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Neisseria SPECIES ISOLATED FROM DOLPHINS[1]

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Neisseria species have been identified as part of the normal microbial flora of several domestic and experimental animals,[1] but they have not however, been observed in marine mammals.[3] We are presently studying on a long-term basis, the total microbial profile (bacteria, viruses, and fungi) of marine mammals in their natural habitat and the inter- and intra-species changes which occur when the animals are brought into contact with man, other animals, and when placed in isolation under husbandry conditions.

The present report describes the isolation of a bacterium *Neisseria mucosa* from dolphins *(Lagenorhynchus obliguidens* and *Delphinus bairdi).* The importance of this observation with regard to the husbandry of marine mammals is discussed.

MATERIALS AND METHODS

Thirty-five dolphins were captured by net approximately 25 miles due west of the Channel Islands (50-60 miles from shore). Divers brought the animals aboard ship and within 10 minutes samples for culture were obtained from the mouth, eye, blowhole, conjunctiva, skin (axilla or flipper), vagina or prepuce, and anus. The animals were then returned to the ocean. The samples were obtained with dacron swabs[4] and placed aseptically in Amies Transport Medium.[5] The transport medium was maintained between 6 and 20C for 24 to 36 hours before plating onto various media for isolation and identification. Samples from the throat, mouth, blowhole, anus, and vagina or prepuce were cultured in duplicate on PPLO medium.[5] One plate was incubated at 37C (10% CO_2) and the other at 37C (20% CO_2). Samples from other body sites were plated on blood agar, nutrient agar, phenylethyl alcohol agar with 5% blood, eosin-methylene blue agar, chocolate agar, thioglycolate broth, Sabaroud dextrose agar, and Dubos' oleic agar.[5] Duplicate plates were incubated aerobically and anaerobically at 6C, 20C, and 37C for 48 hours. All dissimilar colonies from all plates were selected from primary cultures and subjected to routine clinical bacteriology procedures.

RESULTS

Only five of several hundred bacterial isolates proved to be gram negative, oxidase positive diplococci. These were initially isolated on PPLO medium aerobically at all temperatures of incubation from the blowhole of two female dolphins *(L. obliguidens)* and the blowhole, mouth, and throat of three female dolphins *(D. bairdi)* respectively.

The colonial appearance after incubation at 35C on nutrient agar overnight was similar for all five isolates, i.e. entire, smooth, 2-3 mm in diameter, slightly raised, butyrous, and containing a buff, yellow pigment.

The colonial morphology and production of acid in media containing 1% carbohydrate indicated that the dolphin isolates belonged to one of three species of chromogenic *Neisseria,* i.e. *N. mucosa, N. sicca,* or *N. perflava.* Prototype

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[5] Difco, Detroit, Michigan.

TABLE 1. Growth and biochemical reaction of the **Neisseria** species from dolphins and selected prototype **Neisseria.**

Tests	Prototype Neisseria			Dolphin Isolates				
				L. obliguidens		*D. bairdi*		
	N. mucosa	*N. sicca*	*N. perflava*	1	2	blow hole	mouth	throat
M-H Blood	3+	4+ beta	4+ beta	4+	4+	4+	4+	4+
M-H Plain	1+	3+	4+	—	—	—	—	1+
N.A.	2+	NG	1+	3+	3+	3+	3+	3+
Nitrate broth	$+NO_2$ G	—	—	$+NO_2$ G	$+NO_2$ G	$+NO_2$ G	$+NO_2$ G	$+NO_2$ G
T.S.I.								
slant/butt	A/NC	A/NC	A/NC	A/NC	A/NC	A/NC	A/NC	A/NC
H_2S	3+	—	3+	—	—	2+	1+	2+
Sellers								
slant/butt	FB/G	NG	NG	FB/NC	FB/NC	FB/NC	FB/NC	FB/G
Sugars								
maltose	+	+	+	+	+	+	+	+
dextrose	+	+	+	+	+	+	+	+
sucrose	+	+	+	+	+	+	+	+
mannitol	—	—	—	—	—	—	—	—
lactose	—	—	—	—	—	—	—	—
fructose	+	+	+	+	+	+	+	+
IM Vi C								
Indole	—	—	—	—	—	—	—	—
Methyl Red	+	—	—	+	+	+	+	+
V-P	—	—	—	—	—	—	—	—
Citrate	—	—	—	—	—	—	—	—

Abbreviations: A = acid; NC = No change; — = No reaction; NG = No growth; FB = faint blue color; G = gas; $+NO_2$ = detectable nitrite; N.A. = nutrient agar (DIFCO); M-H blood = Mueller-Hinton Agar (DIFCO) containing 5% defibrinated sheep blood.

strains of these three species were compared to the dolphin isolates by growth and biochemical characteristics. Results are shown in Table 1. Although as expected, there were minor differences among all of the species tested, the dolphin isolates and *N. mucosa* could readily be distinguished from *N. sicca* and *N. perflava*. The latter species produced beta hemolysis on blood agar, did not grow in Seller's Medium and did not produce acid in the methyl red test. The colonies of the animal isolates were similar in consistency to *N. perflava* but were distinctly different from *N. sicca* which are dry, crinkled, and easily pushed along the agar surface.

