

# Bacterial flora of wild black (*Ctenosaura similis* Gray, 1831) and green (*Iguana iguana* Linnaeus, 1758) iguanas from a Mexican Caribbean atoll

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**Abstract.** Black (*Ctenosaura similis*) and green iguanas (*Iguana iguana*) occur in a great variety of habitats and could be a source of zoonotic diseases. Herein, we characterized the bacterial flora in oral and cloacal cavities of these species from Banco Chinchorro atoll, Mexico, where they are in close contact with humans. We obtained samples from 19 green and 10 black iguanas. We performed bacterial cultures on different growth media and identified bacteria by gross examination and biochemical tests. We isolated 25 bacteria species (15 genera from six families) from all samples. Twelve bacteria (48%) were common to both iguana species and 12 (48%) and one (4%) were only detected in green and black iguanas, respectively. *Iguana iguana* shows a greater richness of bacteria in cloaca (n = 15 vs n = 7) and oral cavity (n = 16 vs n = 11) than *C. similis*, likely due to different feeding habits. 72% of the detected bacteria have been associated with infections in humans. This information on oral and cloacal bacteria of wild green and black iguanas could help to provide adequate clinical assistance to sick animals and ideal antimicrobial therapy in cases of bites or infections.

**Keywords.** Banco Chinchorro, Iguanidae, cloacal bacteria, island, Mexico, oral bacteria

## Introduction

Black (*Ctenosaura similis* Gray, 1831) and green (*Iguana iguana* Linnaeus, 1758) iguanas occur in a great variety of habitats and substrates throughout Southeast Mexico and the Caribbean islands (Campbell, 1998; Alberts et al., 2004). They are also common pet reptiles and could be a source of zoonotic diseases (Bibbs et al., 2001; Alves et al., 2019; Bautista-Trujillo et al.,

2019). However, few studies have been conducted to characterize their oral and cloacal microflora.

Some bacteria from conjunctiva, oral cavity, choanas and cloaca of healthy captive green iguanas are considered potential health hazard to humans and malnourished reptiles (Hsieh and Babl, 1999; Harris and Rogers, 2001; Barten, 2002; Mehler and Bennett, 2003; Johnson-Delaney, 2006; Mader, 2006; Jacobson, 2007; Taddei et al., 2010; Grim et al., 2010; Romero et al., 2015; Guyomard-Rabenirina et al., 2019). Furthermore, *Serratia marcescens* and *Staphylococcus aureus* have been reported in infected wounds from iguana bites (Hsieh and Babl, 1999; Grim et al., 2010). Wildlife living closer to human settlements also carry more resistant bacteria than those from protected areas (Allen et al., 2010; Wheeler et al., 2012). Exposure to iguana faeces may increase the risk of salmonellosis, the principal and potentially lethal zoonotic disease transmitted by reptiles (Mitchell and Shane, 2000; Bibbs et al., 2001; Mermin et al., 2004; Guyomard-Rabenirina et al., 2019). Guyomard-Rabenirina et al. (2019) found several serovars of *Salmonella enterica* in faeces of *I. iguana* and *Iguana delicatissima* Laurenti, 1768 in Guadeloupe and conclude that human infections could result from indirect or direct contact with those animals. Another

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risk is through meat consumption, since iguana meat is a traditional dish in different countries of Central and South America, including Mexico; with *I. iguana* and *Ctenosaura* spp. the most commonly consumed species (Magnino *et al.*, 2009; CONABIO, 2019; Ramos-Roca and Rodríguez-Castañeda, 2019).

Banco Chinchorro Biosphere Reserve (BCBR) is an atoll 30.8 km far from the nearest continental point of the Yucatan Peninsula in the Mexican Caribbean (INE, 2000). The atoll is composed of a very large reef lagoon (53,379 ha) 50 km long and 18 km wide, delimited by 115 km of coral reef (INE, 2000). Four cays of different sizes are present in the lagoon, covering a total of 582 ha. In the extreme south is Cayo Lobos, the smallest cay (0.4 ha). In the extreme north, two cays, Cayo Norte menor and Cayo Norte mayor, sum an area of 40 ha, and at the centre of the atoll there is Cayo Centro, the largest cay with 541 ha (INE, 2000). Thirteen species of reptiles are present in the atoll, including black and green iguanas, which occur in Cayo Centro and Cayo Norte (Charruau *et al.*, 2015). Populations of both iguanas are abundant at fishermen camps and the field station of the Comisión Nacional de Areas Naturales Protegidas (CONANP) in Cayo Centro (Charruau *et al.*, 2015). Iguanas occasionally feed on organic wastes produced by fishermen. Moreover, tourists that arrive to Cayo Centro to rest and eat as part of their tour to BCBR often get close to green iguanas to take photographs. These close contacts between iguanas and humans could result in zoonotic infections. For these reasons it is important to characterize the microflora of iguanas to help providing correct antimicrobial therapy in case of incidents and infections. Thus, the objectives of this study were to characterize the bacterial flora of the oral cavity and cloaca of green and black iguanas from Cayo Centro, BCBR, and to assess their potential threats to iguanas and humans health.

