1	Clinical Bacterial Isolates and Antibacterial Susceptibility Patterns of Clinical Bacterial
2	<b>Isolates</b> from Reptiles (2005-2016)
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4	Pak Kan Tang BVetMed; Stephen Divers BVetMed; Susan Sanchez PhD
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6	Royal Veterinary College, North Mymms, Hertfordshire, AL9 7TA, UK (Tang), Department
7	of Small Animal Medicine and Surgery (Divers, Tang), and Department of Infectious
8	Diseases (Sanchez), College of Veterinary Medicine, University of Georgia, GA, 30602,
9	USA.
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11	Dr. Tang's present address is School of Veterinary Medicine, University College Dublin,
12	Belfield, Dublin 4, Ireland.
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14	Email address correspondence to Dr. Tang (ptang1@rvc.ac.uk)
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**Objective** – To identify the bacterial isolates and their corresponding sensitivity patterns

# 26 STRUCTURED ABSTRACT

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29 from clinical cases and establish appropriate empirical antimicrobial agents in reptiles. 30 **Design** – Single-institutional retrospective study. **Sample populations** – 96 reptiles, 127 samples, 61 positive bacterial culture with a total of 31 32 129 identified organisms. Procedures - A retrospective analysis of medical records to identify bacterial culture 33 34 submissions and sensitivity results from reptiles presented between January 2005 and December 2016 to the Veterinary Teaching Hospital at the University of Georgia were 35 evaluated. Prevalence of bacterial genera and species were analysed. Results were 36 subcategorised into Gram-negative bacteria and Gram-positive bacteria, and their 37 38 susceptibility patterns against antimicrobial agents were reviewed. **Results** -48% of the submitted samples were cultured positive with a total of 129 bacterial 39 40 isolates. Pseudomonas spp. and Enterococcus spp. were the most frequently identified Gramnegative and Gram-positive bacteria in reptiles, respectively. Our isolated Gram-negative 41 bacteria demonstrated high sensitivities towards gentamicin (95.2%), tobramycin (91.5%), 42 amikacin (86.1%) and trimethoprim-sulfamethoxazole (82.9%). Majority of Gram-positive 43 bacteria were highly susceptible to doxycycline (100%), gentamicin (100%), 44 45 chloramphenicol (91.7%) and ampicillin (83.3%). Gram-positive bacteria were consistently resistant to ceftazidime in our study population. 46 Conclusions and clinical relevance – This study highlights the different antimicrobial 47 48 susceptibility results in aerobic Gram-negative and Gram-positive bacteria. Our results demonstrated that aminoglycosides, particularly amikacin, and potentiated sulphonamides are 49

50	appropriate	empirical	antibiotic	choices in	the presence of	Gram-negative	bacteria, while
						0	,

- 51 doxycycline or ampicillin are preferred initial choices for Gram-positive bacteria.
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### 53 Abbreviations

- 54 AMR Antimicrobial resistance
- 55 AAVLD American Association of Veterinary Diagnosticians
- 56 CLSI Clinical and Laboratory Standards Institute
- 57 EUCAST European Committee on Antimicrobial Susceptibility Testing
- 58 MALDI-TOF MS Matrix assisted laser desorption ionization-time of fliughtflight mass

59 spectrometry

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The discovery of antimicrobial agents was a major breakthrough in medicine in the twentieth 61 century<sup>1</sup>. Since then, antimicrobials have become fundamental therapeutic agents for treating 62 bacterial infections in both human and animals. Although the phenomenon of intrinsic and 63 64 acquired antimicrobial resistance (AMR) has long been recognised, a rising trend of prevalence has become critically apparent<sup>2,3</sup>. To a large extent this has been driven by the 65 selection pressures provoked by the increased popularity of antimicrobial drugs in both 66 humans and animals<sup>4</sup>. Widespread AMR reduces the effectiveness of antimicrobial therapies 67 68 against bacterial infection, results in treatment failure and increases clinical complications, leading to increased morbidity and mortailiy<sup>5</sup>. AMR is considered a global crisis affecting 69 70 humans, animals and the environment.

