**Rothia dentocariosa**: taxonomy and differential diagnosis

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**ABSTRACT**

As recent external quality control results have shown, the diagnosis of *Rothia dentocariosa* infection still presents problems for clinical laboratories. This review describes the taxonomy, as well as the chemotaxonomic, morphological and biochemical characteristics, of this organism, and surveys bacteria that may be confused with *Rothia dentocariosa*.

**Keywords** Identification, Rothia dentocariosa, taxonomy

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**INTRODUCTION**

In 2001, the Swiss External Quality Assessment Scheme in Bacteriology and Mycology, which operates under the direction of the author [1], sent out a strain of *Rothia dentocariosa* to 50 laboratories participating in the scheme. The organism was described as a bacterium isolated from a case of vertebral osteomyelitis, similar to a case reported in the literature [2]. Only 36 (72%) of the participants arrived at a correct diagnosis, mostly by use of the API Coryne system, v.2 (bioMérieux, La Balme-les-Grottes, France). This score was below the average for the yearly performance of all the participating laboratories [3], and was similar to scores obtained for ‘problem organisms’ such as *Lactobacillus* spp., *Clostridium tertium*, *Neisseria weaveri*, *Capnocytophaga* spp., *Eikenella corrodens* and *Moraxella* spp. [1,3]. Five participants would have sent the strain to a reference laboratory; four participants identified it as a species of coagulase-negative staphylococci by means of the API Staph or ID32 Staph systems; three participants identified it as *Stomatococcus mucilaginosus* (which is an organism now classified as *Rothia mucilaginosa*) with ID32 Staph; and three participants identified it, respectively, as *Propionibacterium* spp., *Actinomyces* spp. and *Rhodococcus equi* by means of API Coryne; however, incubation times, inoculum and/or the catalase test may not have been correct in individual cases. Thus, it was obvious that *R. dentocariosa* was also a problem organism.

These observed difficulties were surprising in view of the fact that *R. dentocariosa* has been known for over 35 years. In 1967, Georg and Brown proposed the genus *Rothia* [4] for a group of coccoid to diphtheroid to filamentous Gram-positive organisms isolated from the human oral cavity, naming the genus after Roth [5], who had performed basic studies on organisms, isolated from carious lesions, that had been described initially by Onisi [6] as *Actinomyces dentocariosus*. Subsequently, similar organisms were isolated in several laboratories from a variety of human sources, mostly from the oral cavity, but also from blood, respiratory secretions, abscesses, wounds, peritoneal dialysates and the eye [7,8]. Reports of human disease caused by *R. dentocariosa* started to be published in the late 1970s [9], and at least 30 case reports can now be found in the literature, many of which describe patients with endocarditis. These reports have been reviewed recently [10,11], and interested readers are referred to these previous reviews for further details.

**TAXONOMY**

The taxonomic position of the genus *Rothia* has undergone a number of changes since Georg and Brown first assigned it to the family Actinomy-
R. dentocariosa is a Gram-positive, non-acid-fast, non-spore-forming, non-pigmented, non-haemolytic, non-motile bacterium. Chemotaxonomically, it is characterised by a guanine-to-cytosine DNA ratio of 47–53 mol%. The main cellular fatty acids are C15:0 ai, C17:0 ai, C16:0 i and C16:0 at. The peptidoglycan is of the A3\textsubscript{a} type; that is, D-alanine is found in position 4, L-lysine is found in position 3, and the inter-peptide bridge consists of L-alanine. The polar lipids are di- and mono-phosphatidylglycerol, and the menaquinones are unsaturated with seven isoprene units. The cell-wall sugars are galactose, fructose, glucose and ribose [17–19]. The bacterium is fermentative, with the main end-products of carbohydrate metabolism being lactic and acetic acids.

Microscopically, the morphology varies from coccoid to diphtheroid (with clavate ends) to filamentous. Coccoid forms are observed predominantly in fluid cultures, and filamentous forms on plates, but mixtures may appear in any culture. Rudimentary branching and loss of the Gram-positive appearance may be seen in ageing cultures. Cells occur singly, in pairs, in clusters or in chains, and colonial pleomorphism can also be observed [7,8].

R. dentocariosa grows faster under aerobic than under anaerobic conditions, and does not need CO\textsubscript{2} or lipids for growth. On non-selective media used in the clinical laboratory, the organism will grow within 24 h to form colonies of 1-mm diameter, which will enlarge to 2–3 mm within 48 h. There is no growth on Sabouraud dextrose agar [6]. In contrast to other diphtheroid organisms, fosfomycin may be inhibitory to R. dentocariosa [20]. Colonies are either of a smooth, convex type with entire edges, or of a rough form which shows either a convoluted, cerebriform or ‘spoke-wheel’ surface with irregular or scalloped edges, or a pebbly surface [7]. Colonies may adhere to the agar surface. Mixtures of colony types also occur. Under anaerobic conditions, small ‘spider’ colonies with filamentous borders are the most common form.

