

Case Report

Isolation of *Bordetella* species from unusual infection sites

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Introduction: *Bordetella hinzii* has been isolated mainly from respiratory specimens and from blood of immunocompromised patients, and *Bordetella trematum* from ear infections or leg, arm and ankle wounds and from diabetic foot ulcers. *Bordetella holmesii* is instead associated with bacteraemia in young adults, mostly with underlying conditions. Only three septic arthritis cases due to this species have been described in the literature.

Case presentation: Herein we describe four cases of infections due to *Bordetella* species that have been recovered from unusual infection sites: two cases of *B. hinzii* infections, one recovered from the urine of a patient with chronic prostatitis and the other from a liver cyst in an immunocompetent patient; one *B. trematum* case from a bone biopsy of a patient with chronic osteomyelitis of the hip; and one *B. holmesii* case isolated from the joint fluid of an immunocompetent patient with diagnosed septic arthritis. The organisms were identified using standard biochemical tests, by API 20 NE version 6.0, by automated system VITEK 2, by mass spectrometry using the Bruker Daltonics MicroFlex LT spectrometer with MALDI Biotyper 3.1, and by PCR amplification of 16S rRNA.

Antibiotic susceptibility testing was performed using the VITEK 2 system, except for *B. holmesii*, for which the epsilometric method (Etest technique; bioMérieux) was used.

Conclusion: We highlight the importance of isolating *Bordetella* species from severe infections and unusual sites, and also of combining both phenotypic and genotypic methods for definitive identification.

Keywords: *Bordetella* infections; species-dependent antibiotic susceptibility; unusual infection sites.

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Introduction

Except for *Bordetella petrii*, which was initially found as a free-living environmental bacterium (von Wintzingerode *et al.*, 2001), all other bordetellae are found only in warm-blooded

animals and humans. *Bordetella avium* is a bird pathogen causing coryza and rhinotracheitis in poultry (Rimler, 1985; Kersters *et al.*, 1984). *Bordetella bronchiseptica* may cause respiratory infections in many animal species and, infrequently, also in humans. *Bordetella ansorpii*, *Bordetella hinzii*, *Bordetella holmesii*, *B. petrii*, and *Bordetella trematum* are found rarely in human infections and mainly cause

Abbreviations: TMP-SMX, trimethoprim-sulfamethoxazole.

disease in immunocompromised patients. *Bordetella parapertussis* is found in sheep and humans, and *Bordetella pertussis* is thought to be a strictly human pathogen (Wirsing von Koning *et al.*, 2011).

Herein we describe four cases of infections due to *Bordetella* species that have been recovered from unusual infection sites: two cases of *B. hinzii* infections, one recovered from the urine of a patient with chronic prostatitis and the other from a liver cyst in an immunocompetent patient; one *B. trematum* case from a bone biopsy of a patient with chronic osteomyelitis of the hip; and one *B. holmesii* case isolated from the joint fluid of an immunocompetent patient with diagnosed septic arthritis.

Case report 1

1 A 55-year-old male with a history of chronic prostatitis was seen at the Urology Department because of mictional urgency, dysuria and a long history of pelvic pain. A urine sample was taken for bacteriological culture, and empirical treatment with trimethoprim-sulfamethoxazole (TMP-SMX) was started. The urinalysis showed a pathological sediment with more than 25 leukocytes per field, pyuria and bacteriuria. The urinary culture yielded $>10^5$ c.f.u. ml⁻¹ of a Gram-negative rod. The organism was identified using standard biochemical tests as *B. hinzii*. Identification results obtained by the VITEK 2 and API 20 NE systems are shown in Table 1. Identification through these two systems yielded the wrong result. Instead, identification carried out using matrix-assisted laser desorption ionization–time-of-flight (MALDI TOF) MS (Bruker Daltonik) showed a spectral score of 2.292 for *B. hinzii*.

Additionally, the 16S rRNA sequencing results showed 99 % identity with the sequences corresponding to the 16S rRNA of *B. hinzii* (AF177667).

Based on the susceptibility test result (Table 2), a 1 month course of TMP-SMX therapy was prescribed.

Urine culture after treatment was negative. However, the patient returned to the hospital 2 weeks later, complaining of urinary symptoms. A new urine sample was collected, demonstrating 25 leukocytes per field. A pure growth of more than 10^5 c.f.u. ml⁻¹ of a microorganism with the same phenotypic characteristics as the previous urine culture isolate was obtained. The isolate was again identified as *B. hinzii*. The antibiotic sensitivity was the same as that of the prior isolate. This time, the same therapy was established for 3 months with good clinical progress.

