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AEROBIC BACTERIAL FLORA OF NESTING GREEN TURTLES (*CHELONIA MYDAS*) FROM TORTUGUERO NATIONAL PARK, COSTA RICA

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Abstract: Bacteriological examination of 70 nesting green turtles (*Chelonia mydas*) from Tortuguero National Park, Costa Rica was performed to investigate nasal and cloacal aerobic bacteria. A total of 325 bacterial isolates were obtained, including 10 Gram-negative and three Gram-positive genera. Two hundred thirty-nine were Gram-negative and 86 were Gram-positive isolates. *Klebsiella pneumoniae* was the most common microbe identified in turtle samples: 27/70 (38.5%) in cloacal, and 33/70 (47.1%) in nasal samples. The *Enterobacteriaceae* family, including *Enterobacter agglomerans*, *E. cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae*, and *Serratia marcescens*, was the largest Gram-negative group of bacteria recovered and comprised 127 of 239 (53.1%) of the Gram-negative isolates. *Staphylococcus* species was the largest Gram-positive bacteria group, including *S. aureus*, *S. cromogenes*, *S. epidermis*, and *S. intermedius*, and made up 63 of 86 (73.2%) of the Gram-positive isolates recovered. The results of this study demonstrate that the aerobic bacterial flora of nesting green turtles at Tortuguero National Park is composed of a very wide spectrum of bacteria, including several potential pathogens.

Key words: Bacterial flora, *Chelonia mydas*, Costa Rica, green turtle, *Klebsiella pneumoniae*, *Staphylococcus*.

BRIEF COMMUNICATION

The green turtle (*Chelonia mydas*), a primarily herbivorous species, lives in tropical and subtropical waters of the Atlantic, Pacific, and Indian oceans. Information on the bacterial flora of free-ranging sea turtles is limited. Only one report exists on bacteria of juvenile green turtles with and without fibropapillomatosis.¹ Some data have been published for loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) nesting females.^{13,16} Results from these studies reported that the bacteria recovered included many potential pathogens that cause diseases in free-living and captive sea turtles.^{5,6,9,10,15}

Baseline information on the composition of normal flora of wild turtles is an extremely useful tool for the correct interpretation of bacteriological culture results and to better understand the role of bacteria as pathogenic agents in disease events among turtles. In this article, the nasal and cloacal aerobic bacterial flora of apparently healthy free-ranging nesting female green turtles from Tortuguero National Park (TNP), Costa Rica was documented.

From August through October 2003, at TNP (10°32'27"N, 83°29'59"W–10°21'17"N, 83°23'29"W)

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on the northeast Caribbean coast of Costa Rica, bacterial specimens were obtained from female green turtles. The TNP contains approximately 26 km of nesting beach. Here, the green turtle nesting season occurs between June and October. Samples were collected along the first northern 5 km of TNP beach, and the study area was divided into three sectors (sites one, two, and three) with a length of approximately 1.65 km each. After nesting, the clinical status of all turtles was evaluated by an external physical examination. The curved carapace length (CCL) at midline was measured and the number identification of the flipper metal tags was recorded. Only bacteriological data from apparently healthy nesting green turtles, with no external lesions or clinical signs of disease, were included in this study. Additionally, three seawater samples per month (one sample from each sampling site) were collected in 10-dl sterile plastic bags, near the shore. This sampling was taken to investigate the effect of seawater contamination on the turtle normal flora composition.

Turtle specimens were obtained during nesting, by inserting a cotton sterile swab into the cloaca and in one nasal conduct. Swabs were then placed in Amies agar gel transport medium (Oxoid, Ltd., Basingstoke, Hampshire, England), placed on ice, and within 24 hr cultured on blood agar (for the growth of most bacteria), MacConkey agar (for the isolation of *Enterobacteriaceae*), manitol salt agar (for the isolation of *Staphylococcus* species), thio-sulfate-citrate-bile-salts-sucrose (TCBS) agar (for the isolation of *Vibrio* species), and xylose-lysine-

desoxycholate (XLD) agar (Oxoid) (for the isolation of *Salmonella* and *Shigella* species). Plates were incubated at 25–27°C aerobically and examined after 24 hr. Additionally, the cloacal samples were placed on enrichment media, Rappaport-Vasiliadis-soy peptone broth (Oxoid), incubated at 37°C for 18 hr and subcultured on XLD agar for *Salmonella* detection. In order to detect diversity among isolates, a maximum of three colonies were selected from each agar plate according to the size, morphology, pigmentation, and hemolysis of the colonies. Isolated colonies were identified by Gram and biochemical reactions, with the use of standard protocols.⁸ Identification of Gram-negative organisms was confirmed by the Api System 20 NE and 20 E (BioMérieux, Marcy-l'Étoile, France). Water samples were processed by conventional methods.²

The chi-square test was used to compare the number of turtle bacteria collected during the 3 mo of sampling. The minimum significance value was $P < 0.05$. Statistical analysis was performed with the use of the Systat version 10 software (SPSS Inc., Chicago, Illinois 60611, USA). Prevalence is defined as the percentage of turtles infected with a bacterium species.

