



# Bacterial Stomatitis in Wild Reticulated Pythons (*Malayopython reticulatus*) in Malaysia

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## ABSTRACT

Bacterial stomatitis is a common clinical form of upper alimentary tract disease in reptiles. The current study aimed to isolate and identify the common aerobes in the oral cavities of wild reticulated pythons and to profile their antimicrobial susceptibility. The need to conduct the current research was deemed in parallel with the increasing demand for snakes as pets and the growing emergence of multiple-drug-resistant organisms. A total of 40 fresh carcasses of the wild-caught reticulated pythons were assessed for the presence or absence of stomatitis. Oral swabs were obtained and cultured on blood and MacConkey agar media. The colony and cellular morphologies of the isolates were evaluated, followed by Gram-positive and Gram-negative bacterial identification. Antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion method against selected antibiotics, namely gentamicin (GEN), amoxicillin (AMX), cephalexin (LEX), azithromycin (AZM), tetracycline (TET), and ciprofloxacin (CIP), commonly used to treat bacterial infection in reptiles. Results indicated that the prevalence of stomatitis was 77.5%. Among 153 isolates identified, 76.47% of bacteria were identified from pythons with stomatitis lesions, while 23.53% of bacteria were identified from pythons without stomatitis. Of 153 isolates, Gram-negative bacteria were shown to be predominant (94.77%). The three most isolated bacterial species were *Aeromonas* spp. (14.38%), *Klebsiella pneumoniae* (11.76%), and *Alcaligenes faecalis* (8.5%). Meanwhile, coagulase-negative *Staphylococcus* spp. (4.58%) and *Corynebacterium* spp. (0.66%) were the only isolated Gram-positive aerobes. Most isolates were observed to be equally susceptible to GEN and CIP (at 95.8%) but highly resistant to AMX (83.3%) and LEX (75.0%). In conclusion, bacterial stomatitis in wild-caught reticulated pythons was highly prevalent and often seen as a mixed bacterial infection (96.8%). The isolated bacteria consistently show susceptibility towards GEN and CIP and thus could be considered the primary line of antibiotics in treating this disease.

**Keywords:** Antimicrobial susceptibility, Bacteria, Malaysia, Reticulated python, Stomatitis

## INTRODUCTION

The reticulated python (*Malayopython reticulatus*), which has been described as the most important species among other pythons from the economic aspects, is the world's longest snake in the family of Pythonidae (Groombridge and Luxmoore, 1991 acquired from Khadiejah et al., 2021). Pythons have been exploited for products sold in fashion, food, and traditional medicine markets (Klemens and Thorbjarnarson, 1995; Kasterine, 2012). In Southeast Asia, approximately 340,000 reticulated python skins are exported annually, making it the most heavily traded species in the trade of python skins. Malaysia is considered one of the main sources of pythons for the skin trade, alongside Indonesia, most of which are wild-caught (Kasterine, 2012).

Bacterial and *Mycoplasma* infections are frequently reported among reptiles. Among bacterial infections, Gram-negative bacteria are more commonly observed in reptilian diseases (O'Rourke and Lertpiriyapong, 2015). Gram-negative bacteria are normally present as part of the normal flora in reptiles. Their presence alone does not necessarily indicate the presence of diseases (O'Rourke and Lertpiriyapong, 2015). Besides, Gram-positive bacteria, anaerobes, and *Mycoplasma* spp. play a notable role in reptilian diseases (Rosenthal and Mader, 1996). A recent study conducted on pythons identified Gram-negative bacteria, including *Aeromonas* spp., *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*), and *Klebsiella pneumoniae* (*K. pneumoniae*), as part of their normal flora (Abba et al., 2017). *Bacteroides* spp. were the most common anaerobic isolates in reptiles, while *Clostridium* spp. has been correlated with gastrointestinal disease and endotoxemia (Schmidt et al., 2013).

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Infectious stomatitis or “mouth rot” is a common disease in snakes kept in captivity (Diaz-Figueroa and Mitchell, 2006). Isolated bacteria from such cases often include *E. coli*, *Citrobacter* spp., *Proteus* spp., and *Salmonella* spp. with *Staphylococcus* spp. being the only isolated Gram-positive bacterium (Pereira et al., 2017). Several risk factors contribute to the development of infectious stomatitis in snakes, including mites’ infestation, malnutrition, oral trauma, poor oral hygiene, neoplasia, inappropriate husbandry, and stress (Kaplan and Jereb, 1995; Mehler and Bennett, 2006). Untreated stomatitis may progress to other diseases, such as osteomyelitis and pneumonia (Mehler and Bennett, 2006; Jacobson, 2007). Osteomyelitis is thought to result from chronic proliferative lesions that are extended into the maxilla or mandible (Mehler and Bennett, 2006), while pneumonia is described to be caused by the presence of cellular debris in the respiratory tract through inhalation of the debris (Jacobson, 2007).