Case report 2

A 14-year-old male, from Formosa (Argentinean province), with a history of left hip septic arthritis caused by *Staphylococcus aureus*, not responding to antibiotic treatment, was referred to the Ricardo Gutierrez Children's Hospital owing to unfavourable progress. On admission he

was febrile and haemodynamically unstable. Wide surgical toilette with bone hip curettage was performed. From the bone biopsy culture, growth of a micro-organism presumptively identified by standard biochemical tests as *B. trematum* together with *Escherichia coli* was obtained. The results of the microorganism identification by the VITEK 2 system and by the API 20 NE system are shown in Table 2. In addition, identification using MALDI TOF MS (Bruker Daltonik) showed a spectral score of 2.206 for *B. trematum*. Moreover, the 16S rRNA sequencing results showed that the isolate had 99 % identity with the 16S rRNA gene from *B. trematum* (GenBank accession no KF601906).

The patient required multiple surgical debridements because of his torpid outcome, and also Steinman nail placement and skeletal traction. The clinical diagnosis was hip pandiaphysitis and femur epiphysiolysis. The patient had hip septic arthritis caused by *S. aureus* with superinfection due to *E. coli* plus *B. trematum*. Pathological examination was consistent with chronic osteomyelitis. Based on antibiotic susceptibility (Table 2), the patient was treated intravenously with meropenem and TMP-SMX for 6 months. Once the antibiotic therapy was complete, the patient was discharged, with oral minocycline administered as an outpatient.

Case report 3

A 16-year-old male was admitted to the Emergency Department of the Austral Hospital, Buenos Aires, Argentina, in March 2009 because of severe pain and functional disability of his right knee and fever of 3 days' progress (102.2 °F). He reported having suffered increasing knee pain after a slight trauma that occurred while he was playing football 4 days before.

On admission, his right knee was oedematous, erythematous, and tender to touch, with limited motion range (with signs of inflammation). Ultrasound of the knee soft tissue showed a thin band of fluid adjacent to the distal insertion of the quadriceps tendon. Surgical drainage of the joint by arthrotomy was indicated.

Laboratory findings on the peripheral venous blood sample on admission were: white blood cell count, $15\,300\text{ mm}^{-3}$ (with 85 % neutrophils); haematocrit, 42 %; haemoglobin count, 13.3 g dl^{-1} ; and platelet count, $232\,000\text{ mm}^{-3}$. At this time, the C-reactive protein level was 24 mg l^{-1} .

Examination of the synovial fluid showed 10^4 white blood cells mm^{-3} with 80 % neutrophils, but no microorganisms were observed on Gram-stained smear. In subcultures on solid media (5 % sheep blood agar plates and chocolate agar plates), incubated in 5 % CO₂, no bacterial growth was obtained, but subcultures in liquid medium (thioglycollate broth) on the same solid media mentioned, grew a microorganism, showing small colonies surrounded by a brownish area. On trypticase soy agar, colonies showed a slight brown diffusible pigment. The microorganism was identified by standard biochemical tests (Wirsing von

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Table 1. Phenotypic identification results of VITEK 2 and API 20 NE systems

Case	Vitek 2 system			API 20 NE		
	Biocode	Identification	Confidence level	Biocode	Identification	Confidence level
1		<i>Achromobacter denitrificans</i> <i>Achromobacter xylosoxidans</i> <i>Cupriavidus pauculus</i>	Low discrimination	0000067	<i>Bordetella avium</i>	Good identification 96.6 %
2	0000001101500011	<i>Bordetella trematum</i>	Excellent identification 99 %	1000067	<i>Achromobacter denitrificans</i> 78.3 % <i>Bordetella bronchiseptica</i> 18.3 %	Low discrimination
3	0631002020 Card NH 0000001100100000 Card GN	<i>Neisseria sicca/Oligella urethralis</i> <i>Acinetobacter Iwoffii</i>	Low discrimination Excellent identification 97 %	1010044	<i>Moraxella lacunata</i>	Good identification 97.6 %
4	4040001101500253	<i>Cupriavidus pauculus</i> <i>Achromobacter denitrificans</i> <i>Achromobacter xylosoxidans</i>	Low discrimination	0000063	<i>Bordetella avium</i>	Good identification 96.2 %

Koning *et al.*, 2011) as *B. holmesii*. The identifications obtained by the VITEK 2 and by API 20 NE systems are shown in Table 1; identification using MALDI TOF MS (Bruker Daltonik) showed a spectral score of 2.073 for *B. holmesii*. Moreover, the amplification and sequence analysis of 16S rRNA showed a 99 % identity with the 16S rRNA from *B. holmesii* (GenBank accession no. DQ409136.1).

