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Turicella otitidis and *Corynebacterium auris*: 20 years on

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Abstract *Turicella otitidis* and *Corynebacterium auris*, described as new species 20 years ago, have been isolated mainly from the external ear canal and middle ear fluid. While their taxonomic position has been clearly established, their diagnosis in the routine laboratory is difficult. The question of their pathogenic potential in otitis is still open but might be elucidated better if corynebacteria are speciated more often.

Keywords *Turicella otitidis* · *Corynebacterium auris* · Otitis

Coryneform bacteria in the skin flora

Coryneform bacteria, that is, non-spore forming Gram-positive rods with irregular outlines, have been known for many years to occur on human skin [1]. A recent study of the skin microbiome showed that “Corynebacteria” predominated on moist skin sites but also occurred together with propionibacteria and staphylococci on sebaceous sites such as the external auditory canal [2]. Other authors have found them in cerumen as well [3]. Among species of coryneforms found on the skin, the most frequent ones

were *Turicella otitidis* and *Corynebacterium auris* [4], the two species more closely described below. A culture-independent molecular analysis of the external ear canal flora found “*Alloiococcus otitis*” [5], “*Corynebacterium otitidis*” (*C. auris*?) and *Staphylococcus auricularis* as the most prevalent DNA sequence types [6].

The new species

Coryneform bacteria, however, were not incriminated as agents of otitis media [7] or otitis externa [8] until 1993 when pure cultures of 19 coryneforms were found in samples collected by tympanocentesis from 19 patients evaluated for acute otitis media [9]. All of these isolates resembled *Corynebacterium afermentans* subsp. *afermentans* (formerly called CDC Group ANF-1) [10], that is, they were non-lipophilic, strictly aerobic, non-fermentative and non-oxidative, catalase-positive Gram-positive rods containing meso-diaminopimelic acid and arabinogalactan in their cell walls. In contrast to this species, however, the new isolates lacked mycolic acids, yielded convex, whitish to creamish colonies, and produced DNAase. The authors, who called them “ANF-1-like bacteria”, assumed that they belonged to an unidentified genus since mycolic acids were, at that time, assumed to be invariable constituents of the genus *Corynebacterium*. In the meantime, however, several species of this genus have been found to lack mycolic acids [11].

In the same year, we encountered in our laboratory eight similar strains from patients with otitis media, five of them in pure culture [12]. The phenotypic and chemotaxonomic characteristics were identical to those described by Simonet et al. [9.] except that the strains showed a strong CAMP reaction and lacked certain enzymes in the

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APIZYM system (bioMérieux, Marcy-l'Etoile, France), probably due to differences in incubation times. The guanine-cytosine content and cellular fatty acids resembled those of non-diphtheria corynebacteria; in addition, tuberculostearic acid was found. A successive study examining partial 16S rRNA gene sequences [13] showed that these bacteria were only remotely related to members of previously described coryneform genera with sequence similarities below 92.0 % and not higher than 93.1 %; furthermore, their principal menaquinones were MK-10 and MK-11, in contrast to MK-8 and MK-9 in the genus *Corynebacterium*. We decided to name this new genus *Turicella* after Turicum, the Latin name for the city of Zürich; the species was called *T. otitidis*.

Another group of coryneforms, also isolated in our laboratory from patients with acute or chronic otitis media, resembled *T. otitidis* in many biochemical characteristics except for lack of DNase and in assimilation of certain carbon compounds as well as production of certain enzymes. Chemotaxonomically and by 16S rRNA gene sequencing, however, they clearly belonged to the genus *Corynebacterium* [14]. Their closest relative was *C. afermentans* subsp. *afermentans* but their colonial morphology (dry, slightly adherent colonies) differed from both *C. afermentans* and *T. otitidis*. We named this species *C. auris*. Our results were confirmed and amplified by Renaud et al. [15] from an isolate in a case of otitis with otorrhea.

Laboratory diagnosis

Microscopically, the two corynebacteria are typical diphtheroids while *T. otitidis* is reported to show diphtheroidal as well as straight Gram-positive rods [11, 15]. Colonial morphology differences between the three (flat and grayish-white in *C. afermentans*, convex and pale yellow in the other two, but adherent in *C. auris*) may be hard to discern. Differentiation in the routine laboratory by use of biochemical tests alone is difficult as well [16]. In the API-Coryne system (bioMérieux) all three yield the code 2100004; thus, additional tests (CAMP, DNase, APIZYM reactions) are required. The VITEK 2 ANC card (bioMérieux) most likely also requires additional tests [18]. The RapID CB Plus system (Remel, Inc., Lenexa, KS, USA) correctly identified *T. otitidis* and *C. auris* but not *C. afermentans* to the species level [17]. The first version of the BIOLOG system (Biolog, Hayward, CA, USA) included only *C. afermentans*, with three of five strains correctly identified at the genus level only [19]. Its Gen III database contains all three species but test runs have not been published. The Andromas matrix-assisted laser desorption ionization–time of flight mass spectrometry system (MALDITOF; Bruker, Bremen, Germany) has been able to

identify *T. otitidis* [20] and, in our experience, both this bacterium and *C. auris* correctly with scores of >2.0. A final diagnosis would still require 16S rRNA gene sequencing [21]; partial or full sequencing of the *rpoB* gene [22] is said to be the most widely used approach [21].