## Materials and Methods

*Study Site.*—The study was made at Cayo Centro, the largest cay of Banco Chinchorro atoll. It presents halophytic vegetation, coastal dune vegetation and mangrove (Charruau *et al.*, 2015). There is no freshwater source on the cay and the only water available presents high salinity rates (Charruau *et al.*, 2005).

*Sampling.*—We captured iguanas during the day using the break-away snare technique in August 2011, June 2012 and August 2013. We physically examined each captured individual for signs of diseases, and took samples by passing sterile culture swab (BBL™ Culture

Swab™ Plus) in their oral cavity and the cloaca. We then preserved swabs at 2 to 8°C in AMIES transport mediums (Becton & Dickinson), ideal for aerobic and anaerobic bacteria. We measured the snout-vent length (SVL) of each iguana with a flexometer ( $\pm 0.1$  cm). Each iguana was marked by notching scales of their dorsal crest to avoid recapture of same individuals in other capture campaigns.

*Bacterium analyses.*—We made bacterial cultures using different bacterium growth medium (Cowan and Steel, 1974; Quinn *et al.*, 1994) including Blood Agar 5%, McConkey Agar, Mannitol Salt Agar, Brilliant Green Agar, and Salmonella – Shigella agar. The inoculation and incubation of the plates, as well as the morphological and microscopic analysis of the bacteria were performed following the method described in Charruau *et al.* (2012). After gross observations, we proceed to the identification of Gram-negative bacteria with biochemical tests in tubes: mobility, indole, decarboxylation of ornithine and lysine, fermentation of lactose, saccharose and dextrose, oxidation of iron, use of citrate as source of carbon, hydrolysis of urea, gas production, deamination of phenylalanine, nitrate reduction and differentiation of oxidative or reductive metabolism of bacteria (Kämpfer *et al.*, 1991; MacFaddin, 2000). We compared these reactions with the characteristics obtained during primary identification, using reference tables of biochemical reactions to obtain the final identification (Cowan and Steel, 1974; Quinn *et al.*, 1994; Murray *et al.*, 1999).

For all special Gram-positive and Gram-negative bacteria we used a miniaturized method with the help of BBL Crystal™ Identification Systems Enteric/Non fermenter Id Kit and BBL Crystal™ Identification Rapid Gram-positive Id Kit, that contain 30 dried biochemical and enzymatic substrates. Fermentation reactions detect the ability of an isolate to metabolize carbohydrates in the absence of atmospheric oxygen, and oxidation reactions are based on the ability of an organism to metabolize the substrate with oxygen as the final electron acceptor. Both reactions are usually detected by the use of a pH indicator in the test substrate (Killian and Bulow, 1976; MacFaddin, 2000).

## Results

We captured and obtained oral and cloacal samples from 19 green iguanas (13 females, six males) and 10 black iguanas (three females, seven males), all clinically healthy. Mean SVL of females and males' green iguanas were  $376 \pm 57$  mm and  $439 \pm 92$  mm, respectively, with

an overall mean SVL of  $396 \pm 74$  mm. Mean SVL of females and males' black iguanas were  $261 \pm 11$  mm and  $305 \pm 42$  mm, respectively, with an overall mean SVL of  $292 \pm 41$  mm.

We isolated 25 bacteria species (15 genera, six families) from the samples (Table 1). Species of the family *Enterobacteriaceae* represented 72.0% of the species. We isolated 12 bacteria species (48.0%) in both iguana species and 12 (48.0%) and one (4.0%) species only in green and black iguanas, respectively. We found 19 bacteria species (13 genera, five families) in oral samples, eight (42.1%) were present in both iguana species, eight (42.1%) only in green iguanas and three (15.8%) only in black iguanas. We found 15 bacteria

species (11 genera, four families) from cloacal samples, seven (46.7%) were present in both iguana species and eight (53.3%) only in green iguanas.