Based on assuming septic arthritis due to *S. aureus*, the patient was empirically treated with clindamycin (1 g every 6 h)/gentamicin (240 mg every 24 h) for 4 days. When the isolation of *B. holmesii* was reported, the patient was making favourable progress with the antibiotic therapy administered empirically; therefore this therapy was continued. Then, owing to having made good clinical progress, he was discharged from hospital with the indication to

receive treatment with TMP-SMX 10 mg kg⁻¹ day⁻¹ for 17 days.

Case report 4

A 58-year-old female, with a history of hypothyroidism and open cholecystectomy 20 years before, consulted the hospital Emergency Room due to abdominal pain in the right upper quadrant. The patient was afebrile and normotensive. On physical examination the abdomen was tender and depressible.

Laboratory findings on the peripheral venous blood sample on admission were: white blood cell count, 18 600 mm⁻³ (with 73.5 % neutrophils); haematocrit, 26.9 %; haemoglobin count, 8.6 g dl⁻¹; and platelet count, 478 000 mm⁻³. She had elevated levels of alkaline phosphatase (230 IU l⁻¹),

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Table 2. Antimicrobial susceptibility of *Bordetella* species (µg ml⁻¹)

	Case 1	Case 2	Case 3	Case 4
Ampicillin	≥32 R	16 I	0.5 S	≥32 R
Ampicillin-sulbactam	≥32 R	16 I	ND	≥32R
Piperacillin-tazobactam	≤4 S	≤4	0.032S	≤4 S
Cefalotin	8 S	8 S	ND	8 S
Cefotaxime	≥64 R	32 I	2 S	≤64 R
Ceftazidime	≥64 R	4 S	0.5 S	8 S
Cefepime	≥64 R	4 S	0.38 S	16 I
Imipenem	2 S	≤1.0 S	0.19 S	≤1.0 S
Meropenem	≤0.25S	≤0.25 S	0.004S	≤0.25S
Amikacin	8 S	16 S	4 S	4 S
Gentamicin	8 I	4 S	0.047 S	2 S
Ciprofloxacin	2 I	2 I	0.032	1 S
Colistin	≤0.5 S	≤0.5 S	≤0.064 S	≤0.5
TMP-SMX	≤2 S	≤2 S	0.023 S	≤2 S

alanine aminotransferase (151 IU l⁻¹), and aspartate aminotransferase (99 IU l⁻¹), with total bilirubin (0.5 mg d l⁻¹) and direct bilirubin (0.1 mg d l⁻¹) within normal range.

Tumour markers, including alpha-fetoprotein, carcinoembryonic antigen, and CA 19-9, were all within normal limits. On magnetic resonance imaging cholangiopancreatography, endoluminal images consistent with gallstones or bile duct dilatation were not observed.

An abdominal computed tomography scan revealed the presence of a 79 × 58 mm cystic image in the right lobe, with the presence of thin walls inside. It was decided to conduct an exploratory laparoscopy with intraoperative ultrasound-guided liver biopsy. The patient received 2 g cefazolin preoperatively as a single dose. A 12 × 10 cm loculated liver cyst in the right hepatic lobe was observed. Two samples of bilious fluid obtained from the punctured cyst were sent for culture. Direct examination of the material revealed the presence of moderate inflammatory reaction, and no bacteria were observed on Gram stain. At 24 h incubation, microbial growth was observed in enriched medium (thioglycollate broth) and Gram stain showed Gram-negative coccobacilli were present. The organism was identified, by conventional biochemical tests, by MS (score 2.220) and by sequencing of 16S rRNA, as *B. hinzii* (GenBank accession no. JX559757). Both the VITEK 2 Compact system (bioMérieux) and API 20 NE version 6.0 yielded an erroneous result (Table 1). The growth of the same microorganism was obtained in both samples submitted. The culture result was reported 5 days after sample culture, but the patient had already been discharged because of good clinical progress (with haemodynamic stability and a soft, depressible and non-tender abdomen); therefore the isolation result was not taken into account.

