CASE REPORT

***Aureobacterium* masquerading as '*Corynebacterium aquaticum*' infection: case report and review of the literature**

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A gram-positive bacillus was isolated repeatedly from blood taken through the lumina of a central venous catheter of a patient with multiple myeloma who developed febrile neutropenia following chemotherapy. The bacterium was identified by the API CORYNE system as '*Corynebacterium aquaticum*'. Gene analysis targeting the 16S rRNA indicated that the organism had a 99.5% identity with *Aureobacterium liquefaciens* although there were two phenotypic characteristics at variance with the description of this species. Problems remain with the routine identification of '*C. aquaticum*' and *Aureobacterium* species. The few clinical reports on patients infected with '*C. aquaticum*' and *A. liquefaciens* indicate that these are rare infections often associated with immunocompromise.

Introduction

Isolates of coryneform gram-positive rods often present difficulties in identification for diagnostic laboratories. One common strategy is to use API CORYNE strips (bioMérieux, Lyon, France) in which a series of 20 biochemical reactions are interpreted with the use of a database. Unfortunately, the discriminating power of this system is limited. This study reports the isolation of an organism from a patient which was identified by this system as '*Corynebacterium aquaticum*'. Subsequent phylogenetic analysis revealed that the organism was in fact similar to *Aureobacterium liquefaciens*, although there were some phenotypic features which militated against this identification.

Only a small number of '*C. aquaticum*' infections has been described, many of which may have been *Aureobacterium* infections. Only two detailed clinical descriptions of *Aureobacterium* infections have been published, although a larger number of clinical isolates have been examined microbiologically. The clinical aspects of these patients and developments for improving microbiological diagnosis are reviewed.

Case history

A 68-year-old man presented with febrile neutropenia in April 1998. He had been diagnosed 9 years earlier as having multiple myeloma and received treatment with six cycles of chemotherapy. Two years later he relapsed and was treated with high dose cyclophosphamide therapy followed by rescue stem cell transplantation. He remained well until 1997 apart from a severe episode of balanitis. In the same year he received another course of six cycles of chemotherapy for rising paraprotein levels which was complicated by an episode of pneumococcal sepsis. In March 1998 he was admitted for insertion of a central venous catheter and initiation of another course of chemotherapy, this time with a combination of etoposide, vincristine, doxorubicin and dexamethasone for 3 days followed by a single dose of cyclophosphamide. Twelve days after the second cycle of chemotherapy he was admitted with febrile neutropenia. At this time his total white cell count was 0.7 cells/ μ l with 0.19 neutrophils (normal range 2.2–6.1), 0.28 lymphocytes (1.3–3.4), 0.22 monocytes (0.2–0.8), albumin 31 g/L (34–48), globulins 55 g/L (22–35), IgG 57.5 g/L (6.5–15), IgA 0.2 g/L (0.6–4.0) and IgM 0.3 g/L (0.5–3.0). Microbiological examination of urine was negative and a chest radiograph showed no evidence of pulmonary disease. *Citrobacter freundii* was grown from two sets of blood cultures taken on the day of admission. The next day six sets of blood cultures taken at different

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times through two lumina of the central venous catheter all yielded growth of a gram-positive bacillus reported as '*Corynebacterium aquaticum*'. Initially the patient was treated empirically with ceftazidime and gentamicin. After 3 days, the ceftazidime was changed to imipenem; vancomycin was added on day 6 and his temperature settled 3 days later. He was discharged from hospital after 8 days, but was re-admitted 6 days later with another episode of febrile neutropenia. On this occasion, he was given vancomycin and ciprofloxacin empirically. *Enterobacter cancerogenus* was grown from blood cultures and the central venous catheter was removed. His temperature settled and he was discharged after 3 days.

Laboratory data

The isolates of *Cit. freundii* and *E. cancerogenus* were identified by the Vitek Automicrobic System (bioMérieux, Hazelwood, MI, USA). A report of '*C. aquaticum*' was issued initially on the basis of the identification provided by the API CORYNE system, version 1.1 (bioMérieux, Lyon, France). This organism was motile and colonies produced a yellow pigment after incubation for 3 days on blood agar. It was lactose non-fermenting on MacConkey agar. Some biochemical reactions are listed in Table 1. In addition, it gave positive results for tyrosine hydrolysis, production of pyrrolidonyl arylamidase, alkaline phosphatase, galactosidase, glucosidase and pyrazinamidase, but negative results for hydrolysis of xanthine, hypoxanthine and adenine, fermentation of mannitol, xylose and ribose, and glucuronidase production. In view of the uncertain identification and the fact that a similar case had been reported from Adelaide [5], an attempt was made to identify the organism by phylogenetic analysis.