According to Mehler and Bennett (2006), stomatitis is often regarded as a secondary condition that arises as a consequence of exposure to various predisposing factors rather than a primary condition. The authors added that snakes are at risk of developing stomatitis, especially those in captivity, usually due to their poor husbandry. Factors, such as traumatic injuries from rubbing on or crashing into barriers, wounds during prey capture, and mite infestations contribute to the development of stomatitis in the reptiles. These conditions can expose the gingiva and lead to desiccation and damage to the mucous membrane, resulting in stomatitis. Furthermore, other predisposing factors like immunosuppression and malnourishment also play a role in increasing the susceptibility of snakes to stomatitis. When infected, the majority of routinely isolated Gram-negative aerobes reported were *Aeromonas hydrophila* (*A. hydrophila*), *Pseudomonas* spp., *E. coli*, *Stenotrophomonas* (*S.*) *maltophilia*, *Salmonella* spp., *Klebsiella* spp., *Serratia* spp., and *Providencia* spp. (Mehler and Bennett, 2006; Jho et al., 2011a; 2011b; Pereira et al., 2017).

The resistance towards antimicrobials used to treat the infection is an important matter to be addressed. Studies have reported the presence of antimicrobial resistance among bacterial isolates from reptiles, among which 9% of *Salmonella* spp. strains showed resistance to ampicillin, amoxicillin (AMX)/clavulanic acid, and streptomycin in reptiles (Romero et al., 2016). Another study showed that reptiles possess a wide variety of *Salmonella* spp. serovars, in which resistance to at least one type of antibiotic was identified in 68% of *Salmonella* spp. when streptomycin, chloramphenicol, gentamicin (GEN), cefoxitin, and tetracycline (TET) antibiotics were investigated (Merkevičienė et al., 2022). Moreover, *A. hydrophila*, *E. coli*, *Pseudomonas* spp., and *Proteus* spp. were routinely isolated from the water and in captive and natural environments, and they were considered to be opportunistic organisms and tended to have extensive resistance to antimicrobial agents (Hilf et al., 1990; Divers and Stahl, 2019). *Stenotrophomonas maltophilia* is another highly virulent pathogen that can be discovered in water and soil and has recently been described as an important nosocomial and community-acquired infection (An and Berg, 2018). The majority of the bacteria described above have a high antimicrobial resistance rate; therefore, an antibiotic sensitivity test (AST) is essential to determine the sensitivity of antibiotics.

It is worth noting that studies on the microbial flora of snakes in Malaysia are scanty. There is also a lack of information on the common aerobic bacteria that cause stomatitis in the wild reticulated python concerning the risk factors of stomatitis in Malaysia. Apart from that, there is also a paucity of reports on the correlation of stomatitis in the wild reticulated pythons with the oral bacteria and their antimicrobial profiles in Malaysia. Therefore, this study aimed to provide the latest insight into the prevalence and antimicrobial susceptibility profile in the case of bacterial stomatitis in snakes.

## MATERIALS AND METHODS

### Ethical approval

This study was approved by the Department of Wildlife and National Parks (PERHILITAN) for the use of protected wildlife species under the Wildlife Conservation (Amendment) October 2022 and complied with the use of animals for scientific purposes in humane and ethical from the Institutional Animal Care and Use Committee (IACUC), University Putra Malaysia (UPM).

### Animal and sample collection

A total of 40 wild-caught reticulated pythons comprised of 10 males and 30 females were included using a convenient sampling technique at a snake abattoir located at Segamat, Johor (southern Peninsular Malaysia). All snakes were originally wild-captured from an oil palm plantation situated at Changkat Jering, Perak, Malaysia (west coast of Peninsular Malaysia) in August 2020. The reticulated pythons recorded an average body weight of 9.07 kg and an average length of 133.78 cm. A general physical examination was carried out by a veterinarian on every selected and freshly decapitated reticulated python, including integument, nares, eyes, ears, oral cavity, and external parasites. The findings were recorded on a form. The photos of their oral cavities were taken using a digital camera. The photos were used to evaluate the presence of signs of stomatitis, such as mucus or pus in or around the mouth, ulcer, foul smell, red color, and inflamed mouth tissue in the reticulated pythons. Oral swabs were obtained from the wild-caught reticulated

pythons with or without stomatitis lesions using Amies sterile transport swabs. The swabbed oral regions included the mandibular area between the teeth and lingual-tracheal ridge as well as the maxillary area between the teeth and lingual-tracheal groove. The sterile transport swabs were stored in an ice box after sampling and during the transportation to the laboratory at the Faculty of Veterinary Medicine, University Putra Malaysia, Malaysia.