Laboratory investigations

The organisms were identified using standard biochemical tests following the scheme proposed by Wauters & Vaneechoutte (2011) and by Wirsing von Koning *et al.* (2011).

The isolates were also analysed on a VITEK 2 Compact system (bioMérieux) using GN and NH Colorimetric Identification Cards and by API 20 NE version 6.0 (numerical profiles were interpreted using the APILAB Software, version 3.3.3; bioMérieux). The VITEK 2 and API 20 NE results are summarized in Table 1. The isolates were also analysed by MS. In addition, PCR amplification of 16S rRNA was performed in order to confirm the species. PCR products of the 16S rRNA, using the primers described by Weisburg *et al.* (1991), were obtained with *Taq* DNA polymerase (Promega) based on the manufacturer's specifications. Sequencing of the 1.4 kb PCR product was performed on both DNA strands at the MacroGen sequencing facility, Seoul, Korea. The sequences obtained for the four isolates were analysed using BLAST version 2.0 software (<http://www.ncbi.nlm.nih.gov/BLAST/>).

The antibiotic susceptibility test was performed using the VITEK 2 system with the AST-079 (GNS susceptibility card) panel. Owing to difficulties in growing *B. holmesii*, in this case only the antibiotic susceptibility test performed using the epsilometric (Etest; bioMérieux) method on Mueller–Hinton agar following the manufacturer's specifications. The MIC breakpoints used in this study were those established by the Clinical and Laboratory Standards Institute (CLSI, 2013) for other non-*Enterobacteriaceae*, except for ampicillin and cephalothin, for which the breakpoints for *Enterobacteriaceae* were used.

Antibiotic sensitivity results (expressed in µg ml⁻¹) are shown in Table 2.

Discussion

B. hinzii was first described by Vandamme *et al.* (1995). Most of the isolates included in their study were from the respiratory tracts of chickens and turkeys. *B. avium* poultry infections (rhinotracheitis) have been well documented (Rimler, 1985; Kersters *et al.*, 1984). However, although *B. hinzii* is capable of colonizing turkeys (Vandamme *et al.*, 1995), experimental infections in turkey have shown that it is non-pathogenic (Jackwood *et al.*, 1985).

B. hinzii has been reported in respiratory samples from three patients. Two of them had an immunosuppressive disease: a patient with cystic fibrosis and another with AIDS. In the first, *B. hinzii* was isolated from sputum repeatedly over a period of 3 years (Funke *et al.*, 1996); in the second, the organism was recovered from a bronchoalveolar lavage together with *Nocardia asteroides* (Gadea *et al.*, 2000). Recently Palacián Ruiz *et al.* (2013) have reported the case of a respiratory infection with *B. hinzii* in an 85-year-old immunocompetent patient. In this case report the organism was isolated from sputum together with *Klebsiella oxytoca* (Palacián Ruiz *et al.*, 2013). *B. hinzii* has also been described as a cause of bacteraemia in immunosuppressed patients: in a patient with myelodysplastic syndrome (Fry *et al.*, 2013), in another patient with Epstein–Barr virus-associated diffuse large B-cell lymphoma (Hristov *et al.*, 2008) and in an AIDS patient (Cookson *et al.*, 1994). Furthermore, a case of chronic cholangitis due to *B. hinzii* has been documented in a liver transplant recipient (Arvand *et al.*, 2004). In this case the organism was isolated in four biliary specimens collected over 6 months from a 29-year-old male receiving immunosuppressive therapy. In addition, a case of fatal bacteraemia after endoscopic retrograde cholangiopancreatography in an immunocompetent patient with cholestasis has been described (Kattar *et al.*, 2000). To our knowledge, no cases of *B. hinzii* from a liver cyst in an immunocompetent patient have been reported. As in the case of Arvand *et al.* (2004), prior contact with birds could not be demonstrated in our patient. In the Kattar case (Kattar *et al.*, 2000), the patient had participated in a cookout at a farm 2 weeks before his illness as the only epidemiological history;

however, no further details regarding contact with poultry or birds were available. As suggested by these authors (Arvand *et al.*, 2004; Kattar *et al.*, 2000), gastrointestinal colonization by *B. hinzii* and a subsequent ascending infection to the liver could be hypothesized in our patient.

B. hinzii isolation from urine in a patient with prostatitis has not previously been described in the literature. The only remarkable epidemiological link for this case was the drinking of unsafe water: the patient documented that he had drunk water and had bathed in an Australian tank pool where often there were dead birds. Again in this case, gastrointestinal colonization could be hypothesized since the ascending path is the most common route of infection of the urinary tract. As gastrointestinal colonization typically occurs through oral transmission, *B. hinzii* could have been acquired by drinking water contaminated with dead poultry.