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Some genera of bacteria are innately resistance to certain classes of antimicrobial agents
through their inherent structural and/or functional characteristics; for example, *Enterococcus*is intrinsically resistant cephalosporins, clindamycin macrolides and fusidic acid and displays

75 low-level resistance to aminoglycoside<sup>6–8</sup>, while *Pseudomonas* is naturally resistant to many 76 antimicrobial agents, including most  $\beta$ -lactams, first generation guinolones, chloramphenicol, tetracycline, macrolides, potentiated sulfonamides and rifampicin<sup>7,9</sup>. Use of antimicrobial 77 78 drugs exerts selective pressure on bacterial populations, allowing an emergence and spread of acquired resistance traits in the community. Many veterinary surgeons commonly treat 79 reptiles empirically with broad-spectrum antibiotics, sometimes in the absence of a bacterial 80 infection. This not only promotes the selection of resistant strains but also delays an accurate 81 diagnosis. In addition, the unnecessary use of antimicrobials may cause adverse effects to the 82 reptile<sup>10</sup>. Indeed, the routine use of broad spectrum antimicrobials implies a low level of 83 expertise on the part of the clinician<sup>11</sup>. 84

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Reptiles have become increasingly popular as domestic pets over the last decades in Europe 86 and United States<sup>12,13</sup>. As a result, captive reptiles are presented more frequently for 87 veterinary attention and many of them have conditions with a bacteriological aetiology, either 88 89 as a primary disease or secondary to husbandry deficiencies, viral infections and immunosuppression<sup>14</sup>. Nevertheless, it is noteworthy that clinically healthy reptiles have a 90 resident gastrointestinal microflora microbiota which plays key roles in digestion and 91 92 immune function; maintenance of commensal microflora is of paramount importance<sup>11</sup>. 93 Therefore, an accurate definitive diagnosis of bacterial infection relies upon the 94 demonstration of both a host pathological effect (e.g. cytology, histopathology) and the causative agent (e.g. bacterial culture). Furthermore, sensitivity testing (e.g. diffusion disc) is 95 96 also necessary to modify drug selection due to resistance. Obviously, administration of 97 empirical antibiotics may be necessary prior to the culture and sensitivity results in critical cases; however, the routine use of Gram stains still provides vital information to guide initial 98 drug selection. 99

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101	The principle objective of this study was to determine the most common bacterial isolates
102	from clinical reptile cases, and their antimicrobial sensitivity patterns. Subsequently, this data
103	was used to modify our service's antimicrobial stewardship policy and help direct empirical
104	drug selection while awaiting culture and sensitivity results. We hypothesised that (1) the
105	antimicrobial susceptibility testing results would be vastly different between Gram-negative
106	and Gram-positive bacteria in reptiles, and (2) commonly advocated first tier drugs (e.g.
107	potentiated sulphonamides, basic penicillins) would be largely ineffective, necessitating the
108	use of more advanced drugs (e.g. fluroquinolones and advanced third or fourth generations of
109	cephalosporins).
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111	MATERIALS AND METHODS
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113	Medical records review
114	The Veterinary Teaching Hospital medical records and the Athens Veterinary Diagnostic
115	Laboratory databases at University of Georgia, College of Veterinary Medicine was searched
116	for all reptiles (lizard, chelonian, snake and crocodilian) presented between 2005 and 2016,
117	inclusive, from which samples were submitted for aerobic bacterial culture, and sensitivity
118	testing. Submitted samples (fresh tissue, fluids, swabs <sup>a</sup> ) were generally processed the same
119	day as collection. Samples collected outside of normal laboratory hours were refrigerated at
120	4°C until the next business day.
121	
122	Bacteriological methods
123	Samples uUpon receipt at the laboratory were processed according to the microbiology
124	laboratory standard operating procedures. The laboratory is accredited (most recent