Table 1 summarises the biochemical reactions of R. dentocariosa. Of note is the recent discovery of urease-positive and catalase-negative strains [15,20] (K. Bernard, personal communication).

<table>
<thead>
<tr>
<th>Biochemical Reaction</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>K/A, H2S-negative</td>
<td>Triple sugar iron agar&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Fermentation: Fructose, Ribose</td>
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<tr>
<td>Fermentation: Glucose</td>
<td>Salicin</td>
<td>Mannitol</td>
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<td>Maltose</td>
<td>Trehalose</td>
<td>Raffinose</td>
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<td>Sucrose</td>
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<td>Rhamnose</td>
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<td>x-Glucuronidase&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Pyrazinamidase&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Catalase</td>
<td>Urea</td>
<td>Indole</td>
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<td>DNase</td>
<td>Oxydase</td>
<td>Lysine</td>
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<td>Asesculin hydrolysis</td>
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<td>decahydroxylic</td>
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<td>Nitrate to nitrite</td>
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<td>decarboxylase</td>
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<td>CAMP test</td>
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<sup>a</sup>Acid (without gas).  
<sup>b</sup>Without gas.  
<sup>c</sup>API ZYM system.

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IDENTIFICATION OF R. DENTOCARIOISA IN THE CLINICAL LABORATORY

Identification of R. dentocariosa in the clinical laboratory starts with the observation of colonial and microscopic features. Biochemical testing should follow [21] (Table 1). As a minimum, fermentation of glucose, sucrose, maltose, mannitol and xylose should be tested, together with nitrate reduction, urease production, aesculin hydrolysis, catalase and motility. Kligler’s or triple sugar iron agar should also be inoculated. In lieu of single tests, commercial identification systems such as API Coryne v.2 [22] or RapID CB Plus (Remel Inc., Norcross, GA, USA) [23] may be used; both contain R. dentocariosa in their data bases. Directions for use must be followed strictly. Misdiagnoses will ensue if identification systems for anaerobes are used [24].

Colonial morphology alone can rule out Nocardia spp. because nocardiae grow more slowly, adhere firmly to the agar, and show, at least after several days, aerial mycelia and, very often, pigmentation. Like other aerobic actinomycetes (e.g., Actinomadura, Nocardiopsis, Gordonia, Tsukamurella, Rhodococcus), they are obligate aerobes. Members of the family Microbacteriaceae (Microbacterium–Aureobacterium, Leifsonia, Curtobacterium) are mostly aerobic and are also pigmented.

Differential diagnosis of most catalase-, aesculin- and nitrate-positive, urease-negative strains involves mainly Actinomyces viscosus, Actinomyces neuii, Propionibacterium avidum, Propionibacterium granulosum, Corynebacterium durum, Corynebacterium matruchotii, and a new black-pigmented corynebacterium [25,26]. Colonial and microscopic features, poor growth under anaerobic conditions, and a negative CAMP test for R. dentocariosa, as well as the end-products of glucose metabolism (succinate in Actinomyces, propionate in Propionibacterium and the corynebacteria mentioned), should lead to the correct diagnosis.

Catalase-negative strains of R. dentocariosa will be more difficult to recognise with traditional tests, since they may mimic the rare Bifidobacterium strains that are able to grow aerobically, as well as Actinomyces and Arcanobacterium spp., Propionibacterium propionicum and catalase-negative Listeria strains [21,27]. However, these organisms can be largely ruled out by their smaller colony size and the poorer anaerobic growth of R. dentocariosa, by end-product analysis (succinate in Actinomyces and Arcanobacterium, propionate in Propionibacterium, and lactate in Listeria), and by CAMP tests. Aesculin- or nitrate-negative strains, as well as urease-positive strains, may suggest Corynebacterium freneyi, Corynebacterium sundsvallense or Dermabacter hominis. Again, observation of morphology and enzymatic reactions (e.g., ornithine and lysine decarboxylase, pyrazinamidase) should lead to the correct identification [28–31].

Only in exceptional circumstances should 16S rDNA sequencing or cellular fatty-acid analysis be necessary to identify a clinical isolate. The presence of > 5% C18.1 omega c9 or C15:0 i will rule out R. dentocariosa [32].

REFERENCES

13. Collins MD, Hutson RA, Baverud V, Falsen E. Characterization of a Rothia-like organism from a mouse: description of Rothia nasimurium sp. nov. & reclassification of...