As regards antibiotic susceptibility, our results are consistent with those published by other authors (Kattar *et al.*, 2000). Interestingly, cephalothin was more active than cefotaxime. Furthermore, as in our work, resistance to fourth-generation cephalosporins has been reported in *B. hinzii*. The organism was also susceptible to aminoglycosides, ciprofloxacin and TMP-SMX; however, quinolone resistance (Arvand *et al.*, 2004) and TMP-SMX resistance (Palacián Ruiz *et al.*, 2013) have been described in this species. Unlike *B. hinzii*, *B. avium* retains sensitivity to most beta-lactam antibiotics: ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cephalothin, ceftriaxone, cefotaxime, cefepime and imipenem (Kattar *et al.*, 2000); consequently, this antibiotic sensitivity profile can be used to differentiate both species.

B. trematum was first described by Vandamme *et al.* (1996). They performed a comprehensive phenotypic and genotypic analysis of 10 atypical or unclassified *Bordetella* strains and found that such strains belonged to a new species of *Bordetella*. All of them had been isolated from ear infections or leg, arm and ankle wounds. Before the species *B. trematum* had been established, Dorittke *et al.* (1995) had described the isolation of a 'B. avium-like organism' from a patient with chronic otitis media, which was later reclassified as *B. trematum* (Vandamme *et al.*, 1996). Although *B. trematum* has been isolated from skin and soft tissue infections, isolation from bone biopsy of a patient with chronic hip osteomyelitis has not previously been reported in the literature. In addition, *B. trematum* usually colonizes or infects ulcers in diabetic patients (Daxboeck *et al.*, 2004), but our patient did not present this underlying disease.

Reports in the literature mention this *Bordetella* species as oxidase-negative (Vandamme *et al.*, 1996; Daxboeck *et al.*, 2004), but the fact that the oxidase activity in these works was tested with a freshly prepared 1 % solution of *N,N'*-dimethyl-*p*-phenylene monochloride, and not with the *N,N,N',N'*-tetramethyl-*p*-phenylenediamine derivative, should

be taken into account. For our *B. trematum* isolate, the oxidase reaction performed with the dimethyl derivative was negative (reading time up to 1 min), whereas, when we used the tetramethyl-*p*-phenylenediamine derivative, an oxidase-positive result was obtained within 15 s. Regarding identification by API 20 NE, other authors (Daxboeck *et al.*, 2004; Hernández-Porto *et al.*, 2013) have reported that this system identified the organism as *B. avium* (biocode 0000063) because *B. trematum* was not present in their database. However, other biocodes could be obtained considering that the nitrate reduction in this species is a dependent strain and that the oxidase test result varies depending on the reagent used. We obtained biocode 1000067, which corresponds to *Achromobacter denitrificans* 78.3 %, *B. bronchiseptica* 18.3 %, because our strain reduced nitrate and was oxidase-positive by using the *N,N,N',N'*-tetramethyl-*p*-phenylenediamine derivative. 6

As regards the sensitivity to beta-lactam antibiotics and in agreement with Hernández-Porto *et al.* (2013), cefotaxime was not very active. Interestingly, as in *B. hinzii* cases, cephalothin was more active than cefotaxime (Table 2); ceftazidime, cefepime carbapenems and piperacillin-tazobactam were also active. Regarding other antibiotics and in accordance with previous reports (Daxboeck *et al.*, 2004; Hernández-Porto *et al.*, 2013), aminoglycosides and TMP-SMX were active. The lack of fluoroquinolone activity observed in our isolate has also been pointed out previously (Daxboeck *et al.*, 2004; Hernández-Porto *et al.*, 2013).