125 accreditation: January 2018) by the American Association of Veterinary Diagnosticians (AAVLD) and fellows. Culture methodology varied depending on the site of collection and 126 type of samples that were submitted for culture<sup>15</sup>. Appropriate temperature and incubation 127 128 conditions (30°C in ambient atmosphere) were used. Samples were incubated for 48 hours before they were determined to be negative for bacterial growth. Bacterial isolates were 129 identified using traditional biochemical reactions until 2014<sup>16–19</sup>, when automated mass 130 spectrometry microbial identification system<sup>b</sup> was used for all isolates. Organisms that were 131 132 unable to be identified by matrix assisted laser desorption ionization-time of flight mass 133 spectrometry (MALDI-TOF MS) were likely to be environmental bacteria. Sensitivities were performed using disc diffusion. Human break point interpretation following Clinical and 134 Laboratory Standards Institute (CLSI) guidelines and European Committee on Antimicrobial 135 136 Susceptibility Testing (EUCAST – updated and reviewed each year over the retrospective period) were used for all antibiotics tested 20,21,30-39,22,40,41,23-29. 137

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## 139 Data Analysis

Digital medical records and microbiologic results were reviewed and tabulated using a 140 computerised spreadsheet<sup>c</sup>. Each specimen was assigned to a specific category depending on 141 the source of origin. Faecal samples were excluded from this study due to the presence of 142 143 commensal microfloramicrobes. All culture results without an antimicrobial susceptibility 144 pattern were also excluded. If multiple samples from the same patient were collected within a 3-month period from the same site, subsequent samples were excluded from this study. 145 Distinctions were made between reptiles treated with antimicrobial agents within a 14-days 146 147 period prior to sample collection ("treated") and the remaining populations ("untreated"). Isolated organisms were initially subcategorized into Gram-negative and Gram-positive 148

149	bacteria and their antimicrobial sensitivities were recorded. In addition, the antimicrobial
150	susceptibility patterns of the five most common bacterial isolates were analyzed individually.
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### 152 Statistical Analysis

153 Descriptive statistics were used to report the bacterial isolates and the susceptibility results. 154 Bacterial culture results (positive or negative) were compared between treated and untreated 155 reptiles using Chi-square ( $\chi$ 2) test. Statistical analyses were performed using commercially 156 available software<sup>d</sup>. For all statistical analyses, values of P < 0.05 were considered 157 significant.

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### 159 **RESULTS**

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Between 2005 and 2016, 131 samples originating from nine different sites (Table 1) of 100 161 reptiles (40 lizards, 30 chelonians, 29 snakes and 1 crocodilian) were initially submitted for 162 163 bacterial culture and sensitivity testing (including two fecal samples which were subsequently excluded from this study). Two repeated submissions (one ocular sample and one external 164 lesion sample from two lizards) were also excluded from the study. The most prominent 165 isolates from each anatomical site are illustrated in Table 1. Out of the remaining 127 166 167 samples, 48% (n=61) cultured positive (21 lizards, 26 chelonians and 14 snakes), in which 168 14.8% (n=9) had come from reptiles that had received antimicrobial therapy, either 169 systemically or topically, within the previous 14-days; no significant difference in the prevalence of positive culture was identified between treated (50%) and untreated groups 170 171 (47.7%) (P=0.857) (Figure 1). Out of the 61 positive cultures, 129 isolates were identified (Table 2). Of those, 74.4% were Gram-negative (n=96) and 25.6% were Gram-positive 172 173 bacteria (n=33). The antimicrobial sensitivity results based upon Gram staining are reported

174 in Table 2. A total of 34 genera of bacteria were identified, and the five most frequently isolated were *Pseudomonas* (n=19), *Enterococcus* (n=15), *Morganella* (n=9), *Staphylococcus* 175 (n=7) and *Escherichia* (n=7) (Table 3). Antimicrobial sensitivity results for each of these five 176 177 genera are reported in Figures 2-6 and Supplementary materials Tables 4-8. Antimicrobials to which an important proportion of each genus of bacteria might be expected to exhibit 178 179 intrinsic resistance are indicated by asterisks<sup>7</sup>. 180 Gram-negative bacteria were generally susceptible to most aminoglycosides (except 181 182 neomycin), second generation fluoroquinolones, advanced  $\beta$ -lactams and third generation cephalosporins. Sensitivity to trimethoprim-sulfamethoxazole (% S = 82.9%) was 183 comparable to enrofloxacin (%S = 81.5%) or ceftazidime (%S = 81.0%). There was 184 185 widespread resistance against penicillins, first and second generation cephalosporins, clindamycin, and azithromycin. 186 187 188 All Gram-positive isolates were susceptible to doxycycline, gentamicin and imipenem. Many isolates were susceptible to chloramphenicol, first and second generation cephalosporins, 189 penicillins, tetracyclines, and bacitracin. However, they displayed resistance to third and 190 fourth generation cephalosporins, clindamycin, fluoroquinolones, amikacin, and 191 trimethoprim-sulfamethoxazole. 192 193 194 *Pseudomonas* spp. (n=19) were isolated from six different sources and were predominantly in external lesion/abscess (n=7) and oral specimens (n=6). They were susceptible to most 195 196 aminoglycosides (except neomycin), second and fourth generation fluoroquinolones, third