### **Isolation and identification of bacteria**

Each oral swab ( $n = 40$ ) was used to inoculate onto 4% blood agar (OXOID, UK) and MacConkey agar (OXOID, UK). The oral swab was rolled onto one side of the agar, followed by the streak plate method to obtain a primary culture. The inoculated blood and MacConkey agar plates were then incubated at 37°C for 24-48 hours under aerobic conditions. The well-isolated colonies were identified on the blood and MacConkey agar plates, and their colony and cell morphologies were described and recorded. The colony morphology was characterized according to the shape, size, color, surface texture, hemolytic activity on blood agar; lactose fermentation on MacConkey agar, and smell (Chew and Smith, 1992). Gram staining was used to identify the cellular morphology of the bacteria using a compound microscope (Nikon Eclipse E200) under 1000x magnification with oil immersion. The Gram reaction, shape, arrangement of cells, and presence or absence of spores were recorded.

The identification of the isolated bacterial colonies was made by biochemical tests (Jang et al., 2008). Depending on the cellular morphologies observed, either cocci or rods, for Gram-positive aerobic bacteria, the tests included catalase test, coagulase test, urease test, glucose test, nitrate reduction test, sucrose test, hemolysis test, trehalose test, motility test, and Christie-Atkins-Munch-Peterson (CAMP) test.

As for Gram-negative bacteria, the biochemical tests comprised of spot oxidase test, Triple Sugar Iron (TSI) test, sulfide-indole-motility (SIM) test, urease test, and citrate test. Other than the aforementioned tests, additional tests were carried out to identify the bacterial isolates. The decision to perform additional tests for the diagnostic evaluation of bacterial and mycological infections in reptiles was based on guidance provided in the book entitled “A Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology” (Jang et al., 2008). This book serves as a reference and provides protocols for various diagnostic tests, such as hanging drop, phenylalanine deaminase (PD) test, Oxidation-Fermentation (OF) test, Lysine Decarboxylase (LDC) test, Ornithine Decarboxylase (ODC) test, O-Nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) test, Polyvalent ‘O’ antisera test, and Polymyxin B.

### **Antimicrobial susceptibility testing**

The Kirby-Bauer disk diffusion method was used to identify the antimicrobial susceptibility or resistance of the bacteria to various selected antimicrobial agents. The Gentamicin (GEN, 10  $\mu$ g), amoxicillin (AMX, 10  $\mu$ g), cephalixin (LEX, 30  $\mu$ g), azithromycin (AZM, 15  $\mu$ g), tetracycline (TET, 30  $\mu$ g), and ciprofloxacin (CIP, 5  $\mu$ g) were chosen as the antimicrobial agents to be tested for the antimicrobial susceptibility among the bacterial isolates which had been identified. The turbidity of bacterial suspension made in sterile distilled water was visually compared to a 0.5 McFarland standard using a Wickerham card as the background. The suspension with comparable turbidity to the standard was used as an inoculum. A sterile cotton swab was dipped into the prepared inoculum and spread thoroughly onto Mueller-Hinton agar.

Using an antibiotic disc dispenser, six selected antibiotic discs mentioned above were dispensed onto the inoculated Mueller-Hinton agar (OXOID, UK) and incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone, the area without bacterial growth around the antibiotic disc, was measured using a digital caliper. The measurement was done for every dispensed antibiotic disc and recorded in millimeters. The results were interpreted as resistant, intermediate, or susceptible using the tables provided in the Clinical and Laboratory Standards Institute AST standards- CLSI documents VET01-S2 (CLSI, 2013) and M100-S20 (CLSI, 2010).

### **Statistical analyses**

A descriptive analysis was done via IBM SPSS (USA, version 29) to obtain the prevalence of stomatitis in the wild-caught reticulated pythons. A chi-square test was run to determine a significant relationship between stomatitis, sex, and ectoparasite infestation. The analysis aimed to determine the relationship between these two findings with the occurrence of bacterial stomatitis in the wild reticulated pythons. The association between the occurrence of stomatitis and the identified corresponding bacterial isolates was also analyzed using a chi-square test. Both tests were analyzed at a significance level of  $p < 0.05$ .

## **RESULTS**

The prevalence of stomatitis in the sampled wild-caught reticulated pythons was 77.5%. The prevalence rates of stomatitis in males and females were 100% and 70.0%, respectively. There was a significant difference between the occurrence of stomatitis in males and females ( $p < 0.05$ , Table 1 A). Among the reticulated pythons with tick infestation,

60% were male and 79.2% were female observed to be suffering from stomatitis. Whereas 75.0% of the reticulated pythons without tick infestation suffered from stomatitis (Table 1 B). The occurrence of stomatitis was comparable in the reticulated pythons with or without tick infestation ( $p > 0.05$ ).