The most critical biochemical reaction used to identify *N. mucosa* is its ability to reduce nitrates with the production of gas.[4] Only the prototype *N. mucosa* and the dolphin isolates reduced nitrates with production of gas. It was noted however, that at higher concentrations of nitrate (1%), growth of the dolphin isolates was inhibited whereas the *N. mucosa* produced positive reactions.

Serology

Hyperimmune rabbit antisera to the prototype *N. mucosa* and one of the dolphin isolates (throat, *D. bairdi*) were produced by daily injections (1.0 ml, intravenously) of 10^7 bacterial/ml for 5 days; rested 3 days; and repeated for another 5 days. The rabbits were bled 5 days following the last injection. Results of cross-agglutination are shown in Table 2. As can be seen, antiserum to *N. mucosa* or the dolphin isolate reacted specifically and did not agglutinate with *N. perflava* or *N. sicca*.

Pathogenicity for mice

It has been reported that some strains of *N. mucosa* are pathogenic for mice. The prototype *N. mucosa* and dolphin isolates were grown in Mueller-Hinton Broth on a rotary shaker at 37C for 4 hours. Two-fold dilutions of the broth culture were made in saline and 1 ml injected intra-peritoneally in each of six mice per dilution. Controls consisted of incubated but uninoculated broth treated as above. An inoculum of 1.0-1.5 x 10^8 bacteria caused all mice to become moribund with 24 hours. An average of 70% of the mice died within 24 hours when inoculated with 1.5-3.0 x 10^9 bacteria. No control mice or those receiving less than 10^8 bacteria showed any ill effects. Necropsy of the moribund animals and those that died revealed an enlarged spleen and liver, fibrinous exudate on the surface of the visceral organs, and a dark-colored, congested mesentery with the omentum appearing "bunched up". These findings were comparable to those of Veron[4] who used strains of *N. mucosa* isolated from children with bronchial pneumonia.

TABLE 2. Agglutination reaction of rabbit anti-**Neisseria** sp. and dolphin isolate No. 1 **(L. obliguidens)** sera with prototype **Neisseria** sp. and dolphin isolates.

Test Antiserum	Agglutinin Titer (Reciprocal of dilution)							
				L. obliguidens		*D. bairdi*		
	N. mucosa	*N. sicca*	*N. perflava*	1	2	blow hole	mouth	throat
N. mucosa	256	<2	<2	128	64	64	32	16
Isolate No. 1 *(L. obliguidens)*	128	<2	<2	256	128	64	64	32

DISCUSSION

Studies currently in progress indicate that marine mammals may have a microbial population indigenous to themselves. Although taxonomically related to isolates from human sources, preliminary studies indicate that upon biochemical and genetic tests, the bacteria from various marine mammals differ significantly. The *N. mucosa* strains in this report were an exception. The prototype strain of human origin compared very closely to the isolates from dolphins even in ability to grow in the presence of increasing amounts of NaCl.

Veron[4] originally described *N. mucosa* as the etiological agent of bacterial pneumonia in children. Recently Berger[2] divided the species into two types based on pigment production, growth on blood agar and biochemical and serological tests. He designated the pigmented type as *N. mucosa var. heidelbergensis* and found these to be present in the nasopharynx of 48 of 100 adult healthy individuals. Because of the pigmented colonies and the biochemical characteristics in Table 1, it appears that the dolphin isolates best fit into the *N. mucosa var. heidelbergensis* classification.

Although *N. mucosa* has been found in healthy individuals, its implication as the etiological agent of pneumonia in children indicates its potential danger. The probable mode of transmission is via the respiratory route. The findings in this report that *N. mucosa* can be isolated from the blowhole of dolphins should be considered by those investigators who work very closely with these marine mammals in their husbandry and training programs.

Studies are currently in progress on experimental infection of marine mammals, particularly sea lions, exposed via the respiratory route in order to better understand environmental and biological procedures to be used for control of infectious agents between animals and from animal to man.

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