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197 generation cephalosporins and advanced  $\beta$ -lactams. Interestingly, our analysis showed

198 reduced efficacy of extended-spectrum penicillins, trimethoprim-sulfamethoxazole and

199	second generation of fluoroquinolones including enrofloxacin and orbifloxacin agains
200	Pseudomonas spp.

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*Enterococcus* spp. (n=15) were isolated from seven remote sources and were principally in
 coelomic cavity (n=4), external lesion/abscess (n=3) and blood (n=3). All isolates were
 susceptible to doxycycline and gentamicin. Many isolates were also susceptible to broad
 spectrum beta-lactam antimicrobial agents. However, they showed resistance to most
 aminoglycosides (except gentamicin), ceftazidime, clindamycin, tetracycline, trimethoprim sulfamethoxazole, and second generation fluoroquinolones.

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209 *Morganella morganii* (n=9) was isolated from four different sample sites with external 210 lesion/abscess (n=4) and oral cavities (n=3) being the prime locations. All isolates were 211 susceptible to most of the antimicrobials, including aminoglycosides, fluoroquinolones, 212 doxycycline, florfenicol and ticarcillin; a few isolates showed reduced sensitivity to 213 trimethoprim-sulfamethoxazole (%S = 87.5%) and tetracycline (%S = 85.7%). However, 214 there was widespread resistance against first generation cephalosporin, aminopenicillin and 215 azithromycin.

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*Escherichia coli* (n=7) was isolated from three different sample sites with external
lesion/abscess (n=5) being the predominant location. The isolates were fully susceptible to
almost all antimicrobial agents tested, including aminoglycosides, many second and third
generation cephalosporins, chloramphenicol, doxycycline, trimethoprim-sulfamethoxazole
and ticarcillin. However, first generation cephalosporin and aminopenicillin demonstrated
antimicrobial resistance.

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Stankylogogous spn (n-7) was isolated from three remote origins with equilar being the

224	Staphylococcus spp. (n=7) was isolated from three remote origins with ocular being the
225	predominant site (n=4). All isolates were generally susceptible to an extensive variety of
226	antimicrobials, except some penicillins, ceftiofur, clindamycin and erythromycin.
227	
228	A total of 22 isolated bacteria were collected from nine reptiles that had received either
229	systemic and/or topical antibiotics, in which eight organisms from four samples displayed
230	resistance to the antimicrobial agent administered: two Pseudomonas sp. and one Monganella
231	morganii isolated from the oral cavity were resistant to azithromycin; one Stenotrophomonas
232	maltophilia isolated from the nasal cavity showed resistance to ciprofloxacin; one
233	Enterococcus sp. and one Gram-positive bacilli isolated from an external abscess/lesion
234	showed resistance to ceftazidime; one Streptococcus sobrinus and one Pseudomonas sp.
235	isolated from the oral cavity were resistant to enrofloxacin.
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#### 237 **DISCUSSION**

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There are currently limited studies and reviews on the use of antimicrobial agents in reptiles 239 240 and to date there is a lack of consensus on empirical antibacterial therapy<sup>42</sup>. Commonly, t<sup>The</sup> 241 rationale and justification on the empirical antibiotic therapy in reptiles is often imprudent and unconsidered<sup>10,42</sup>. Broad-spectrum antibiotics, especially enrofloxacin (second generation 242 243 fluoroquinolone) and ceftazidime (third generation cephalosporin), have been overused as first-line antibiotics by primary veterinarians and it is common practice to employ 244 antimicrobial therapy in the absence of a diagnosed infection<sup>10</sup>. Therefore, an appropriate 245 antimicrobial stewardship program is of paramount importance in minimizing the 246 development and implication of AMR. Despite position statements and publications on 247 antibiotic stewardship from various domesticated animal veterinary groups and organizations, 248