We isolated 24 bacterial species from green iguanas (Table 1), of which seven (29.2%) were in both oral and cloacal cavities and eight (33.3%) and nine (37.5%) were only found in the cloaca and the oral cavity, respectively. Ten (62.5%) bacteria species from oral samples were Gram-negative and six (37.5%) were Gram-positive (Table 2). *Enterobacter aerogenes* and *Streptococcus uberis* were the most frequent isolates from oral samples (Table 2). Two (13.3%) bacteria species from cloacal samples of green iguanas were Gram-positive and 13 (86.7%) were Gram-negative

**Table 1.** Species of bacteria isolated in oral (O) and cloacal (C) cavities of iguanas of Banco Chinchorro.

| Family                    | Species                       | Green iguana<br>( <i>Iguana iguana</i> ) | Black iguana<br>( <i>Ctenosaura similis</i> ) |
|---------------------------|-------------------------------|--|---|
| <i>Aeromonadaceae</i>     | <i>Aeromonas hydrophila</i>   | C  | O,C   |
| <i>Alcaligenaceae</i>     | <i>Alcaligenes faecalis</i>   | C  | -   |
| <i>Bacillaceae</i>        | <i>Bacillus licheniformis</i> | O  | -   |
|                           | <i>Bacillus megaterium</i>    | O,C                                      | O   |
| <i>Enterobacteriaceae</i> | <i>Citrobacter koseri</i>     | O,C                                      | O   |
|                           | <i>Cronobacter sakazakii</i>  | C  | -   |
|                           | <i>Edwardsiella tarda</i>     | C  | O,C   |
|                           | <i>Enterobacter aerogenes</i> | O  | O   |
|                           | <i>Enterobacter cloacae</i>   | O,C                                      | C   |
|                           | <i>Escherichia coli</i>       | O,C                                      | O,C   |
|                           | <i>Klebsiella oxytoca</i>     | O,C                                      | -   |
|                           | <i>Klebsiella pneumoniae</i>  | O,C                                      | O,C   |
|                           | <i>Kluyvera cryocrescens</i>  | O  | -   |
|                           | <i>Kluyvera</i> sp.           | O  | -   |
|                           | <i>Pantoea agglomerans</i>    | O  | -   |
|                           | <i>Proteus vulgaris</i>       | C  | -   |
|                           | <i>Providencia rettgeri</i>   | C  | -   |
|                           | <i>Serratia marcescens</i>    | C  | -   |
| <i>Serratia rubidaea</i>  | O,C                           | O,C                                      |   |
| <i>Enterococcaceae</i>    | <i>Enterococcus durans</i>    | O  | -   |
|                           | <i>Enterococcus faecalis</i>  | C  | C   |
|                           | <i>Enterococcus faecium</i>   | O  | O   |
| <i>Staphylococcaceae</i>  | <i>Staphylococcus lentus</i>  | O  | -   |
|                           | <i>Streptococcus</i> sp.      | -  | O   |
|                           | <i>Streptococcus uberis</i>   | O  | O   |

**Table 2** List of bacteria species isolated from the oral cavity of iguanas at Banco Chinchorro and number of swab samples from which each bacteria species was isolated with the prevalence (% in parenthesis) of that bacteria within each species. FA: facultative anaerobic, A: aerobic, SD: Standard deviation.