**Table 1.** The relationship between the occurrence of stomatitis in different sexes of phytons (A), and the relationship between the occurrence of stomatitis with the presence of tick infestation (Band relationship between the occurrence of stomatitis with isolation of *Klebsiella oxytoca*) in Malaysia 2020

<b>Stomatitis * sex crosstabulation</b>						
Count (number-percentage)						
A		Sex		Total	Pearson chi-square value	P value
		Male	Female			
Stomatitis	without stomatitis	0 (0%)	9 (30%)	9 (22.5%)	3.871	0.049
	with stomatitis	10 (100%)	21 (70%)	31 (77.5%)		
Total		10	30	40		

<b>Stomatitis * ectoparasitic infestation crosstabulation</b>						
Count (number-percentage)						
B		ectoparasitic infestation		Total	Pearson chi-square value	P value
		Present	Absent			
Stomatitis	without stomatitis	5 (20.8%)	4 (25%)	9 (22.5%)	0.096	0.757
	with stomatitis	19 (79.2%)	12 (75%)	31 (77.5%)		
Total		24	16	40		

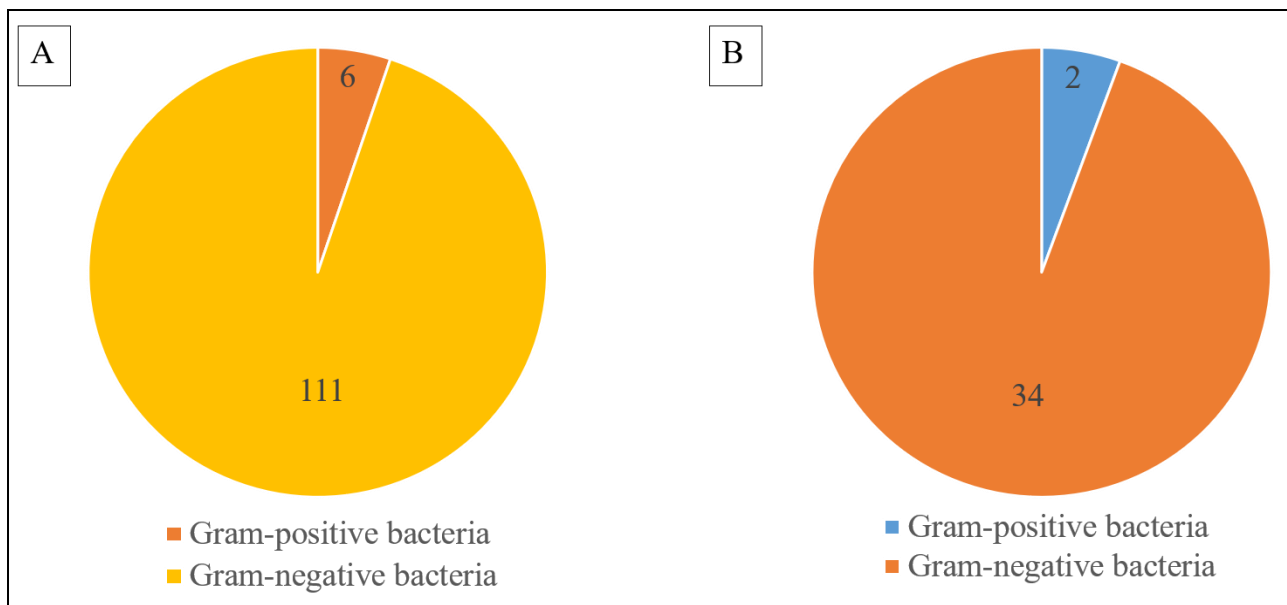
<b>Stomatitis * <i>Klebsiella oxytoca</i> crosstabulation</b>						
Count (number-percentage)						
C		<i>Klebsiella oxytoca</i>		Total	Pearson chi-square value	P value
		Negative	Positive			
Stomatitis	-ve	9 (30%)	0 (0%)	9 (22.5%)	3.871	0.045
	+ve	21 (70%)	10 (100%)	31 (77.5%)		
Total		30	10	40		

### Bacterial isolates

A total of 153 bacterial isolates were identified. There were 24 bacterial species from 18 genera among these bacterial isolates. Of the 24 bacterial species, only two (8.3%) were Gram-positive aerobic bacteria, while the others (91.7%) were Gram-negative aerobic bacteria. The most predominant bacterial isolates in the oral cavities of the reticulated pythons were *Aeromonas* spp. (14.4%), followed by *K. pneumoniae* (11.8%), and *Alcaligenes faecalis* (8.5%). Table 2 shows the prevalence of bacteria in the oral cavities of the reticulated pythons.

In the oral cavities of the reticulated pythons with lesions of stomatitis, 5.1% (6/117) of the bacterial isolates were Gram-positive aerobes, and 94.9% (111/117) of the bacterial isolates were Gram-negative aerobes (Figure 1a). Regarding the oral cavities of the reticulated pythons without lesions of stomatitis, 5.6% (2/36) of the bacterial isolates were Gram-positive aerobes, and 94.4% (34/36) of the bacterial isolates were Gram-negative aerobes (Figure 1b).