249	the zoological field has been notably deficient in addressing this problem. To the authors'
250	knowledge, this study is the first to report the clinical isolates and the corresponding
251	antimicrobial susceptibility patterns from reptilian samples in the United States.
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253	In the study reported here, a large proportion (52%) of samples submitted were cultured
254	negative; although there are numerous factors that could have influenced the culture results
255	including the locations and techniques of sampling, this may also reflect the
256	inappropriateness of bacterial culture requested by the attending veterinarians. Of the positive
257	<u>cultures</u> , Gram-negative bacteria are more commonly identified than Gram-positive bacteria,
258	with a prevalence of 74.4% and 25.6%, respectively. While this predominance of Gram
259	negatives has been well appreciated previously, it is important to note that isolated bacteria
260	vary based on the source of sample (reptile species and anatomical location) due to their
261	predilection for different environments <sup>14</sup> . Therefore, the prevalence of isolated bacteria
262	cannot be determined and is beyond the scope of this study.
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264	The findings from our study demonstrated that the Gram-negative isolates displayed high
265	levels of sensitivities towards aminoglycosides, especially gentamicin, tobramycin and
266	amikacin; of which the top three identified Gram-negative bacteria (Pseudomonas spp.,
267	Morganella morganii and Escherichia coli.) were all fully susceptible. Despite the well-
268	known risk of renal injury associated with aminoglycosides they remain crucial first-line
269	bactericidal agents against Gram-negative bacterial infections <sup>43–45</sup> . Gentamicin has a narrow
270	margin of safety and has been reported to be more nephrotoxic than amikacin <sup>45,46</sup> .
271	Consequently, when confronted with an initial Gram-negative result, the empirical use of
272	tobramycin, amikacin or trimethoprim-sulfamethoxazole (83.6%S) may be safer and
273	effective. However, it is noteworthy that the most frequently identified Gram-negative

bacteria (*Pseudomonas* sp.) and Gram-positive bacteria (*Enterococcus* sp.) are both innately
resistant to trimethoprim-sulfamethoxazole<sup>7</sup>; empirical use of trimethoprim-sulfamethoxazole
should be reconsidered in the presence of *Pseudomonas* sp. or *Enterococcus* sp. and
antimicrobial therapy should be adjusted according to the sensitivity testing results. Despite
the small number of *Salmonella* sp. isolated in the study, this organism is of importance
regarding human health due to its nature of zoonotic risk<sup>12,13</sup>. We found that all isolates were
all fully susceptible to trimethoprim-sulfamethoxazole, tobramycin and gentamicin.

282 Gentamicin was also highly effective *in-vitro* against Gram-positive bacteria in this study. However, it is worth noting that *Enterococcus* is considered innately resistant to 283 aminoglycosides<sup>7</sup> and the results obtained from using the high concentration discs in 284 285 antibiograms may not be applicable as monotherapy clinically. Of the effective alternatives, doxycycline or ampicillin are obvious choices for a first line drug, because imipenem 286 (carbapenems) and vancomycin (glycopeptides) should be avoided and reserved for more 287 serious life-threatening and multi-resistant infections<sup>47–50</sup>. Chloramphenicol should also be 288 reserved given its bacteriostatic nature and only used when required by sensitivity results<sup>10</sup>. 289 Surprisingly, all Gram-positive isolates were resistant to ceftazidime (third generation 290 cephalosporin), a semi-synthetic, broad-spectrum, bactericidal, beta-lactam antibiotic 291 commonly used in reptile medicine<sup>51,52</sup>. This drug should be reserved for refractory infections 292 to decrease the risk of beta-lactamase development and resistance<sup>53,54</sup>. As a result, the routine 293 use of ceftazidime as a first-line drug is discouraged, unless specifically indicated by Gram 294 stain or sensitivity results. 295

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297 In this study, samples were obtained from both untreated and antibiotic-treated reptiles.