B. holmesii was described by Weyant *et al.* (1995) as *Bordetella* species associated with bacteraemia in young adults, most with underlying conditions (sickle cell anaemia, diabetes, lymphoma, Hodgkin's disease, and prior splenectomies). Recently, Tartof *et al.* (2014) have made the first report of temporally related cases of *B. holmesii* in bacteraemia in the United States, from April 2010 to January 2011. Twenty-two cases of invasive *B. holmesii* infection were identified from six states. The median age of the patients was 17.1 years, and 64 % had functional or anatomical asplenia. These cases occurred during a peak in pertussis outbreaks, with documented cases of *B. holmesii*/*B. pertussis* respiratory co-infection; pulsed-field gel electrophoresis profiles of a sample of isolates were identical; however, whether there is a link between *B. holmesii* respiratory and bloodstream infection is unknown since no epidemiologic links between the patients were identified (Tartof *et al.*, 2014). Only three cases of septic arthritis caused by *B. holmesii* have been described in the literature. The first report of septic arthritis caused by *B. holmesii* in an asplenic patient was described by Moissenet *et al.* (2011). They described a case of septic arthritis caused by this *Bordetella* species in a 15-year-old boy with chronic haemolytic anaemia, but, recently, others authors (Abouanaser *et al.*, 2013) reported the first two published cases of septic arthritis caused by this organism in apparently immunocompetent patients and unaccompanied by bacteraemia. One of these patients was a 54-year-old woman who consulted owing to

suspected right prosthetic knee infection but no history of frequent or unusual infections. As in our case, the other case was a previously healthy 15-year-old boy who developed post-traumatic knee arthritis with no preceding history of respiratory symptoms and no significant past medical history, including no previous invasive bacterial infections and no adverse reactions to vaccines (Abouanaser *et al.*, 2013). Unlike our case, where the patient was initially treated with clindamycin plus gentamicin (and then switched to TMP-SMX, assuming septic arthritis due to *S. aureus*), in the two cases described by Abouanaser *et al.* (2013), initial treatment was with ceftriaxone, and then oral fluoroquinolone (ciprofloxacin in the first case; levofloxacin in the second case), as per literature reports documenting low fluoroquinolone MICs for most *B. holmesii* isolates. As in our case, in both Abouanaser cases, clinical progress was favourable. In our case, the organism was highly susceptible to beta-lactam (ampicillin, piperacillin-tazobactam, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem), gentamicin, ciprofloxacin and TMP-SMX antibiotics. However, resistance to cefotaxime (Shepard *et al.*, 2004; Panagopoulos *et al.*, 2010; Monnier *et al.*, 2010; Nguyen *et al.*, 2013; Jonckheere *et al.*, 2012; Bassetti *et al.*, 2012), ceftriaxone (Monnier *et al.*, 2010; Van Balen *et al.*, 2012; Stoddard, 2013) and TMP-SMX has been reported (Monnier *et al.*, 2010; Nguyen *et al.*, 2013); resistance to third-generation cephalosporins should be considered since sometimes the initial treatment for septic arthritis by *B. holmesii* has been ceftriaxone (Abouanaser *et al.*, 2013).

As has previously been noted, *B. holmesii* may be an under-recognized pathogen owing to its slow growth and difficult identification. In our case, the *B. holmesii* isolate was misidentified as *Acinetobacter lwoffii* by the VITEK 2 Compact system (bioMérieux) using the GN Colorimetric Identification Card; using the NH Colorimetric Identification Card, the organism was erroneously identified as *Neisseria sicca/Oligella urethralis*. There have been reports of repeated misidentifications of this organism as *A. lwoffii* using standard automated laboratory systems (Abouanaser *et al.*, 2013; Panagopoulos *et al.*, 2010). The misidentification as *Moraxella lacunata* by API 20 NE (Table 1) has already been reported (Moissenet *et al.*, 2011). These misidentifications by the VITEK 2 automated and API 20 NE systems can be attributed to the fact that this species is not found in the databases. An excellent review about microbiology, epidemiology, clinical features, diagnosis and treatment of *B. holmesii* has recently been published by Pittet *et al.* (2014).

Cumulative evidence suggests that these *Bordetella* species can cause clinical diseases other than those recognized to date. Further studies are needed to establish pathogenicity of these *Bordetella* species in these infection sites.

We wish to highlight the importance of isolating *Bordetella* species from severe infections and unusual sites, and also the importance of polyphasic identification to address definitive identification.