Most reticulated pythons with or without stomatitis had multiple microbial isolates in their oral cavities. Only 2.5% (1/40) of the python had a single bacterial species isolated, which was also presented with stomatitis. The number of oral bacterial isolates ranged from two to six from a single python. Three pythons (3/40) had 2 isolates, 10 pythons had 3 bacterial isolates, 17 pythons had 4 isolates, 6 pythons had 5 isolates and 3 pythons had 6 isolates, isolated from their oral cavities. *Corynebacterium* spp. (0.7%), *Enterobacter aerogenes* (0.7%), *Klebsiella (K.) oxytoca* (6.5%), *Salmonella* spp. (2.0%), and *S. maltophilia* (1.3%) were only isolated from the oral cavities of reticulated pythons with stomatitis lesions. There was an association between the presence of *K. oxytoca* in the oral cavity and the occurrence of stomatitis ( $p < 0.05$ ) (Table 1 C). Additionally, it was noticed that the highest percentage of bacteria isolated from the mouth of the subject belonged to the *Enterobacteriaceae* family, which consists of genus *Escherichia*, *Salmonella*, *Citrobacter*, *Yersinia*, *Klebsiella*, *Serratia*, *Pseudomonas*, *Proteus* and *Vibrio* (Janda and Abbot, 2021).



**Figure 1.** The proportion of bacteria in the oral cavities of the reticulated pythons with stomatitis (A) and the proportion of bacteria in the oral cavities of the reticulated pythons without stomatitis (B). The wild reticulated pythons captured at an oil palm plantation in Changkat Jering, Perak, Malaysia in August 2020.

**Table 2.** Prevalence of bacteria in the oral cavities of the reticulated pythons wild-captured in an oil palm plantation in Changkat Jering, Perak, Malaysia in August 2020 expressed in percentage

Bacteria	Number
<b>Gram-positive isolates</b>	
Coagulase-negative <i>Staphylococcus</i>	7 (4.6%)
<i>Corynebacterium</i> spp.	1 (0.7%)
<b>Gram-negative isolates</b>	
<i>Acinetobacter baumannii</i>	9 (5.9%)
<i>Acinetobacter lwoffii</i>	3 (2.0%)
<i>Aeromonas</i> sp.	22 (14.4%)
<i>Alcaligenes faecalis</i>	13 (8.5%)
<i>Bordetella bronchiseptica</i>	6 (3.9%)
<i>Citrobacter freundii</i>	4 (2.6%)
<i>Citrobacter</i> spp.	2 (1.3%)
<i>Escherichia coli</i>	4 (2.6%)
<i>Enterobacter aerogenes</i>	1 (0.7%)
<i>Enterobacter cloacae</i>	8 (5.2%)
<i>Enterobacter</i> spp.	8 (5.2%)
<i>Klebsiella oxytoca</i>	10 (6.5%)
<i>Klebsiella pneumoniae</i>	18 (11.8%)
<i>Klebsiella</i> spp.	6 (3.9%)
<i>Plesiomonas shigelloides</i>	3 (2.0%)
<i>Proteus</i> spp.	10 (6.5%)
<i>Pseudomonas aeruginosa</i>	3 (2.0%)
<i>Salmonella</i> spp.	3 (2.0%)
<i>Serratia</i> spp.	4 (2.6%)
<i>Stenotrophomonas maltophilia</i>	2 (1.3%)
<i>Yersinia pestis</i>	3 (2.0%)
<i>Vibrio cholerae</i> -01	3 (2.0%)

### Antimicrobial susceptibility testing

Table 3 indicates the results of AST performed on the identified bacterial isolates. Table 4 tabulates the percentages of antimicrobial susceptibility and resistance of the antimicrobial agents tested. Most bacterial species were equally susceptible to GEN and ciprofloxacin, with a rate of 95.8%. Besides, some bacterial isolates were resistant to TET (20.8%). Of the investigated bacterial species, 20 (83.3%) and 18 (75.0%) were reportedly resistant to AMX and LEX, respectively, showing a high resistance profile. The results indicate the susceptibility of various bacterial species to different antimicrobial agents, with AZM showing the highest number of intermediate results in the test, followed by cephalexin (8.3%). Out of the bacterial species tested, 10 (41.7%) showed an intermediate response to AZM, indicating that the effectiveness of this antimicrobial agent against these bacteria is not fully clear and falls in the middle of susceptibility and resistance.

A high proportion (75.0%) of the bacterial isolates tested was multiple-drug resistant (MDR), demonstrating antimicrobial resistance to at least one antimicrobial agent in three or more antimicrobial categories. Coagulase-negative *Staphylococcus*, *E. coli*, *K. oxytoca*, *K. pneumoniae*, *S. maltophilia*, and *Yersinia pestis* were among the isolated isolates that were not MDR.