A total of 325 isolates, including 10 Gram-negative and 3 Gram-positive genera, were identified from nasal and cloacal samples of 70 female green turtles (mean of CCL was 111.5 cm). Two hundred thirty-nine isolates were Gram-negative and 86 isolates were Gram-positive microorganisms. One hundred sixteen Gram-negative and 57 Gram-positive isolates were identified from nasal samples. One hundred twenty-three Gram-negative and 29 Gram-positive isolates were found in cloacal samples. The bacteria isolated are listed in Table 1. No bacterial growth was reported from two nasal and three cloacal samples.

Klebsiella pneumoniae was the most common microorganism identified in turtle samples (33/70 or 47.1% in nasal ducts and 27/70 or 38.5% in cloaca, respectively) and the most prevalent (65.7%). *Enterobacteriaceae*, including *Enterobacter agglomerans*, *E. cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae*, and *Serratia marcescens*, was the largest Gram-negative group of bacteria cultured, and comprised 127 of 239 (53.1%) of the Gram-negative isolates. *Staphylococcus* species, including *S. aureus*, *S. cromogenes*, *S. epidermis*, and *S. intermedius*, was the largest Gram-positive group and included 63 of 86 (73.2%) of the Gram-positive isolates. However, in turtle samples, *Enterobacteriaceae* decreased in October (61 in August, 60 in September, and 40 in October), and *Staphylococcus* increased in the same sampling months (9 in Au-

gust, 22 in September, and 32 in October). The total number of bacterial isolates recovered in each of the 3 mo of sampling was similar ($P = 0.54$).

Seawater samples did not have visible bacterial fecal contamination. However, during October a high presence of total coliforms was detected in water sampling sites 1 and 3 (Table 2).

During the last two decades, there has been an increasing interest in infectious diseases of wild sea turtles. Reports of bacterial infections in free-ranging stranded turtles include valvular endocarditis and septicemia by *Vibrio damsela* infection in a leatherback (*Dermochelys coriacea*); *Corynebacterium* encephalitis and *Aerococcus viridans*-associated esophageal infection in loggerheads; *Mycobacterium chelonae* osteoarthritis in a Kemp's ridley (*Lepidochelys kempii*); and mixed generalized infections in green, leatherback, and loggerhead turtles.^{4,7,9,10,14}

The results of our study demonstrated that the aerobic bacterial flora of nesting female green turtles that were apparently healthy was composed of a very wide microbe spectrum, including several potential pathogens.

In this study, *Klebsiella pneumoniae* was the most common Gram-negative microbe. In many reptiles, it has been considered a resident flora.¹¹ However, it has also been associated with ulcerative stomatitis in captive hatchling and juvenile green and loggerhead turtles; and with abscess, hypopyon, necrotizing dermatitis, and pneumonia in captive terrestrial reptiles.^{3,6,11}

Aeromonas sp., *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Pasteurella* sp., *Proteus* sp., *Pseudomonas* sp., *Serratia marcescens*, *Staphylococcus* sp., and *Vibrio* sp. have been associated with abscesses of the salt-secreting gland and peritoneal wall, bronchopneumonia, integumental lesions, obstructive rhinitis, traumatic ulcerative dermatitis, ulcerative shell disease, and ulcerative stomatitis of captive and wild sea turtles.^{5,6,10,15}

The impact and the role of these bacteria on free-ranging green turtle populations are not well known. The finding of potential pathogenic bacteria in free-living turtle tissues is not synonymous with illness and should be evaluated relative to the potential impact on a specific animal group, considering numerous stressor conditions. During courtship and mating, the superficial tissues of the female turtles are frequently injured by male bites and claws. Dermal lesions and seawater aspiration are the most important routes by which the microbes may enter the sea turtles.⁴ Most of the microbes found in this study are usually considered to be opportunistic pathogens. It is possible that con-

Table 1. Number of aerobic isolates recovered from nasal (N) and cloacal (C) samples of the nesting green turtles at Tortuguero National Park ($n = 70$), August–October 2003.

