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A new species of *Neisseria* from iguanid lizards, *Neisseria iguanae* sp. nov.

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S.J. BARRETT, L.K. SCHLATER, R.J. MONTALI AND P.H.A. SNEATH. 1994. A new species of the genus *Neisseria* is proposed, *Neisseria iguanae* sp. nov. The organism is associated with septic lesions in the common iguana (*Iguana iguana*) and the rhinoceros iguana (*Cyclura cornuta*) but is also a commensal. It resembles *N. animalis* and *N. canis* phenotypically but is distinguished from these by exhibiting pronounced tetrad arrangement and alpha haemolysis and by fermenting gluconate.

INTRODUCTION

An unusual outbreak of infection, caused by a species of *Neisseria*, occurred in iguanid lizards at the National Zoological Park, Washington, DC, in 1984 and 1985 (Plowman *et al.* 1987). The organism was first isolated from a septicaemic rhinoceros iguana (*Cyclura cornuta*) with multiple granulomas and later from chronic tail abscesses in common iguanas (*Iguana iguana*). It was also isolated from the oral cavities of healthy lizards of these two species.

The organism was seen as a Gram-negative diplococcus within the granulomas and abscesses. In some lesions, the bacteria were surrounded by dense eosinophilic amorphous material radiating from the periphery, similar to 'sulphur-granules' in actinomycotic lesions in mammals; such changes have been reported in humans as Splendore-Hoeppli reactions, caused by certain fungi, bacteria and parasites (Johnson 1976; Jones and Hunt 1983). The organism was identified at the National Veterinary Services Laboratories (NVSL) as a species of *Neisseria* (Plowman *et al.* 1987), but was not then formally proposed and named as a new species of that genus.

Some of these strains (Provided by LKS) were included in a recent survey by one of us (SJB) and were examined in an extensive set of bacteriological tests, followed by numerical taxonomic computer analysis.

MATERIALS AND METHODS

Strains

The strains from lizards had NVSL numbers as follows: 85297, 85728, 85737, 85738 and 85739. NVSL 85728 was isolated from a liver granuloma in a rhinoceros iguana; the others were from tail abscesses in common iguanas. A collection of about 300 other neisserias was studied in parallel; they included representative (including type) strains of *Neisseria gonorrhoeae*, *N. meningitidis* and most other currently recognized species, including *N. animalis* and *N. canis* (paper in preparation).

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Morphology of colonies and cells at 35°C

Colonial morphology was determined by observing 72 h growth on blood agar (heart infusion agar base; Difco Laboratories, Detroit, MI) containing 5% (v/v) defibrinated bovine blood. Growth from the bovine blood agar plate was removed with an inoculating loop and examined against white filter paper for the presence of pigment. Haemolytic activity was determined on bovine, sheep and horse blood agars (5% v/v) after 48 h at 35°C. Gram staining was done by the method of Koppeloff (Cottral 1978) on smears prepared from 72 h growth on bovine blood agar. Motility was determined by examination of wet mount preparations, using phase-contrast microscopy. Wet mounts were prepared using growth from bovine blood agar plates incubated aerobically for 72 h at 25°C.

Biochemical tests

Dehydrated media from commercial sources were used whenever possible. Inoculated media for all tests were incubated aerobically at 37°C for 72 h, then allowed to incubate at room temperature for an additional 4 d. Catalase, deoxyribonuclease and oxidase were recorded at 72 h. All other biochemical tests were recorded at 1 week and considered negative if no reaction was observed at that time. Unless otherwise indicated, methods described by MacFaddin (1980) were used for the conventional biochemical tests.

The test for indole production was done by using Ehrlich reagent following extraction with xylene. Oxidase was detected on filter paper, using the tetramethyl-*p*-phenylenediamine dihydrochloride reagent (Marion Scientific Division, Marion Laboratories, Inc., Kansas City, MO). Hydrolysis of aesculin was tested on heart infusion agar slants containing 1% aesculin and 0·5% ferric chloride (Phillips and Nash 1985). Nitrite reduction was performed by the method of Cowan (1974) in addition to that of MacFaddin (1980). Acid production from carbohydrates was examined in API 50CH galleries (Analytab Products, Montalieu-Vercieu, France) and read after 24 h at 35°C and on cystine-tryptic digest agar slants containing 2% (w/v) of carbohydrate (Morello *et al.* 1985) read after 48 h at 37°C. Enzyme profiles were performed in ATB FGN galleries (Analytab Products) according to the manufacturer's instructions. Growth was also tested on 5% (v/v) horse blood chocolate agar containing 0·5% (w/v) sodium taurocholate and on nutrient agar (Oxoid Ltd, Basingstoke, UK) after incubation at 35°C for 48 h.

Antimicrobial susceptibility tests

Antibiograms were determined by the disk diffusion method of Bauer *et al.* (1986), using Mueller-Hinton agar (Difco) supplemented with 5% (v/v) sheep blood and 1% (v/v) IsoVitalex (BBL Microbiological Systems, Cockeysville, MD). The antibiotics and the concentrations used were: penicillin, 10 units; chloramphenicol, 30 μg; gentamicin, 10 μg streptomycin, 10 μg; tetracycline, 30 μg; novobiocin, 5 μg; and trimethoprim, 5 μg. Zone sizes were interpreted as resistant, intermediate or susceptible according to the National Committee for Clinical Laboratory Standards (1975).