298 Although this may limit the validity of results, it reflects the reality of clinical reptile cases

299 presented to referral hospitals or for second opinions. Our results also demonstrated that the prevalence of positive culture did not differ significantly between treated and untreated 300 reptiles, which raises questions of appropriate drug dosing and efficacy. Repeated 301 302 submissions from the same animal collected from the same site within a 3-month period were excluded from this study to avoid selection bias of organisms and the corresponding 303 susceptibility patterns. Nevertheless, any samples collected from different body sites and/or 304 obtained at least three months apart were included; however, the occurrence of multiple 305 306 submissions from the same animal was 6.3% (n=8).

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The most important limitation of this study pertains to its retrospective nature, including the 308 309 variation of drug discs used in antibiograms for susceptibility testing across the study timeframe according to the recommended methods for disk diffusion susceptibility 310 testing<sup>32,33</sup>. Some drug discs appear in antibiograms intermittently and infrequently; this 311 reduced the validity of the susceptibility results from those antimicrobial drugs. Another 312 313 limiting factor was the small number of culture and sensitivity results obtained from most genera. Additional limitation includes the absence of Gram-staining results in this study and 314 the current lack of published agreement between Gram-staining results and bacterial culture 315 in reptiles. Nevertheless, Gram staining remains an easy and rapid technique for the detection 316 and differentiation of Gram-positive and Gram-negative bacteria in clinical practice, and 317 318 should be used to help direct initial drug selection while pending culture and sensitivity results. 319

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Initial selection of antimicrobials cannot be guided by the results of bacterial culture and
sensitivity assays. In critical cases where bacterial infections are strongly indicated, such as
septicemia and other acute, life-threatening infections, empirical selection of antimicrobial

agent should be directed by direct Gram smears as well as the likely organisms found at
specific anatomical locales, spectrum of antimicrobial activity, potential adverse effects,
pharmacokinetics, and practicality of administration<sup>53,55</sup>. Antibacterial drugs with the
narrowest spectrum of activity should always be considered first; not only does this allow
veterinarians to select antimicrobials that are most likely to be effective, but also limits the
collateral damage to the commensal microflora and minimizes the development of resistant
pathogens<sup>53,55</sup>.

331 Prospective investigations are required to further evaluate the pharmacokinetics and

332 pharmacodynamics of the first-line antimicrobial agents in different species of reptiles.

333 Future study to investigate the trends of antimicrobial susceptibility in bacterial isolates from

reptiles is warranted and standardization of antimicrobial drug discs used in antibiograms, or

a move to a more quantifiable approach (e.g. minimal inhibitory concentration) is

recommended. Further prospective studies are also needed to evaluate the correlation

between results from bacterial cultures and Gram's stains in reptiles as only fair agreement

338 was shown in amazon parrots.<sup>56</sup> In addition, further investigation of clinical antibiotic drug

efficacy is required, since the *in vitro* susceptibility results may not always predict the clinical

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response.

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In conclusion, the findings from this retrospective study highlight the different antibacterial susceptibility results from Gram-negative and Gram-positive bacteria cultured from clinical reptiles cases. It is recommended to perform Gram staining technique on direct smears when considering the initial use of empirical antibiotic therapy. As such, the presence of Gramnegative bacilli (most likely *Pseudomonas* spp., *Morganella morganii*. and/or *Escherichia coli*.) may indicate that amikacin, tobramycin or trimethoprim-sulfamethoxazole are appropriate first-line choices, while awaiting bacterial culture and sensitivity results. In

349	addition,	doxycycline c	or ampicillin	appears to	be the most	suitable firs	st-line c	choices ag	gainst
	,		1	11					-

- 350 Gram-positive infections (most likely Enterococcus spp. and/or Staphylococcus spp.). Our in-
- *vitro* susceptibility results demonstrate the complete ineffectiveness of ceftazidime against
- 352 Gram-positive bacteria and it has no superior efficacy against Gram-negative bacteria
- 353 compared with other antimicrobial agents (including trimethoprim-sulfamethoxazole). Future
- reptile pharmacokinetic studies should focus on first line drugs like doxycycline,
- 355 trimethoprim-sulfamethoxazole and basic penicillins, rather than the newest
- 356 fluoroquinolones, cephalosporins or advanced penicillins.
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### 358 FOOTNOTES