Bacterial isolates	Aug ($n = 23$)		Sep ($n = 22$)		Oct ($n = 25$)		Total isolates		Positive turtles	
	N	C	N	C	N	C	n	%	n	%
Gram-positive bacteria										
<i>Bacillus</i> sp.	1	0	0	0	2	0	3	4.2	3	4.2
<i>Bacillus cereus</i>	5	0	0	0	3	0	8	11.4	8	11.4
<i>B. firmus</i>	1	0	0	0	0	0	1	1.4	1	1.4
<i>Micrococcus</i>	2	1	2	2	2	2	11	15.7	11	15.7
<i>Staphylococcus aureus</i>	4	0	17	0	3	0	24	34.2	24	34.2
<i>S. cromogenes</i>	1	0	0	3	0	5	9	12.8	9	12.8
<i>S. epidermis</i>	1	0	0	0	0	0	1	1.4	1	1.4
<i>S. intermedius</i>	1	2	0	2	12	12	29	41.4	23	32.8
Gram-negative bacteria										
<i>Aeromonas hydrophila</i>	0	3	0	0	0	2	5	7.1	5	7.1
<i>A. salmonicida</i>	5	2	0	2	2	0	11	15.7	11	15.7
<i>Bordetella avium</i>	3	3	0	2	0	4	12	17.1	11	15.7
<i>B. bronchiseptica</i>	0	0	0	0	1	0	1	1.4	1	1.4
<i>Enterobacter agglomerans</i>	9	1	4	2	5	2	23	32.8	22	31.4
<i>E. cloacae</i>	0	1	0	0	1	0	2	2.8	2	2.8
<i>Escherichia coli</i>	2	1	0	0	2	2	7	10	6	8.5
<i>Klebsiella oxytoca</i>	5	8	2	5	1	3	24	34.2	22	31.4
<i>K. pneumoniae</i>	14	8	13	9	6	10	60	85.7	46	65.7
<i>Pasteurella</i> sp.	0	0	0	0	2	0	2	2.8	2	2.8
<i>Proteus mirabilis</i>	1	2	2	8	0	3	16	22.8	15	21.4
<i>P. vulgaris</i>	2	7	1	5	2	1	18	25.7	16	22.8
<i>Pseudomonas aeruginosa</i>	0	2	1	2	5	5	15	21.4	13	18.5
<i>P. fluorescens</i>	0	1	0	0	0	0	1	1.4	1	1.4
<i>P. mendocina</i>	0	0	1	1	0	1	3	4.2	3	4.2
<i>P. putida</i>	1	1	0	0	2	1	5	7.1	4	5.7
<i>Serratia marcescens</i>	0	0	3	6	1	1	11	15.7	10	14.2
<i>Vibrio alginolyticus</i>	8	3	1	0	2	0	14	20	13	18.5
<i>V. fluvialis</i>	1	1	1	0	1	2	6	8.5	5	7.1
<i>V. hollisae</i>	0	0	1	0	1	0	2	2.8	2	2.8
<i>V. parahaemolyticus</i>	0	0	1	0	0	0	1	1.4	1	1.4
Total isolates	67	47	50	49	56	56	325			
Culture with no growth	0	1	1	1	1	1				

comitant diseases and/or environment-associated, reproductive, or many other stressing conditions, may affect the immune system of the hosts. Hence, the animal would be more susceptible to infection from these opportunistic bacteria that are normally present in healthy turtles. Gestation is a critical time for female reptiles, and may have an inhibitory effect on their immune system; the immunosuppression due to reproductive effort might affect the delicate microflora balance.¹²

Several bacterial genera cultured in this study have been associated with egg failure and correlated with lower hatching success in natural and relocated nests of loggerheads.¹⁶

Environmental influences and sources of contamination must be considered in the interpretation of

bacterial cultures from the mucous membranes of sea turtles. Many bacteria recovered from sea turtles are microorganisms commonly found in the environment; thus contamination by sea water and/or beach sand may occur.⁸ In addition, some bacteria species and other microbes may be carried to the sea by sources of pollution, such as effluent and runoff. Recently, it was noted that pollution along the Caribbean coastline of Costa Rica influenced the leatherback bacterial flora during the nesting season (unpublished data). It was hypothesized that water quality influences the composition of turtle microflora and predicted a positive correlation between seawater coliform levels and Gram-negative constituents of sea turtle flora. That hypothesis was not supported in this study, as high numbers of co-

Table 2. Most probable number of seawater microbe presence and rain precipitation at Tortuguero National Park, August–October 2003.

Site	Indicator trait	Months of sampling, 2003		
		Aug	Sep	Oct
Site 1	Total coliforms	23/100 ml	23/100 ml	540/100 ml
	Fecal coliforms	2/100 ml	2/100 ml	<2/100 ml
	Enterococci	4/100 ml	4/100 ml	4/100 ml
Site 2	Total coliforms	8/100 ml	8/100 ml	8/100 ml
	Fecal coliforms	8/100 ml	8/100 ml	<2/100 ml
	Enterococci	4/100 ml	4/100 ml	<2/100 ml
Site 3	Total coliforms	8/100 ml	8/100 ml	350/100 ml
	Fecal coliforms	8/100 ml	8/100 ml	2/100 ml
	Enterococci	4/100 ml	4/100 ml	4/100 ml
	Rain precipitation (mm)	675.2	282.4	331.4

liforms were only observed in sea water from sampling sites 1 and 3 during October, which corresponded to lower coliform presence in turtle samples. It also appeared that monthly rain precipitation did not influence the results of turtle samples. Monthly rainfall levels from the TNP obtained by the Caribbean Conservation Corporation (unpublished data) are reported in Table 2. This suggests there was no relationship between bacteria from turtles and from seawater. Thus, the cause of the monthly variation of *Enterobacteriaceae* and *Staphylococcus* proportions in turtle isolates observed in this study remains unknown.

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