Antimicrobial susceptibility

T. otitidis has so far been susceptible to many antimicrobials, with surprisingly low MIC 90 values for penicillins, cephalosporins, carbapenems, chloramphenicol, ciprofloxacin, aminoglycosides, rifampicin, tetracyclines, linezolid, teicoplanin, and vancomycin; the only exception being clindamycin and erythromycin [4, 23–25]. The latter is probably associated with mutations at positions 2058 and/or 2059 (*Escherichia coli* numbering) [26]. In contrast, *C. auris* and *C. afermentans* showed occasional resistance to penicillin, ampicillin, and cefazolin as well as to clindamycin and erythromycin [4, 14, 23, 27].

Pathogenic potential

The question whether *T. otitidis* and *C. auris* are mere colonizers or potential pathogens in cases of otitis cannot be answered unequivocally at this time. All cases have so far been seen in children. Mastoiditis was reported in three publications. In the first one, *T. otitidis* was isolated from the right and left middle ear fluid of one patient with mastoiditis [28]. In the other, a series of 13 cases of otitis media with mastoiditis, *T. otitidis* was cultured from ear fluid together with other potential pathogenic bacteria in 12 patients. Only in one case was a pure culture of *T. otitidis* obtained but Gram stain also disclosed Gram-positive cocci. Isolation and identification methods were not described [29]. In the third publication, a retroauricular abscess in a case suggestive of mastoiditis, the aspirate showed abundant leukocytes and Gram-positive rods. The latter grew only in enrichment culture [30] and were identified as *T. otitidis* by 16S rRNA gene sequencing. *T. otitidis* has also been isolated from a cervical abscess [31]. Furthermore, in one pediatric patient with acute lymphoblastic leukemia and symptoms of right external otitis, *T. otitidis* grew in a swab from the ear as well as in two blood cultures [32]. These cases are suggestive of *T. otitidis* as a potentially extraotic pathogen (*C. auris* has never been isolated from extraotic sites). In determining the pathogenic potential of *T. otitidis* and *C. auris*, Holzmann et al. [33], using appropriate sampling techniques, were able to isolate *T. otitidis* in 23 of 205 (11.2 %) and *C. auris* in 32 of 205 (15.6 %) of external auditory canal swabs of healthy children. Of 60 (23.3 %) children with otitis media, 14 yielded *T. otitidis* in the external ear canal and 6 of 60

(10 %) in both external ear canal and middle ear effusion. *C. auris* was isolated in 2 of 60 (3.3 %) patients from the external ear canal only and in 1 of 60 (1.7 %) from both external ear canal and middle ear fluid. None of the otitis media patients grew either bacterium exclusively from the middle ear fluid. The authors concluded that neither bacterium causes otitis media with effusion in children. In a later study, Gomes-Garces et al. [34] isolated *T. otitidis* in middle ear fluids of seven children, five of which originating from spontaneous drainage, and two from tympanocentesis. One of the latter and four of the former yielded additional staphylococci or *A. otitidis*. Thus, in only one case was *T. otitidis* isolated in monoculture. Identification had been established by APICoryne, APIZYM, and API 50 systems plus phenotypic tests. The susceptibility patterns of *T. otitidis* resembled the pattern listed above. The case presented by Poulter et al. [35] is even more difficult to interpret. The adult patient had a history of myringotomies and one-sided deafness. Admitted for nephrectomy, he developed hearing loss on the contralateral side with no symptoms of otitis but a middle ear effusion whose culture yielded a CAMP-positive, irregularly staining Gram-positive rod with an API code of 2100004 which formed whitish-creamy colonies and was diagnosed as *T. otitidis*, although the diagnosis of *C. afermentans* could have been possible as well. It should be mentioned here that the isolation of *C. afermentans* subsp. *afermentans* has, in almost all cases, not been related to an infection [27].

It seems strange that, 20 years after *T. otitidis* and *C. auris* were outlined as species, only few cases of isolation have been reported in the literature. This may be due to the difficulty to diagnose these species but it could also be ascribed to the still widely held opinion that diphtheroids are not worth being speciated. In this line is the recent consensus statement of a British group that coagulase-negative staphylococci, diphtheroids, and enterococci isolated from patients with otitis externa should not be reported by name, generic terms should be used, and susceptibilities not be reported [36]. We are, however, of the opinion that our program of identifying (and possibly detecting new species of) diphtheroids, at least if they occur in pure culture, has paid off nicely in terms of clinical significance [11–14, 16, 18, 19, 21, 23, 24, 33].

Conflict of interest None

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