| Bacteria species                                     | Gram/type | <i>Iguana iguana</i> (n=19)     | <i>Ctenosaura similis</i> (n=10) |
|--|-----------|---------------------------------|----------------------------------|
| <i>Aeromonas hydrophila</i>                          | -/FA      | 0 (0.0)                         | 1 (10.0)                         |
| <i>Bacillus licheniformis</i>                        | +/FA      | 1 (5.3)                         | 0 (0.0)                          |
| <i>Bacillus megaterium</i>                           | +/A       | 2 (10.5)                        | 1(10.0)                          |
| <i>Citrobacter koseri</i>                            | -/FA      | 1 (5.3)                         | 3 (30.0)                         |
| <i>Edwardsiella tarda</i>                            | -/FA      | 0 (0.0)                         | 1 (10.0)                         |
| <i>Enterobacter aerogenes</i>                        | -/FA      | 7 (36.8)                        | 3 (30.0)                         |
| <i>Enterobacter cloacae</i>                          | -/FA      | 1 (5.3)                         | 0 (0.0)                          |
| <i>Enterococcus durans</i>                           | +/FA      | 1 (5.3)                         | 0 (0.0)                          |
| <i>Enterococcus faecium</i>                          | +/FA      | 5 (26.3)                        | 4 (40.0)                         |
| <i>Escherichia coli</i>                              | -/FA      | 4 (21.1)                        | 5 (50.0)                         |
| <i>Klebsiella oxytoca</i>                            | -/FA      | 2 (10.5)                        | 0 (0.0)                          |
| <i>Klebsiella pneumoniae</i>                         | -/FA      | 5 (26.3)                        | 5 (50.0)                         |
| <i>Kluyvera</i> sp.                                  | -/FA      | 3 (15.8)                        | 0 (0.0)                          |
| <i>Kluyvera cryocrescens</i>                         | -/A, FA   | 1 (5.3)                         | 0 (0.0)                          |
| <i>Pantoea agglomerans</i>                           | -/FA      | 1 (5.3)                         | 0 (0.0)                          |
| <i>Serratia rubidaea</i>                             | -/FA      | 3 (15.8)                        | 2 (20.0)                         |
| <i>Staphylococcus lentus</i>                         | +/FA      | 2 (10.5)                        | 0 (0.0)                          |
| <i>Streptococcus</i> sp.                             | +/ND      | 0 (0.0)                         | 1 (10.0)                         |
| <i>Streptococcus uberis</i>                          | +/FA      | 7 (36.8)                        | 3 (30.0)                         |
| <b>Total species</b>                                 |           | <b>16</b>                       | <b>11</b>                        |
| <b>Mean (<math>\pm</math> SD) species per iguana</b> |           | <b>2.4 <math>\pm</math> 0.6</b> | <b>2.9 <math>\pm</math> 0.3</b>  |

(Table 3). *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were the most frequent bacteria in cloacal samples (Table 3).

We isolated 13 bacterial species from black iguanas (Table 1), of which five (38.5%) were in both oral and cloacal cavities and two (15.4%) and six (46.1%) only in the cloaca and the oral cavity, respectively. Seven (63.6%) bacteria species from oral samples of black iguanas were Gram-negative and four (36.4%) were Gram-positive (Table 2). *Escherichia coli* and *Klebsiella pneumoniae* were the most frequent isolates from oral samples (Table 2). One (14.3%) bacteria species from cloacal samples of black iguanas was Gram-positive and six (85.7%) Gram-negative (Table 3). *Enterobacter cloacae* and *E. coli* were the most frequent bacteria in cloacal samples (Table 3).

## Discussion

In recent decades, research about iguanas has been focused in improving health care under captive conditions and few studies have explored their oral and cloacal bacterial flora (Boyer, 1991; Donoghue, 1994; Frye, 1995; Barten, 2002; Martínez-Silvestre *et al.*, 2003; Mader, 2006; Burghardt, 2013; Romero *et al.*, 2015). The present study is the first record of oral and cloacal bacteria of wild *C. similis* and *I. iguana*. Although we detected *Salmonella* sp. and anaerobic bacteria in a precedent study on oral and cloacal microflora of American crocodiles (*Crocodylus acutus*) in Banco Chinchorro (Charruau *et al.*, 2012), we surprisingly did not detect those bacteria in iguanas of the atoll during this present work.

*Iguana iguana* presents greater bacteria richness in cloaca (n = 15 vs. n = 7) and oral cavity (n = 16 vs. n

**Table 3** List of bacteria species isolated from the cloacal cavity of iguanas at Banco Chinchorro and number of swab samples from which each bacteria species was isolated with the prevalence (% in parenthesis) of that bacteria within each species. FA: facultative anaerobic; A: aerobic, SD: Standard deviation.