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References

- Abouanaser, S. F., Srigley, J. A., Nguyen, T., Dale, S. E., Johnstone, J., Wilcox, L., Jamieson, F., Rawte, P. & Pernica, J. M. (2013). *Bordetella holmesii*, an emerging cause of septic arthritis. *J Clin Microbiol* **51**, 1313–1315.
- Arvand, M., Feldhues, R., Mieth, M., Kraus, T. & Vandamme, P. (2004). Chronic cholangitis caused by *Bordetella hinzii* in a liver transplant recipient. *J Clin Microbiol* **42**, 2335–2337.
- Bassetti, M., Nicco, E., Giacobbe, D. R., Marchese, A., Coppo, E., Barbieri, R. & Viscoli, C. (2012). *Bordetella holmesii* endocarditis in a patient with systemic lupus erythematosus treated with immunosuppressive agents. *J Chemother* **24**, 240–242.
- CLSI (2013). *Performance Standards for Antimicrobial Susceptibility Testing*; 23rd Informational Supplement M100-S23. Wayne, PA: Clinical and Laboratory Standard Institute.
- Cookson, B. T., Vandamme, P., Carlson, L. C., Larson, A. M., Sheffield, V. L., Kersters, K. & Spach, D. H. (1994). Bacteremia caused by a novel *Bordetella* species, *B. hinzii*. *J Clin Microbiol* **32**, 966–969.
- Daxboeck, F., Goerzer, E., Apfalter, P., Nehr, M. & Krause, R. (2004). Isolation of *Bordetella trematum* from a diabetic leg ulcer. *Diabet Med* **21**, 1247–1248.
- Doritke, C., Vandamme, P., Hinz, K. H., Schemken-Birk, E. M. & Wirsing von König, C. H. (1995). Isolation of a *Bordetella avium*-like organism from a human specimen. *Eur J Clin Microbiol Infect Dis* **14**, 451–454.
- Fry, N. K., Duncan, J., Edwards, M. T., Tilley, R. E., Chitnavis, D., Harman, R., Hammerton, H. & Dainton, L. (2007). A UK clinical isolate of *Bordetella hinzii* from a patient with myelodysplastic syndrome. *J Med Microbiol* **56**, 1700–1703.
- Funke, G., Hess, T., von Gravenitz, A. & Vandamme, P. (1996). Characteristics of *Bordetella hinzii* strains isolated from a cystic fibrosis patient over a 3-year period. *J Clin Microbiol* **34**, 966–969.
- Gadea, I., Cuenca-Estrella, M., Benito, N., Blanco, A., Fernández-Guerrero, M. L., Valero-Guillén, P. L. & Soriano, F. (2000). *Bordetella hinzii*, a “new” opportunistic pathogen to think about. *J Infect* **40**, 298–299.
- Hernández-Porto, M., Cuervo, M., Miguel-Gómez, M. A., Delgado, T. & Lecuona, M. (2013). Diabetic leg ulcer colonized by *Bordetella trematum*. *Rev Esp Quimioter* **26**, 72–73.
- Hristov, A. C., Auwaerter, P. G., Romagnoli, M. & Carroll, K. C. (2008). *Bordetella hinzii* septicemia in association with Epstein-Barr virus viremia and an Epstein-Barr virus-associated diffuse large B-cell lymphoma. *Diagn Microbiol Infect Dis* **61**, 484–486.
- Jackwood, M. W., Saif, Y. M., Moorhead, P. D. & Dearth, R. N. (1985). Further characterization of the agent causing coryza in turkeys. *Avian Dis* **29**, 690–705.
- Jonckheere, S., De Baere, T., Schroyers, P., Soetens, O., De Bel, A. & Surmont, I. (2012). Prosthetic valve endocarditis caused by *Bordetella holmesii*, an *Acinetobacter* lookalike. *J Med Microbiol* **61**, 874–877.
- Kattar, M. M., Chavez, J. F., Limaye, A. P., Rassouljian-Barrett, S. L., Yarfitz, S. L., Carlson, L. C., Houze, Y., Swanzy, S., Wood, B. L. & Cookson, B. T. (2000). Application of 16S rRNA gene sequencing to