**Table 3.** Antimicrobial profiles of the isolated bacteria derived from samples collected from wild reticulated pythons in an oil palm plantation in Changkat Jering, Perak, Malaysia in August 2020 which were expressed as susceptible (S), intermediate (I), and resistant (R)

Bacteria/ antibiotics	GEN	AMX	LEX	AZM	TET	CIP
<b>Gram-positive isolate</b>						
Coagulase-negative <i>Staphylococcus</i>	S	S	I	R	S	S
<i>Corynebacterium</i> spp.	R	R	R	R	R	R
<b>Gram-negative isolate</b>						
<i>Acinetobacter baumannii</i>	S	R	R	I	S	S
<i>Acinetobacter lwoffii</i>	S	R	R	R	S	S
<i>Aeromonas</i> spp.	S	R	R	I	S	S
<i>Alcaligenes faecalis</i>	S	R	R	R	S	S
<i>Bordetella bronchiseptica</i>	S	R	R	I	S	S
<i>Citrobacter freundii</i>	S	R	R	R	S	S
<i>Citrobacter</i> spp.	S	R	R	I	S	S
<i>Escherichia coli</i>	S	R	S	R	S	S
<i>Enterobacter aerogenes</i>	S	R	R	R	S	S
<i>Enterobacter cloacae</i>	S	R	R	R	R	S
<i>Enterobacter</i> spp.	S	R	R	I	S	S
<i>Klebsiella oxytoca</i>	S	R	S	R	S	S
<i>Klebsiella pneumoniae</i>	S	R	S	R	S	S
<i>Klebsiella</i> spp.	S	R	R	I	S	S
<i>Plesiomonas shigelloides</i>	S	R	I	I	S	S
<i>Proteus</i> spp.	S	S	R	R	R	S
<i>Pseudomonas aeruginosa</i>	S	R	R	R	S	S
<i>Salmonella</i> spp.	S	R	R	I	S	S
<i>Serratia</i> spp.	S	R	R	R	R	S
<i>Stenotrophomonas maltophilia</i>	S	S	R	S	S	S
<i>Yersinia pestis</i>	S	S	S	I	S	S
<i>Vibrio cholerae</i> -01	S	R	R	I	R	S

AMX: Amoxicillin, AZM: Azithromycin, CIP: Ciprofloxacin, GEN: Gentamicin, LEX: Cephalexin, TET: Tetracycline, S: Susceptible, R: Resistant

**Table 4.** Percentages of antimicrobial susceptibility and resistance of the tested antimicrobial agents against the isolated bacteria (24 different bacterial species) originated from samples of wild reticulated pythons at an oil palm plantation in Changkat Jering, Perak, Malaysia in August 2020

	GEN	AMX	LEX	AZM	TET	CIP
<b>Susceptible</b>	23 (95.8%)	4 (16.7%)	4 (16.7%)	1 (4.2%)	19 (79.2%)	23 (95.8%)
<b>Intermediate</b>	0	0	2 (8.3%)	10 (41.7%)	0	0
<b>Resistant</b>	1 (4.2%)	20 (83.3%)	18 (75.0%)	13 (54.2%)	5 (20.8%)	1 (4.2%)

AMX: Amoxicillin, AZM: Azithromycin, CIP: Ciprofloxacin, GEN: Gentamicin, LEX: Cephalexin, TET: Tetracycline

## DISCUSSION

In the current study, a high proportion of the wild-caught reticulated pythons were presented with stomatitis, accounting for 77.5% of all the pythons. This agrees with the description of infectious stomatitis by Mehler and Bennett (2006), stating that it is the most common clinical form of upper alimentary tract disease in reptiles. The high occurrence of stomatitis in these pythons could be attributed to the wild environment of their habitats. Stomatitis in reptiles can be triggered by various factors, including an environment with poor quality, traumatic injury, or bite wounds (Jho et al., 2011b). Male pythons may be more prone to injuries due to their involvement in fighting for dominance or mates. Therefore, the prevalence of stomatitis in male pythons is higher than in female pythons. An uncondusive wild environment could also lead to stress in the pythons, causing them to be immunocompromised and more prone to developing diseases.

Gram-negative aerobic bacteria were mainly isolated in this study. This was comparable to the findings of the aerobes found in the oral cavities of Lancehead snakes (*Bothrops atrox*) with evidence of stomatitis (Pereira et al., 2017). The predominant gram-negative aerobes were *Aeromonas* spp., *K. pneumoniae*, *Alcaligenes faecalis* and *K. oxytoca*. Another study indicated that *Aeromonas* spp. was the most isolated organism from the oral cavity of snakes, followed by *Pseudomonas* spp., *Proteus* spp., and *E. coli* (Cooper and Leakey, 1976). Yak et al. (2015) conducted a more recent study to detect the bacterial microflora of the oral cavities of free-living reticulated pythons in Singapore. The results showed that the most commonly identified bacterial species was *Pseudomonas* spp., followed by *Staphylococcus sciuri*. *Mammaliicoccus sciuri* was not isolated in the oral cavities of the reticulated pythons in the present study.