- a. BBL culture swab, Becton Dickinson, Franklin Lakes, NJ.
- 360 b. Vitek® MS, BioMérieux, Durham, NC.
- 361 c. Excel version 15.38 for Mac, Microsoft Corporation, Redmond, WA.
- d. SPSS Version 24, IBM Corporation, Armonk, NY.
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# **364 AUTHOR NOTE**

- 365 Data from this study was presented in abstract form at ExoticsCon 2018, Atlanta, GA.
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to Review Only

Sample Location	Number	Isolates	Most common isolates
External	21	20	Pseudomonas sp. (15.2%, n=7)
lesion/abscess			
Ocular	10	13	Staphylococcus sp. (18.2%, n=4)
Oral cavity	11	14	Pseudomonas sp. (24%, n=6)
Respiratory	8	11	Stenotrophomonas sp. (21.4%, n=3)
Coelomic cavity	6	7	Enterococcus sp. (36.4%, n=4)
Blood	2	4	Enterococcus sp. (50%, n=3)
Miscellaneous	2	3	Salmonella sp. (50%, n=2)
Bone	1		Clostridium sp. (100%, n=1)

### Table 1. Summary of the origin of samples and the predominant isolates.

**Figure 1. Proportion of reptiles in retrospective study classified by culture results and status of prior antimicrobial treatment.** The prevalence of positive culture did not differ significantly between reptiles received antibiotics 14-days prior to sample collection (50%) and untreated reptiles (47.7%) (P=0.857).



Table 2. Antimicrobial susceptibility patterns of Gram-negative and Gram-positivebacteria isolated from reptilian samples obtained from the Zoological Medicine Servicein the Veterinary Teaching Hospital, University of Georgia (2005-2016).

Antimicrobial	Gram-negative Bacteria (n=96)				Gram-positive Bacteria			
				(n=33)				
	Total Number	% S	% R	% I	Total Number	% S	% R	% I
Amikacin	79	86.1	10.1	3.8	16	43.8	56.3	0.0
Amox/Clav	70	38.6	60.0	1.4	26	80.8	7.7	11.5
Ampicillin	50	36.0	62.0	2.0	18	83.3	16.7	0.0
Azithromycin	22	13.6	72.7	13.6	9	44.4	44.4	11.1
Bacitracin	11	18.2	81.8	0.0	8	87.5	12.5	10.0
Carbenicillin	9	66.7	22.2	11.1	-	-	-	-
Cefazolin	12	33.3	58.3	8.3	9	66.7	33.3	0.0
Cefotaxime	12	25.0	58.3	16.7	-	-	-	-
Cefotetan	10	40.0	50.0	10.0	5-	-	-	-
Cefpodoxime	22	59.1	36.4	4,5	1	0.0	0.0	100.0
Ceftazidime	42	81.0	16.7	2.4	11	0.0	100.0	0.0
Ceftiofur	24	70.8	25.0	4.2	14	64.3	28.6	7.1
Cephalothin	53	37.7	60.4	1.9	6	83.3	16.7	0.0
Chloramphenicol	34	82.4	5.9	11.8	12	91.7	0.0	8.3
Ciprofloxacin	31	74.2	19.4	6.5	11	36.4	18.2	45.5
Clindamycin	19	0.0	100.0	0.0	17	11.8	82.4	5.9
Doxycycline	19	68.4	26.3	5.3	6	100.0	0.0	0.0
Enrofloxacin	81	81.5	9.9	8.6	26	34.6	23.1	42.3