- identify *Bordetella hinzii* as the causative agent of fatal septicemia. *J Clin Microbiol* **38**, 789–794.
- Kersters, K., Hinz, K. H., Hertle, A., Segers, P., Lievens, A., Siegmann, O. & De Ley, J. (1984). *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. *Int J Syst Bacteriol* **34**, 3456–3470.
- Moissenet, D., Leverger, G., Mérens, A., Bonacorsi, S., Guiso, N. & Vu-Thien, H. (2011). Septic arthritis caused by *Bordetella holmesii* in an adolescent with chronic haemolytic anaemia. *J Med Microbiol* **60**, 1705–1707.
- Monnier, S., Therby, A., Couzon, B., Doucet-Populaire, F. & Greder-Belan, A. (2010). *Bordetella holmesii* bacteremia in a 26-year-old patient with sickle cell disease. *Med Mal Infect* **40**, 299–301.
- Nguyen, L. B., Epelboin, L., Gabarre, J., Lecso, M., Guillot, S., Bricaire, F., Caumes, E., & Guiso, N. (2013). Recurrent *Bordetella holmesii* bacteremia and nasal carriage in a patient receiving rituximab. *Emerg Infect Dis* **19**, 1703–1705.
- Palacián Ruiz, M. P., Vasquez Martinez, M. A. & Lopez Calleja, A. I. (2013). Respiratory infection caused by *Bordetella hinzii*. *Arch Bronconeumol* **49**, 409–410.
- Panagopoulos, M. I., Saint Jean, M., Brun, D., Guiso, N., Bekal, S., Ovetchkine, P. & Tapiero, B. (2010). *Bordetella holmesii* bacteremia in asplenic children: report of four cases initially misidentified as *Acinetobacter lwoffii*. *J Clin Microbiol* **48**, 3762–3764.
- Pittet, L. F., Emonet, S., Schrenzel, J., Siegrist, C. A. & Posfay-Barbe, K. M. (2014). *Bordetella holmesii*: an under-recognised *Bordetella* species. *Lancet Infect Dis* **14**, 510–519.
- Rimler, R. B. (1985). Turkey coryza: toxin production by *Bordetella avium*. *Avian Dis* **29**, 1043–1047.
- Shepard, C. W., Daneshvar, M. I., Kaiser, R. M., Ashford, D. A., Lonsway, D., Patel, J. B., Morey, R. E., Jordan, J. G., Weyant, R. S. & Fischer, M. (2004). *Bordetella holmesii* bacteremia: a newly recognized clinical entity among asplenic patients. *Clin Infect Dis* **38**, 799–804.
- Stoddard, J. M. (2013). A case of *Bordetella holmeseii* endocarditis in an asplenic pediatric patient. *Clin Lab Sci* **26**, 171–174.
- Tartof, S. Y., Gounder, P., Weiss, D., Lee, L., Cassidy, P. K., Clark, T. A., Briere, E. C. & *Bordetella holmesii* Working Group (2014). *Bordetella holmesii* bacteremia cases in the United States, April 2010–January 2011. *Clin Infect Dis* **58**, e39–e43.
- Van Balen, T., Nieman, A. E., Hermans, M. H. A., Schneeberger, P. M. & de Vries, E. (2012). *Bordetella holmesii* meningitis in a 12-year-old anorectic girl. *Pediatr Infect Dis J* **31**, 421–422.
- Vandamme, P., Hommez, J., Vancanneyt, M., Monsieurs, M., Host, E. B., Cookson, B., Wirsing von König, C. H., Kersters, K. & Blackall, P. J. (1995). *Bordetella hinzii* sp. nov., isolated from poultry and humans. *Int J Syst Bacteriol* **45**, 37–45.
- Vandamme, P., Heyndrickx, M., Vancanneyt, M., Hoste, B., De Vos, P., Falsen, E., Kersters, K. & Hinz, K. H. (1996). *Bordetella trematum* sp. nov., isolated from wounds and ear infections in humans, and reassessment of *Alcaligenes denitrificans* Rügger and Tan 1983. *Int J Syst Bacteriol* **46**, 849–858.
- von Wintzingerode, F., Schatke, A., Siddiqui, R. A., Rösick, U., Göbel, U. B. & Gross, R. (2001). *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. *Int J Syst Evol Microbiol* **51**, 1257–1265.
- Wauters, G. & Vanechoutte, M. (2011). Approaches to the identification of aerobic Gram-negative bacteria. In *Manual of Clinical Microbiology*, pp. 539–558. Edited by J. Versalovic, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry & D. W. Warnock, Washington, DC: ASM Press.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, L. D. (1991). 16S-ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Weyant, R. S., Hollis, D. G., Weaver, R. E., Amin, M. F., Steigerwalt, A. G., O'Connor, S. P., Whitney, A. M., Daneshvar, M. I., Moss, C. W. & Brenner, D. J. (1995). *Bordetella holmesii* sp. nov., a new Gram-negative species associated with septicemia. *J Clin Microbiol* **33**, 1–7.
- Wirsing von König, C.-H., J. J. Riffelmann, M. & Coenye, T. (2011). *Bordetella* and related genera. In *Manual of Clinical Microbiology* 10th edn, pp. 739–750. Edited by J. Versalovic, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry & D. W. Warnock, Washington, DC: ASM Press.

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