Use of 16S rRNA gene-based sequencing for identification of *Oligella urethralis* that was misidentified as *Fransciella tularensis* by an automated system

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Introduction: *Oligella* infections are rare and have been only rarely reported in the literature. This may be due to the misidentification of *Oligella* as a *Moraxella*-like organism. To the best of our knowledge, we present what we believe to be the first case report on *Oligella urethralis* bacteraemia in India.

Case presentation: A 65-year-old female patient with compromising underlying illness presented with signs and symptoms of bacteraemia. The organism was initially identified as *Fransciella tularensis* by a bioMérieux colorimetric VITEK 2 Compact GN ID card, but 16S rRNA gene sequencing confirmed the isolate as *O. urethralis*.

Conclusion: The case emphasizes the importance of *O. urethralis* as an emerging opportunistic pathogen. Although automated systems allow accurate and rapid identification of commonly isolated bacterial organisms, they are less likely to correctly identify slow-growing, fastidious, rare or biochemically inert organisms. Therefore, it is good to confirm such isolates with a second method such as 16S sequencing and/or matrix-assisted laser desorption/ionization time-of-flight MS.

Keywords: 16S rRNA gene sequence; bacteraemia; *Fransciella tularensis*; imipenem; piperacillin/tazobactam; *Oligella urethralis*; VITEK 2 Compact.

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Introduction

The genus *Oligella*, so named due to the small size of the bacilli on Gram staining, comprises two species: *Oligella ureolytica* and *Oligella urethralis*. *O. urethralis*, formerly *Moraxella urethralis* and Centers for Disease Control group M4, belongs to a group of taxonomically diverse non-fermentative Gram-negative bacilli (Rossau *et al.*, 1987; Winn *et al.*, 2006).

These organisms are commensals of the genitourinary tract, and most clinical isolates are from urethral samples and are often associated with an indwelling Foley catheter. Rare causes of bacteraemia, septic arthritis and pyelonephritis have been reported in the literature (Mesnard *et al.*, 1992).

Automated systems are used in the majority of clinical microbiology laboratories and are heavily relied on to promptly identify organisms. These systems provide rapid identification, thus reducing turnaround times and improving efficiency and cost-effectiveness (Snyder *et al.*, 2008). However, they are less likely to correctly identify clinically relevant non-fermenting Gram-negative bacilli.

We report a case of *O. urethralis* bacteraemia that was misidentified by a VITEK 2 compact system as *Fransciella tularensis*.

Case report

A 65-year-old female patient who lived in a rural area of Bangalore presented to the Emergency Medical Department of our hospital with predominant complaints of breathlessness for 1 day, swelling of both lower limbs and cough with expectoration for 3 days duration. The patient was a known case of diabetes mellitus, had hypertension for the past 15 years and had ischaemic heart disease 1 year previous; stage 4 chronic kidney disease was diagnosed. She was on regular treatment. On the same day of hospital admission she was shifted to the Intensive Care Unit and intubated in view of tachypnea, reduced pO₂ on arterial blood gas analysis and acidosis.

At the time of admission her pulse rate was 80 min⁻¹, respiratory rate 40 min⁻¹ and blood pressure 180/90 mmHg. On chest examination, bilateral rhonchi and lower zone fine crepts were present. Abdominal examination revealed distension with free fluid. Bilateral lower limb swelling

with pitting pedal oedema was seen. An ulcer was noted on the plantar aspect of the left foot extending up the lateral aspect. No significant lymphadenopathy was noted.

Investigations revealed a white blood cell count of $20\,000\ \mu\text{l}^{-1}$ with 90 % polymorphonuclear cells, haemoglobin $8.9\ \text{g dl}^{-1}$, platelets $276\,000\ \mu\text{l}^{-1}$, general random blood sugar $335\ \text{mg dl}^{-1}$ and HbA1C 10.5 %. Electrolyte imbalance was noted with increased serum potassium ($7.7\ \text{mEq l}^{-1}$), with low sodium ($130\ \text{mEq l}^{-1}$) and calcium ($8.4\ \text{mg dl}^{-1}$). The patient was found to have raised renal parameters (serum creatinine $5.29\ \text{mg dl}^{-1}$, blood urea $143.1\ \text{mg dl}^{-1}$) and was started on haemodialysis. Chest X-ray was noted to be consistent with pneumothorax. Serial arterial blood gases demonstrated the progression of metabolic acidosis. The electrocardiogram demonstrated sinus tachycardia with no ST-T changes. Echocardiography revealed a dilated left-sided chamber, reduced left valve global function, ejection fraction of 42 %, mild mitral regurgitation, sclerotic aortic valve and a trivial aortic regurgitation.