Erythromycin	1	0.0	0.0	100	17	41.2	35.3	23.5
Florfenicol	22	59.1	31.8	9.1	11	81.8	9.1	9.1
Gatifloxacin	7	85.7	14.3	0.0	7	71.4	14.3	14.3
Gentamicin	62	95.2	4.8	0.0	6	100.0	0.0	0.0
Gentamicin	1	100.0	0.0	0.0	7	100.0	0.0	0.0
(120mcg)								
Imipenem	7	85.7	14.3	0.0	4	100.0	0.0	0.0
Marbofloxacin	1	100.0	0.0	0.0	2	50.0	0.0	50.0
Neomycin	19	52.6	26.3	21.1	9	66.7	33.3	0.0
Nitrofurantoin	5	20.0	80.0	0.0	1	0.0	100.0	0.0
Ofloxacin	19	94.7	5.3	0.0	9	55.6	33.3	11.1
Orbifloxacin	32	53.1	25.0	21.9	20	10.0	60.0	30.0
Oxacillin	1	0.0	100.0	0.0	6	33.3	66.7	0.0
Penicillin	23	4.3	95.7	0.0	22	77.3	22.7	0.0
Polymyxin B	19	78.9	21.1	0.0	9	55.6	22.2	22.2
Rifampin	-	-	-	-	5	80.0	0.0	20.0
Tetracycline	55	72.7	23.6	3.6	19	78.9	21.1	0.0
Ticarcillin	62	75.8	19.4	4.8	-	-	-	-
Tobramycin	71	91.5	8.5	0.0	9	66.7	33.3	0.0
Trimethoprim/Sulfa	70	82.9	11.4	5.7	17	52.9	41.2	5.9
Vancomycin	3	100.0	0.0	0.0	1	100.0	0.0	0.0

Table 3. Genera of bacteria isolated from all reptilian samples submitted for cultureand sensitivity from the Zoological Medicine Service in the Veterinary TeachingHospital, University of Georgia (2005-2016). The top five identified genera of bacteria arehighlighted in bold. *Pseudomonas* sp. (n=19) and *Enterococcus* sp. (n=15) were the mostcommon Gram-negative and Gram-positive bacteria isolated, respectively.

Genus	Number
Pseudomonas	19
Enterococcus	15
Aerobic Gram-negative Bacilli (non-specific)	10
Morganella	9
Escherichia	7
Staphylococcus	7
Corynebacterium	5
Proteus	5
Stenotrophomonas	5
Salmonella	4
Enterobacter	4
Providencia	4
Aeromonas	3
Chryseobacterium	3
Citrobacter	3
Klebsiella	3
Pasteurella	3
Acinetobacter	2
Moraxella	2

Streptococcus	2
Aerobic Gram-positive Bacilli (non-specific)	1
Actinomycete	1
Alcaligenes	1
Bordetella	1
Chryseomonas	1
Comamonas	1
Clostridium	1
Empedobacter (Flavobacterium)	1
Aerobic Gram-positive Cocci	1
Ochrobactrum	1
Pantoea	1
Psychrobacter	1
Serratia	1
Vibrio	$\mathbf{O}^1$
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Figure 2. Percentage sensitive (% S) and percentage resistance (% R) of *Pseudomonas* spp. isolated from reptilian samples obtained from the Zoological Medicine Service in the Veterinary Teaching Hospital, University of Georgia (2005-2016). Antimicrobials to which an important proportion of *Pseudomonas* spp. might be expected to exhibit intrinsic resistance are indicated by asterisks.



Figure 3. Percentage sensitive (% S) and percentage resistance (% R) of *Enterococcus* spp. isolated from reptilian samples obtained from the Zoological Medicine Service in the Veterinary Teaching Hospital, University of Georgia (2005-2016). Antimicrobials to which an important proportion of *Enterococcus* spp. might be expected to exhibit intrinsic resistance are indicated by asterisks.



# Figure 4. Percentage sensitive (% S) and percentage resistance (% R) of *Morganella morganii*. isolated from reptilian samples obtained from the Zoological Medicine Service in the Veterinary Teaching Hospital, University of Georgia (2005-2016).

Antimicrobials to which an important proportion of *Morganella morganii* might be expected to exhibit intrinsic resistance are indicated by asterisks.



Figure 5. Percentage sensitive (% S) and percentage resistance (% R) of *Escherichia Coli* isolated from reptilian samples obtained from the Zoological Medicine Service in the Veterinary Teaching Hospital, University of Georgia (2005-2016).



Figure 6. Percentage sensitive (% S) and percentage resistance (% R) of *Staphylococcus* spp. isolated from reptilian samples obtained from the Zoological Medicine Service in the Veterinary Teaching Hospital, University of Georgia (2005-2016). Antimicrobials to which an important proportion of *Staphylococcus* spp. might be expected to exhibit intrinsic resistance are indicated by asterisks.