A 1333-bp fragment of the 16S rRNA was sequenced following 'heat lysis' DNA extraction and gene amplification [6] with dye terminator chemistry (Per-

kin-Elmer, Applied Biosystems Division, Foster City, CA, USA) as described previously [7], but with the following primers: forward primer, 5'-GGCTCAGATTGAACGCTGGCGG-3'; reverse primer, 5'-ACCCACTCCCATGGTGTGACGG-3'; reverse primer, 5'-TGTTACCGACTTTCATGACTTGACGG-3'. The forward primer was used with each of the reverse primers to produce and sequence two amplicons, and a contiguous sequence was constructed with the Gene-Compar program (Applied Maths, Kortrijk, Belgium). A Basic BLASTn Search (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, USA; <http://www.ncbi.nlm.nih.gov>) was performed, followed by a comparison and alignment of sequences retrieved by the search with the GeneCompar program. The GenBank accession numbers for the 16S rRNA sequences were as follows: *A. liquefaciens*, X77444; *A. keratanolyticum*, Y14786; *A. testaceum*, X77445; *Microbacterium lacticum*, X77441; *C. aquaticum*, X77450. The sequence for 1333 bases is shown in Fig. 1. There was a 99.5% identity with *A. liquefaciens*. The next closest species were *A. keratanolyticum* (98.12%), *M. lacticum* (97.45%), *A. testaceum* (95.42%) and *C. aquaticum* (94.37%).

The susceptibility of this organism to various antibiotics was tested by the E test method (AB Biodisk, Solna, Sweden) on Mueller-Hinton blood agar plates. The minimum inhibitory concentrations (mg/L) were penicillin, 3; amoxycillin/clavulanic acid, 3; oxacillin, 64; piperacillin, 1.5; timentin, 8; cephalothin, 16; cefotaxime, 8; imipenem, 16; ciprofloxacin, 2; clarithromycin, 0.05; gentamicin, 2; trimethoprim/sulphamethoxazole, 0.02; vancomycin, 12 and teicoplanin, 1.5. There are no National Committee for Clinical Laboratory Standards or other standards available for susceptibility testing of coryneform bacteria. The study isolate appeared to be most susceptible to clarithromycin, trimethoprim/sulphamethoxazole, penicillin, amoxycillin/clavulanic acid, piperacillin, gentamicin and ciprofloxacin.

Table 1. Motility and biochemical reactions of the study isolate and available information for closely related organisms (adapted from [1-4])

Characteristic	Study isolate	<i>A. liquefaciens</i>	<i>A. keratanolyticum</i>	<i>M. lacticum</i>	<i>A. testaceum</i>	<i>C. aquaticum</i>
Motility	+	-	+	-	+	+
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-
Nitrate red	-	v	+	+	v	+
Urease	-	-	-	-	-	-
Hydrolysis of						
Aesculin	+	+	+	v	+	+
Gelatin	+	+	+	v	+	-
Casein	+	+	-	-	+	-
Starch	-	+	-	+	v	-
DNAase	+	-	-	+	+	-
Fermentation of						
Glucose	-	-	-	+	-	-
Maltose	-	-	-	+	-	-
Sucrose	-	-	-	-	-	-
Lactose	-	-	-	+	-	-

V, variable.

■ 1 ■ 10 ■ 20 ■ 30 ■ 40 ■ 50
A. liquefaciens
Clinical isolate
GAT GAACGCTGGCGGCGT GCTT AACACATGCAAGT CGAACGGT GAACACGGAGCTT GCT
----- AACACATGCAAGT CGAACGGT GAACACGGAGCTT GCT

■ 60 ■ 70 ■ 80 ■ 90 ■ 100 ■ 110
A. liquefaciens
Clinical isolate
CTGT GGGATCAGTGGCGAACGGGTGAGT AACACGTGAGCAACCTGCCCTGACTCTGGG
CTGTGGGATCAGTGGCGAACGGGTGAGT AACACGTGAGCAACCTGCCCTGACTCTGGG

■ 120 ■ 130 ■ 140 ■ 150 ■ 160 ■ 170
A. liquefaciens
Clinical isolate
ATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCTGCGTCT
ATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGATCGCATGGTCTGCGTCT

■ 180 ■ 190 ■ 200 ■ 210 ■ 220 ■ 230
A. liquefaciens
Clinical isolate
TGGAAAGAATTTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTGGTGAGGTAAATG
TGGAAAGAATTTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTGGTGAGGTAAATG

■ 240 ■ 250 ■ 260 ■ 270 ■ 280 ■ 290
A. liquefaciens
Clinical isolate
GCTCACCAAGGCGT CGACGGGTAGCCGGCCTGAGAGGGT GACCGGCCACACTGGGACTG
GCTCACCAAGGCGT CGACGGGTAGCCGGCCTGAGAGGGT GACCGGCCACACTGGGACTG

■ 300 ■ 310 ■ 320 ■ 330 ■ 340 ■ 350
A. liquefaciens
Clinical isolate
AGACACGGCCCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGCACAATGGGCGCAA
AGACACGGCCCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGCACAATGGGCGCAA

■ 360 ■ 370 ■ 380 ■ 390 ■ 400 ■ 410
A. liquefaciens
Clinical isolate
GCCTGATGCAGCAACGCCGCGTGAGGGA CGACGGCCTTCGGGTGTAAACCTCTTTTAG
GCCTGATGCAGCAACGCCGCGTGAGGGA CGACGGCCTTCGGGTGTAAACCTCTTTTAG

■ 420 ■ 430 ■ 440 ■ 450 ■ 460 ■ 470
A. liquefaciens
Clinical isolate
CAGGGAAGAAGCGAAAGT GACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAG
CAGGGAAGAAGCGAAAGT GACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAG

■ 480 ■ 490 ■ 500 ■ 510 ■ 520 ■ 530
A. liquefaciens
Clinical isolate
CAGCCGCGGTAAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTC
CAGCCGCGGTAAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTC

■ 540 ■ 550 ■ 560 ■ 570 ■ 580 ■ 590
A. liquefaciens
Clinical isolate
GTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGCAGTGG
GTAGGCGGTTTGTGCGCTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGCAGTGG

■ 600 ■ 610 ■ 620 ■ 630 ■ 640
A. liquefaciens
Clinical isolate
GTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATG
GTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATG

■ 650 ■ 660 ■ 670 ■ 680 ■ 690 ■ 700
A. liquefaciens
Clinical isolate
CGCAGATATCAGGAGGAACACCGATGT CGAAGACAGATCTCTGGGCCGTAACGACGCT
CGCAGATATCAGGAGGAACACCGATGT CGAAGACAGATCTCTGGGCCGTAACGACGCT

■ 710 ■ 720 ■ 730 ■ 740 ■ 750 ■ 760
A. liquefaciens
Clinical isolate
GAGGAGCGAAAGGGTGGGGAGCAACACAGGCTTAGATACCCTGGTAGTCCACCCGTA
GAGGAGCGAAAGGGTGGGGAGCAACACAGGCTTAGATACCCTGGTAGTCCACCCGTA

■ 770 ■ 780 ■ 790 ■ 800 ■ 810 ■ 820
A. liquefaciens
Clinical isolate
CGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAA
CGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAA

■ 830 ■ 840 ■ 850 ■ 860 ■ 870 ■ 880
A. liquefaciens
Clinical isolate
GTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGACCC
GTTCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGACCC

■ 890 ■ 900 ■ 910 ■ 920 ■ 930 ■ 940
A. liquefaciens
Clinical isolate
GCACAAGCGGCGGAGCATGCGGATTAAATCGATGCAACGCGAAGAACCTTACCAAGGCT
GCACAAGCGGCGGAGCATGCGGATTAAATCGATGCAACGCGAAGAACCTTACCAAGGCT

■ 950 ■ 960 ■ 970 ■ 980 ■ 990 ■ 1000
A. liquefaciens
Clinical isolate
TGACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGG
TGACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGG

■ 1010 ■ 1020 ■ 1030 ■ 1040 ■ 1050 ■ 1060
A. liquefaciens
Clinical isolate
TGCATGGTTGTGCTCAGCTCGTGTGCTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCA
TGCATGGTTGTGCTCAGCTCGTGTGCTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCA

■ 1070 ■ 1080 ■ 1090 ■ 1100 ■ 1110 ■ 1120
A. liquefaciens
Clinical isolate
ACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACCTCATGGGATACTGCCGGGGTC
ACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGAACCTCATGGGATACTGCCGGGGTC

■ 1130 ■ 1140 ■ 1150 ■ 1160 ■ 1170 ■ 1180
A. liquefaciens
Clinical isolate
AACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCA
AACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTTGGGCTTCA

■ 1190 ■ 1200 ■ 1210 ■ 1220 ■ 1230
A. liquefaciens
Clinical isolate
CGCATGCTACAATGGCCGGTACAAAGGGCTGCAATACCGCGAGGTGGAGCGAATCCCAA
CGCATGCTACAATGGCCGGTACAAAGGGCTGCAATACCGCGAGGTGGAGCGAATCCCAA

■ 1240 ■ 1250 ■ 1260 ■ 1270 ■ 1280 ■ 1290
A. liquefaciens
Clinical isolate
AAAGCCGGTCCCAGTTCGGATTGAGGTCTGCAACTCGACCTCATGAAGTCGGAGTCGCT
AAAGCCGGTCCCAGTTCGGATTGAGGTCTGCAACTCGACCTCATGAAGTCGGAGTCGCT

■ 1300 ■ 1310 ■ 1320 ■ 1330 ■ 1340 ■ 1350
A. liquefaciens
Clinical isolate
AGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGC
AGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGTCTTGTACACACCGC

■ 1360 ■ 1370 ■ 1380 ■ 1390 ■ 1400 ■ 1410
A. liquefaciens
Clinical isolate
CGTCAAGTCATGAAAGTCGGTAACACCTGAAGCCGGTGGCCTAACCTTGTGGAGGGAG

■ 1420 ■ 1430 ■ 1440 ■ 1450 ■ 1460 ■ 1470
A. liquefaciens
Clinical isolate
CCGTGGAAGGTGGGATCGGTAAATTAGGACTAATTCGTAAACAAGGTAGCCGTACCGGAA

Fig. 1. A comparative alignment of the sequence determined for the clinical isolate compared with a segment of 16S rRNA sequence for *A. liquefaciens*, GeneBank accession no. X77444.

Discussion

Identification of coryneform bacteria to genus, let alone species level, is complex and difficult [8]. The study isolate was identified as '*C. aquaticum*' by the API CORYNE system. This grouping does not differentiate true *C. aquaticum* from a number of other bacteria, particularly various species of *Aureobacterium*.

The first description of '*C. aquaticum*' was produced by Leifson in 1962 for gram-positive rods, isolated from distilled water, which exhibited many of the characteristics of motile plant-pathogenic corynebacteria [9]. On the basis of cell-wall antigens and other criteria, Collins *et al.* re-classified these organisms and created a new genus, *Aureobacterium*, with six species [1]. In 1993, Yokawa and colleagues added a further seven species [2]. Since then, Funke *et al.* have described a new species, *A. resistens*, resistant to vancomycin but susceptible to teicoplanin [10]. Although the present isolate had differential susceptibilities to teicoplanin and vancomycin (1.5 cf 12 mg/L), it was less marked than that reported for *A. resistens* (1.5 cf 48 mg/L). *Aureobacterium* species have been found in dairy products such as milk and cheese, and in the environment.

Funke and colleagues reported that of 11 clinical specimens identified tentatively as '*C. aquaticum*', four strains were true *C. aquaticum* whereas seven were species of *Aureobacterium* on biochemical and peptidoglycan analysis [11]. They also commented that many species descriptions of *Aureobacterium* are based upon only one strain, so that phenotypic species level identification within this genus is almost impossible at present [8]. On the basis of ribosomal gene sequence analysis, the present isolate was most closely related to *A. liquefaciens*, with 99.5% identity. However, *A. liquefaciens* is recorded as being non-motile [1]. The isolate in the present study is motile and has a very long single polar flagellum (Fig. 2). On the other hand, the limited biochemical parameters were consistent with *A. liquefaciens* apart from hydrolysis of starch. The next

closest species (*A. keratanolyticum*) is motile, but differs with regard to nitrate reduction and starch hydrolysis. Similarly, there are discrepancies with the next closest species, *M. lacticum*, *A. testaceum* and *C. aquaticum*. Either the original description of *A. liquefaciens* as being non-motile is not mandatory or the study isolate is another organism, possibly a new species.

Identification of coryneform bacteria to species level is valuable to detect and describe new species and to ascribe potential pathogenicity to species thought previously to be non-pathogenic. This is difficult for most routine diagnostic laboratories to perform with the tools generally available. The API CORYNE system version 1.1 labels *C. aquaticum* and a number of other organisms, including species of *Aureobacterium*, as '*C. aquaticum*'. A recent multicentre study has reported the result of classifying 407 strains of coryneform bacteria with the same biochemical system but with an updated and extended database, version 2.0 [12]. This database provided an improved overall performance and now divides '*C. aquaticum*' into *C. aquaticum* and *Aureobacterium* species. Although *Aureobacterium* species are identified to genus level, the authors state that it is most unlikely that there will ever be a commercial identification system with a reliable database for these organisms. Therefore, organisms such as the present isolate will continue to require extended characterisation.