Another study also revealed that *Pseudomonas* spp. had the highest incidence rate of the bacteria isolated from the oral cavity of snakes (Jho et al., 2011b). Although coagulase-negative *Staphylococcus* only accounted for 4.6% among all the identified bacterial isolates, it was the most isolated bacteria in a study conducted in Iran by Dehghani et al. (2015), with a percentage of 34.5% while *Pseudomonas* (3.1%) was the least isolated bacteria. The findings of the oral bacteria from snakes were different in different studies. There was an absence of a noticeable trend of the specific bacterial species isolated from the oral cavity of snakes. This can possibly be attributed to the differences in the snakes in terms of their locations of habitats, predation strategies, and prey types (Shek et al., 2009). Nevertheless, Gram-negative isolates were still predominated in most of the previous studies (Blaylock, 2001; Lam et al., 2010; Dipineto, 2014; Lukač et al., 2017), which was similar to the findings of the current study. Additionally, a high proportion of the bacteria isolated from the oral cavities of the reticulated pythons sampled for this study was in the family of *Enterobacteriaceae*. The feeding behaviors of snakes could be a reason for this phenomenon. The snakes would first eat the head of prey, leading to the colonization of the oral cavity by the fecal flora of the prey (Goldstein et al., 1979).

Among 31 reticulated pythons presented with stomatitis in this study, 30 (96.8%) had a mixed infection of bacterial stomatitis. This was in line with the findings in a study conducted by Pereira et al. (2007). Most of the bacterial isolates in this study are considered part of the normal flora of the oral cavity of snakes (Jho et al., 2011a; Artavia-León et al., 2017). However, many of these bacteria can serve as opportunistic pathogens that can result in clinical diseases by invading the visceral organs when the snake itself as a host is immunocompromised. Besides, they also pose a public health concern as numerous of them are zoonotic bacteria that can cause human infections. One way through which humans acquire bacteria from snakes is through snakebites. After a snakebite occurs, there is a high chance that the wound will become infected, and multiple bacteria could be isolated (Yak et al., 2015; Artavia-León et al., 2017).

In the current study, *Aeromonas* spp. was the most isolated organism. The *A. hydrophila* is known to cause severe infections in humans after snakebites which can be fatal (Mukhopadhyay et al., 2008). It can also lead to death in snakes due to bacteremia (Orozova et al., 2012). Moreover, it can result in diarrhea and soft tissue infection following minor trauma exposure to fresh water containing the organism. *Salmonella* spp. is another important bacterial species as it has a wide host range and can cause diseases in both humans and animals. According to Hardy (2004), *Salmonella* has been an unresolved problem for over a hundred years in public health, epidemiology, and microbiology. It is an opportunistic organism in immunocompromised lizards and snakes (Sting et al., 2013). Due to the increased ownership of exotic pets in the present society, the issue of salmonellosis should be emphasized. Reptiles as exotic pets do harbor *Salmonella* and shed the organism in their feces, and various *Salmonella* serotypes have been identified from these reptilian pets; therefore, humans with immature or poor immune systems are advised to refrain from having contact with reptiles (Woodward et al., 1997).

Pathogenic strains of *E. coli* can cause intestinal and extra-intestinal diseases in both humans and animals, such as diarrhea, cystitis, and meningitis (Ramos et al., 2019). Wild animals can serve as a reservoir of pathogenic strains of *E. coli* after their intestinal microbiota has changed in the populations of *E. coli* due to living closely with humans (de Oliveira Iovine et al., 2015). *Pseudomonas aeruginosa* has been known to cause skin and soft tissue infections in humans, such as folliculitis, ecthyma gangrenosum in neutropenic patients, and burn wounds (Wu et al., 2011). *Aeromonas faecalis* was found to be the third most common bacterial isolate from the oral cavities of the pythons. It can also cause skin and soft tissue infections in humans (Tena et al., 2015). The second most isolated organism was *K. pneumoniae*, an important nosocomial agent that can lead to *pneumonia* in patients with alcoholism or diabetes mellitus, as well as urinary tract infections in humans (Marques et al., 2019; Ashurst and Dawson, 2020). *Yersinia pestis* was isolated in this study as well. It can cause plague primarily in rodents and is transmitted by fleas that carry the organism from the infected wild rodents to humans, resulting in bubonic plague (Falcão, 2014). The presence of this organism could be due to the ingestion of the infected rats before the pythons were captured since the pythons were caught from wild habitats. However, more studies are needed in the future to validate this statement. *Vibrio cholerae-01* is the causative agent of cholera that thrives in aquatic habitats. Humans and animals can acquire this organism through water sources contaminated by fecal materials from infected individuals (Laviad-Shitrit et al., 2019). Concerning this, reticulated python naturally is an excellent swimmer, which could acquire the organism while it is in the water.