| Bacteria species                      | Gram/type | <i>Iguana iguana</i> (n=19) | <i>Ctenosaura similis</i> (n=10) |
|---------------------------------------|-----------|-----------------------------|----------------------------------|
| <i>Aeromonas hydrophila</i>           | -/FA      | 1 (5.3)                     | 1 (10.0)                         |
| <i>Alcaligenes faecalis</i>           | -/A       | 5 (26.3)                    | 0 (0.0)                          |
| <i>Bacillus megterium</i>             | +/A       | 1 (5.3)                     | 0 (0.0)                          |
| <i>Citrobacter koseri</i>             | -/FA      | 1 (5.3)                     | 0 (0.0)                          |
| <i>Cronobacter sakazakii</i>          | -/FA      | 1 (5.3)                     | 0 (0.0)                          |
| <i>Edwardsiella tarda</i>             | -/FA      | 1 (5.3)                     | 1 (10.0)                         |
| <i>Enterobacter cloacae</i>           | -/FA      | 9 (47.4)                    | 8 (80.0)                         |
| <i>Enterococcus faecalis</i>          | +/FA      | 1 (5.3)                     | 1 (10.0)                         |
| <i>Escherichia coli</i>               | -/FA      | 11 (57.9)                   | 5 (50.0)                         |
| <i>Klebsiella oxytoca</i>             | -/FA      | 1 (5.3)                     | 0 (0.0)                          |
| <i>Klebsiella pneumoniae</i>          | -/FA      | 7 (36.8)                    | 3 (30.0)                         |
| <i>Proteus vulgaris</i>               | -/FA      | 1 (5.3)                     | 0 (0.0)                          |
| <i>Providencia rettgeri</i>           | -/FA      | 1 (5.3)                     | 0 (0.0)                          |
| <i>Serratia marcescens</i>            | -/FA      | 1 (5.3)                     | 0 (0.0)                          |
| <i>Serratia rubidaea</i>              | -/FA      | 1 (5.3)                     | 2 (20.0)                         |
| <b>Total species</b>                  |           | <b>15</b>                   | <b>7</b>                         |
| <b>Mean (± SD) species per iguana</b> |           | <b>2.3 ± 0.7</b>            | <b>2.1 ± 0.3</b>                 |

= 11) than *C. similis*. This could be due to differences in feeding habits, which influence the bacterial composition of intestinal communities (Lankau et al., 2012). Although green iguanas can eat carrion and insects, they are primarily herbivorous, and depend on their gastrointestinal microbial communities to improve the efficiency of plant digestion (Loftin and Tyson, 1965; van Marken Lichtenbelt and Albers, 1993; Durtsche, 2000). On the contrary, *C. similis* presents an ontogenic shift from an insectivorous diet in juveniles to an herbivorous-omnivorous diet in adults (Montanucci, 1968; Van Devender, 1982; Fitch and Hackforth-Jones, 1983). We assumed that the insectivorous diet during the juvenile stage influences the composition and structure of the gastrointestinal microbe community, since insects are easily digestive food containing more protein and energy than plants (Golley, 1961; Donoghue, 1995; Durtsche, 2000). Once black iguanas become adults they not only shift their diet, apparently, they also shift their oral, gastrointestinal and cloacal microbes.

Of the 23 species and two genera of bacteria isolated from *I. iguana* and *C. similis* in this study, 18 species (*Aeromonas hydrophila*, *Citrobacter koseri*, *Cronobacter*

*sakazakii*, *Edwardsiella tarda*, *Enterobacter cloacae*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Kluyvera cryocrescens*, *Pantoea agglomerans*, *Proteus vulgaris*, *Providencia rettgeri*, *Serratia marcescens*, *Serratia Rubidaea*, *Staphylococcus lentus*) and both genera (*Kluyvera*, *Streptococcus*) have been associated with infections in humans (Neter and Farrar, 1943; Von Graevenitz and Mensch, 1968; Wilfert et al., 1968; Janda and Abbott, 1993; Tarr, 1995; Ursua et al., 1996; Doran, 1999; Harbarth et al., 1999; Lai, 2001; Sarria et al., 2001; Cruz et al., 2007; Hammerum, 2012; Vijaykrishnan and Rapose, 2012; Hagiwara et al., 2013; Rivera et al., 2014; Persichino et al., 2015; Sagar et al., 2017). *Serratia marcescens* has been especially identified to cause wound infections after iguana bites (Hsieh and Babl, 1999; Grim et al., 2010). This provides valuable information about the potential zoonotic pathogens that could be transmitted by iguanas in case of bite or consumption of contaminated food in Banco Chinchorro.

Although *I. iguana* and *C. similis* are among the most widely distributed lizards in Central America (ITWG, 2011), little is known about the health status of wild populations. To our knowledge, this is the first study documenting the bacterial agents from the oral cavity and cloaca of these species in the wild. These bacteria can be a potential threat to malnourished and stressed individuals, but also to human health. The information provide by this study could help to establish adequate clinical veterinary assistance for sick animals and to choose ideal antimicrobial therapy in cases of bites or infections due to contact with iguanas.

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