There are nine reports in the literature of patients infected with '*C. aquaticum*' (Table 2), although some of these are likely to have been *Aureobacterium* infections. Most adult patients had an underlying chronic disease – particularly diabetes, renal disease or a haematological disorder. The most common presentation was with cellulitis, although two patients undergoing chronic ambulatory dialysis had peritonitis and one patient had a localised infection following a high-pressure water injury. A wide range of antibiotics were tried – including first-, second- and third-generation cephalosporins, penicillin, vancomycin and various aminoglycosides. All patients responded, although one patient had multiple relapses and another

Fig. 2. Scanning electron photomicrograph of the study isolate showing a single, long polar flagellum. Bar = 0.5 μ m.

Table 2. Clinical reports of patients with '*C. aquaticum*' infections

Reference no.	Year	Diagnostic system	Country	Age	Sex	Underlying condition	Presentation	Antimicrobial therapy	Outcome
13	1975	In-house	USA	85	F	Insulin-dependent diabetes	Septicaemia	Cephalexin	Resolution
14	1986	In-house	New Zealand	33	F	Insulin-dependent diabetes; peritoneal dialysis	Peritonitis	Cephalexin, vanco-mycin, co-trimoxazole	Multiple relapses
15	1986	In-house	USA	4 weeks	F	Neonate	Meningitis	Ampicillin + gentamicin	Resolution
16	1988	Not stated	USA	1	M	Chronic granulomatous disease	Septicaemia	Vancomycin	Resolution
17	1989	In-house	USA	8 days	M	Neonate	Urinary tract infection	Ceftriaxone	Resolution
18	1994	API Coryne	Germany	13	M	Acute lymphoblastic leukaemia	Septicaemia	Ceftriaxone, cefaclor	Resolution
5	1995	API Coryne	Australia	74	F	Pancytopenia post- ³² P	Septicaemia	Penicillin	Resolution
19	1996	API Coryne	Sweden	24	M	High-pressure water injury	Cellulitis	Cefuroxime, cefadroxil	Resolution
20	1997	API Coryne	Korea	55	M	Chronic ambulatory peritoneal dialysis	Peritonitis	Cefazolin + tobramycin, vancomycin + amikacin	Resolution after removal of catheter

In-house indicates assessment by conventional culture, Gram's stain, motility and a range of biochemical tests.

Table 3. Clinical reports of patients with *Aureobacterium* infections

Reference no.	Year	Diagnostic system	Country	Age	Sex	Underlying condition	Presentation	Antimicrobial therapy	Outcome
21	1996	In-house, API ZYM, cell wall analysis	USA	39	M	Acute myeloid leukaemia	Cellulitis, bacteraemia	Vancomycin; amikacin + erythromycin; imipenem + rifampicin	Died
22	1996	Phylo-genetic	Germany	75	M	Nil	Septicaemia	Imipenem, penicillin, ciprofloxacin	Died
23	1998	In-house, cell wall analysis	Belgium	73	F	Haemodialysis	Septicaemia	Tetraplanin, netilmicin, amoxycillin	Responded

In-house indicates assessment by conventional culture, Gram's stain, motility and a range of biochemical tests.

required removal of a peritoneal catheter. Antibiotic susceptibility patterns were measured by disk diffusion, E test or unstated methods, but these are difficult to interpret because there are no generally accepted criteria for breakpoints.

As the genus *Aureobacterium* has been described relatively recently and in view of the difficulty of making a bacteriological identification, there appear to have been only three reports in which clinical details have been provided (Table 3). All three patients had bacteraemia and one patient also had severe cellulitis. One patient was on haemodialysis and another had acute leukaemia. Various cocktails of antibiotics were given, but two of the three patients died. The patient reported here fits with this general pattern. He was an elderly man with underlying myeloma who developed febrile neutropenia in the context of polymicrobial infections. The organism was isolated on multiple occasions from blood drawn through a central venous catheter, suggesting colonisation of the catheter with bacteraemia. He responded to combination antibiotic therapy. In addition to these detailed reports, Funke *et al.* indicated that the 11 isolates in their taxonomic study were obtained from blood (4), soft tissues (2), maxillary sinus (1), drain (1), cerebrospinal fluid (1), peritoneal fluid (1) and an epidural abscess, but clinical details were minimal [11].

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