The obtained results of the study indicated that *S. maltophilia* was isolated from only two pythons, one with stomatitis and the other without stomatitis. Although this organism is part of the normal flora of the oral cavity of snakes, it was described to be involved in cases of ulcerative stomatitis in snakes (Hejnar et al., 2007). It is usually a nosocomial infection in humans, leading to diseases such as *pneumonia*, blood-stream infection, wound, and urinary tract infection (Looney et al., 2009). Coagulase-negative *Staphylococcus* is the most isolated gram-positive aerobes in this study. Yak et al. (2015) stated that coagulase-negative *Staphylococcus* is a common organism in the oral cavity of snakes, and it can cause infections in humans. Coagulase-negative *Staphylococcus* is also among the common bacterial isolates from infected wounds due to snakebites in humans (Garg et al., 2009).

The results of AST surprisingly demonstrated that most of the bacteria isolated from this study were MDR organisms, accounting for 75% of the total isolates. The presence of antimicrobial resistance in wild reticulated pythons, even without apparent exposure to antibiotics, raises concerns for both animal and human health. This resistance is likely influenced by the environment in which they inhabit. The role of the environment in the spread of antimicrobial resistance has been well-documented (Prestinaci et al., 2015). The environment might be contaminated with bacteria that carry the resistance genes and antimicrobial residues. Antimicrobial-resistant organisms and antimicrobial residues could still be present in the sewage from human neighborhoods and the animal production industry, even if the sewage had been treated in wastewater treatment plants (Da Costa et al., 2013). A poor sewage system might drain into the wild environment, causing it to be contaminated with resistant bacteria and antimicrobial residues. The antimicrobial residues are usually of sub-inhibitory concentrations, with which the abundance of microbiota in the environment can interact, eventually forming antimicrobial-resistant organisms (Da Costa et al., 2013).

In the present study, AMX and LEX were ineffective against the bacterial isolates from the oral cavities of reticulated pythons. The resistance rates were high for both AMX (83.3%) and LEX (75.0%). The AMX results were comparable to those in a study conducted by Lam et al. (2010). However, the majority of the isolates were sensitive to CIP and GEN, accounting for 95.8% in both. This agrees with the results of a study conducted by Garg et al. (2009). In the treatment of bacterial stomatitis, fluoroquinolones, and aminoglycosides are the common choices of first-line antibiotics while waiting for the results of AST (Mehler and Bennett, 2006). Therefore, CIP and GEN would be good options for antibiotics for bacterial infection in snakes, snakebites, or other snake-related bacterial diseases in humans.

*Salmonella* spp., *Klebsiella* spp., *E. coli*, *Serratia* spp., *Providencia* spp., and *Proteus* spp. are in the family of *Enterobacteriaceae*, and all of them are Gram-negative rod-shaped bacteria. In reptiles, the most common subspecies of *Salmonella* spp. is *Salmonella enterica* subspecies *enterica*, followed by *diarizonae* and *arizonae* subspecies. It can cause salmonellosis in animals and humans who keep reptiles as their pets (Romero et al., 2016). *Salmonella* spp. also resists various antimicrobial agents (Chen et al., 2010). One of the species of *Klebsiella* spp. that is of public health concern is *K. pneumoniae*. It is capable of causing various diseases in humans, including *pneumonia* and septicemia. Additionally, it is a Gram-negative opportunistic bacterium and has been reported to have an extensive resistance profile (Wang and He, 2018). Snakes have a higher frequency of isolation *E. coli*, compared to other reptiles as all snakes are carnivorous animals. The type of diet and contact with other animals greatly influence the frequency of organism isolation (Ramos et al., 2019). The *Enterobacteriaceae* was also reportedly resistant to antibiotics, such as AMX/clavulanic acid and TET (Casey et al., 2015).

## CONCLUSION

The bacterial stomatitis in wild-caught reticulated pythons was highly prevalent, especially in males. It is often seen as a mixed infection in which most are consistently sensitive to gentamicin and ciprofloxacin. Hence, these two antibiotics can be considered as the first-line treatment of stomatitis caused by reptile bacteria.

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### Availability of data and materials

All data underlying the results are available as part of the article by authors of the present study.

### Authors' contributions

All authors equally contributed to sample collection, data collection and analysis, and the write-up of the manuscript. The final manuscript was read and approved by all authors. Sharina Omar advised and supervised on bacteriology and antimicrobial susceptibility testing section, data analysis, and preparation of the manuscript for the



journal, editing the manuscript. Azlan Che Amat was the veterinarian who helped with the diagnosis of pythons and editing the manuscript. Ho Shao Jian sampled and conducted the laboratory work, data analysis, and preparation of the manuscript for the final year project report.

### Conflict of interests

The authors have not declared any conflict of interest.

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