectable on cytology. One example of this is Mycoplasma spp. which are hard to culture but can act as primary pathogens in respiratory infections in cats and dogs.33,34

Broad-spectrum coverage is recommended for patients suspected of bacterial pneumonia due to the multiplicity of agents that can be involved.1,2,5,11,16,35 From our laboratory results, amoxicillin–clavulanate showed excellent in vitro activity against B. bronchiseptica and Pasteurella spp., but the levels of susceptibility were however lower in isolates of other bacterial species. For example, over 20% of E. coli were classified as resistant which is in agreement with similar studies.2,28,30,31 Nevertheless, good to moderate in vitro activity of amoxicillin–clavulanate against respiratory pathogens (except Pseudomonas spp.) was reported in previous studies.2,5,30,31

Fluoroquinolones were the antibiotics that showed better in vitro activity against the isolates tested as shown by a similar retrospective study of medical records between 1989 and 2011 in Germany.30 This could be due to their minimal use in veterinary medicine; possibly attributed to successful responsible antibiotic use messaging and education. The highest levels of resistance to this antibiotic class were observed in Pseudomonas spp., particularly those obtained from canine samples. The in vitro susceptibility of Pseudomonas spp. to ciprofloxacin (100%) was significantly higher than enrofloxacin (50%) and slightly superior to marbofloxacin (87%), this susceptibility trend to fluoroquinolones was similar for all isolates. The majority of enrofloxacin and/or marbofloxacin-resistant Pseudomonas isolates (>90%) detected were susceptible to aminoglycosides (gentamicin, amikacin and/or tobramycin) and ticarcillin. As with humans, antipseudomonal coverage should be considered in the treatment of patients with risk factors for P. aeruginosa infection such as pulmonary comorbidities.36 fluoroquinolones such as ciprofloxacin are one of the options as they penetrate well into the respiratory tract.1 In this study we used breakpoints that were the standard of practice at the time of the study (CLSI 2008). Since then new guide-lines (CLSI 2018) have developed with changes in certain breakpoints such as those for ciprofloxacin which would present differences in perceived antibiotic susceptibilities.

According to this and previous reports, anaerobes can be isolated from 1%–48% of bLRTI cases, either as a single agent or as part of a polymicrobial infection.2,28,37 Their role in bacterial pneumonia is not wholly understood, but it is prudent to provide anaerobic coverage in the empirical treatment of these infections. The spectrum of activity of amoxicillin–clavulanate encompasses most of the species of anaerobic bacteria that have been implicated in bLRTIs in cats and dogs. Enrofloxacin, on the other hand, is not active against obligate anaerobes but its spectrum can be broadened, for example, by the addition of a penicillin, clindamycin or metronidazole. Metronidazole monotherapy is, however, not recommended due its lack of activity against aerobic bacteria. Amoxicillin (non-potentiated) is also not recommended for the empiric use against respiratory bacterial infections given its limited spectrum and observed resistance levels against the most common respiratory pathogens, as previously reported.30

The value of antimicrobial treatment in uncomplicated cases of canine infectious respiratory disease (CIRD) is thought to be limited.36 It is indicated if deeper respiratory or systemic bacterial infection develops, particularly if fever, lethargy or inappetence is present alongside mucopurulent discharges.11,38 Doxycycline is regarded as a first-line antibiotic for the treatment of infections caused by B. bronchiseptica,39,42 the primary bacterial cause of CIRD.38,40 Resistance to this drug has been identified in other settings in the USA (1989–1995, 2001) and Germany (2010–2012);28,41,42 however the great majority (99.3%) of B. bronchiseptica isolates included in our study were susceptible to doxycycline. Unfortunately, Steinfeld et al have indicated that only a low proportion of other Gram-negative bacteria were susceptible to doxycycline, and a moderate proportion of Staphylococcus spp. Although B. bronchiseptica seems to be highly susceptible to this drug in vitro, some cases may be refractory and doxycycline may not be recommended for the empirical treatment of other bLRTIs.43,30,31,44 Based on our susceptibility data, amoxicillin–clavulanate is also a good choice for the treatment of B. bronchiseptica infections, which is in agreement with previous reports.28,41

Studies in humans show that the validity of bacterial culture in the diagnosis of bLRTIs can be significantly affected by just a few hours of effective treatment suggesting that the collection of airway samples for microbiological investigation should precede the initiation of empirical antibiotics.45,46 Furthermore, prior antibiotic therapy was associated with false-negative rates of 10%–40% in human patients with bacterial pneumonia,47 and several strategies have been proposed to increase diagnostic accuracy in such cases, including lowering the threshold for clinically significant growth or stopping antibiotic treatment.46,48

Waiting for the inhibitory effects of the antibiotics to
‘wash off’ can pose undue risks to the patients. In previous reports (USA, 2000–2008) concurrent antibiotic use did not seem to interfere with culture results\(^5,37\) as the pathogens were likely to have been refractory to the antibiotics administered. The optimal strategy for the collection of respiratory specimens when the patient is receiving antibiotics is yet to be defined, as are the thresholds for clinically significant bacterial growth. Therefore, microbiological results should always be interpreted with caution and in conjunction with other clinical and laboratory findings.

In this study the majority (82\%) of samples processed at NWL were collected by TW, either performed blindly or with the aid of a bronchoscope/endoscope. Surprisingly, there was a higher number of Pseudomonas isolates in TW samples than BAL samples even though Pseudomonas is frequently found to contaminate endoscopes.\(^49\) Veterinary laboratories usually perform semi-quantitative cultures of LRT samples therefore the most common clinical practice seems to be the semi-quantitative analysis of TW specimens. Furthermore, the lower airways in healthy dogs and cats are not considered sterile and the passage of an endoscope during BAL sampling can introduce oropharyngeal contamination.\(^32,50\) This highlights the importance of cytology in the evaluation of these patients.

This study used methods that were the standard of practice at the time of the study (2012) including CLSI breakpoints (CLSI 2008) and identification methods. New and improved standards have since been developed (CLSI 2018) which may have presented differences in the antibiotic susceptibility results. In addition, as the analysis of the study was based on laboratory submissions, there was no clinical information such as previous antibiotic treatments, symptoms, type of practice the samples were submitted from (first opinion or referral) or other factors that may have influenced the evaluation of the results.

5 | CONCLUSION

The results from our study provide evidence on the aetiology and antibiotic susceptibility patterns of bLRTIs to antibiotics commonly used in their treatment in cats and dogs. These results reflect the complex nature of such infections and the existence of several unsettled controversies in their diagnosis and treatment which highlights the importance of performing cytology and antimicrobial susceptibility testing when possible. Continued monitoring of the susceptibility profiles of bLRTIs is necessary to guide empirical treatment protocols as well as maintain antimicrobial efficacy and aid combat antibiotic resistance in veterinary and human medicine.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the substantial contribution to this study of Dr. Joana Fonseca, formerly of UCL. Unfortunately, we have been unable to contact Dr. Fonseca and so are unable to include her as an author.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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