Numerical analysis

Test results were coded as positive or negative except for those from API and ATB galleries, which were coded on a scale from 0–2 for the former and 0–3 for the latter, to yield 155 characters. Similarities calculated by the Gower coefficient (including negative matches) and UPGMA clustering were performed as described by Sneath and Stevens (1985).

RESULTS AND DISCUSSION

The numerical taxonomic analysis showed that the strains from lizards formed a compact cluster at 96·2% similarity (Fig. 1). The strains shared many characteristics with *Neisseria animalis*, *N. canis* and neisserias isolated from dental plaque of mammals, but they were clearly distinct from all of these (which were allied to the lizard strains at about the 89% level). The lizard strains, together with *N. animalis*, *N. canis* and the dental strains, belonged phenotypically to the 'true neisserias' rather than the 'false neisserias' that are closely allied to *Branhamella* and *Moraxella* (Vedros 1984). We consider the evidence is sufficient to support the proposal of a new species of *Neisseria* for the isolates from iguanid lizards.

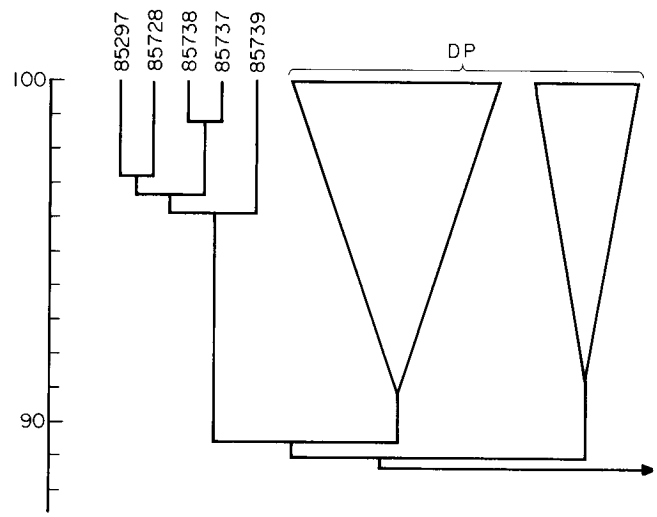

Fig. 1 Relevant portion of UPGMA phenogram, showing strains from iguanid lizards with NVSL numbers, and relationship to nearby neisserias. DP indicates 11 strains from dental plaque. The arrow indicates the branch to other neisserial species. The scale is percentage similarity on the Gower coefficient

The major characteristics distinguishing the new species from other *Neisseria* species are listed in the description below. Additional characteristics may be found in Plowman *et al.* (1987). There were few discrepancies in the results of tests performed by slightly different methods. The API 50 CH tests appeared more sensitive than the carbohydrate slants, and showed weak, though definite, acidity from a few carbohydrates. It is well known that saccharolytic neisserias commonly give weak or negative sugar reactions (Cowan 1974; Vedros 1984; Morse and Knapp 1992). The other discrepancy is in reduction of nitrite; the lizard strains were positive in the sensitive method of Cowan (1974).

Description of *Neisseria iguanae*

Neisseria iguanae sp. nov. (M.L. fem. gen. sing. noun *iguanae*, of the iguana lizard, from Spanish fem. noun, iguana; *Neisseria* sp., Plowman *et al.* 1987). Cells are small cocci about 0·8 μm in diameter; they are largely arranged as diplococci but show numerous tetrads in culture, nonmotile. Gram reaction is negative. Endospores were not observed. Growth is aerobic, no growth under anaerobic conditions, mesophilic; growth occurs between 25°C and

37°C, chemo-organotrophic. Surface colonies grown aerobically on 5% (v/v) sheep or horse blood agar are non-pigmented, round, transparent, domed, about 1 mm in diameter after 72 h at 35°C, and showed marked zones of alpha haemolysis. Oxidase-positive, indole-negative, catalase-positive, urease- and deoxyribonuclease-negative. Nitrates usually reduced, nitrite reduction variable. Sensitive to penicillin, chloramphenicol, gentamicin, streptomycin and tetracyclin, resistant to novobiocin and trimethoprim. Weak acidity usually from glucose, sucrose and gluconate, sometimes trehalose, not from fructose, maltose, lactose or galactose (API 50 CH). Aesculin not hydrolysed. Gelatin not liquified, acetate and citrate not utilized. Grows on GC chocolate agar containing 0·5% (w/v) sodium taurocholate, but not on MacConkey or nutrient agar.

Notable among neisserias for being gluconate-positive, strongly positive for alkaline phosphatase 2 (FGN) and strongly alpha haemolytic on horse and sheep blood agar. Distinguished from *Neisseria animalis* and *N. canis* by these properties, and in forming tetrads and failure to grow on nutrient agar although similar to these species in sometimes acidifying glucose and sucrose but not fructose or maltose, and in usually reducing nitrate and synthesizing polysaccharide.

Associated with septicaemia and abscesses in iguanid lizards, and may be isolated from the oral cavity of healthy lizards (*Iguana iguana* and *Cyclura cornuta*).

The type strain is NVSL 85737.

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