Blood culture was performed in BacT/ALERT 3D (bioMérieux) aerobic adult blood culture bottles. As the patient was intubated, sputum sample could not be obtained. However, a tracheal aspirate yielded no growth. After debridement of the ulcer on the left foot, the pus sample was sent for culture and yielded *Klebsiella pneumoniae*.

Based on the above clinical and laboratory findings, a primary diagnosis of sepsis and pneumothorax with acute respiratory distress syndrome, and a secondary diagnosis of type II diabetes mellitus, left diabetic foot, with ischaemic heart disease and hypertension, was made.

After 24 h incubation in aerobic blood culture bottles, bacterial growth was detected (based on spectrophotometric CO_2 detection). Gram staining revealed a Gram-negative coccobacillus. Subculture performed on 5 % sheep blood agar yielded confluent growth of a single type of grey-white, opaque, non-haemolytic colonies after 48 h incubation. No growth was noted on MacConkey agar plates. The organism was identified as *F. tularensis* by an automated VITEK 2 Compact GN ID card (bioMérieux) bacterial identification system with 99 % probability. This identification result was surprising, as this bacteria species has never been identified and reported in India from known publications. Antimicro-

bial susceptibility testing was not performed due to inadequate laboratory safety facilities for handling this category A biological agent. To confirm the isolate, we sent it for 16S rRNA gene sequencing at VRF Referral Laboratory, Chennai, India. A second blood culture sample collected on day 5 remained sterile even after extended incubation.

The patient was treated empirically with injectable piperacillin/tazobactam 2.25 mg every 8 h and injectable imipenem 250 mg every 12 h for 6 days. From the blood cultures, a single bacterial species was isolated and identified as *F. tularensis*. On day 5, injectable streptomycin $4\ \text{mg kg}^{-1}$ was started on alternate days in modified dose as per renal clearance.

The patient's general condition remained stable with no clinical improvement throughout the hospital stay; the patient finally deteriorated and succumbed to death on day 14. On the day of patient demise, the report of 16S rRNA gene sequencing was received and the isolate was identified as *O. urethralis*. Only partial sequencing was performed, submitted to GenBank and given GenBank accession number KF280285.1.

Although the patient had expired, an antibiotic sensitivity test was still performed on this *O. urethralis* isolate for research purposes. As there are no validated testing methods and based on little documentation available in the literature, an antibiogram was performed by the Kirby Bauer disc diffusion method (Alireza *et al.*, 2006). The isolate was found to be sensitive to imipenem, meropenem, piperacillin/tazobactam, tobramycin, ceftazidime and amikacin, and resistant to penicillin, ampicillin and cotrimoxazole.

Discussion

A review of previously published case reports yielded only five cases of *Oligella* bacteraemia; four cases caused by *Oligella ureolytica* and only one case reported of *Oligella urethralis* bacteraemia (Baqi & Mazzulli, 1996; Baruah *et al.*, 2014). The clinical significance of *Oligella urethralis* bacteraemia is unknown and the mechanism of infection remains unclear, but it appears to occur in conjunction with compromising underlying illness, as seen in the cases reported so far. Available literature also suggests that *Oligella* infec-

Table 1. *O. urethralis* identification by conventional phenotypic methods

Conventional ID	No. of isolates	16S rRNA gene sequence ID	GenBank accession no.	Reference
<i>Comamonas</i> spp.	1	<i>O. urethralis</i>	KC866170	de Melo Oliveira <i>et al.</i> (2013)
<i>O. urethralis</i>	2	<i>O. urethralis</i>	KC866214, KC866215	de Melo Oliveira <i>et al.</i> (2013)
<i>F. tularensis</i>	2	<i>O. urethralis</i>	–	Bosshard <i>et al.</i> (2006)
No identification	2	<i>O. urethralis</i>	–	Zbinden <i>et al.</i> (2007)
No identification	1	<i>O. urethralis</i>	AF133538	Drancourt <i>et al.</i> (2000)
<i>F. tularensis</i>	1	<i>O. urethralis</i>	KF280285.1	Present study

tion responds quickly to antibiotics. Amongst the reported cases, it is seen that only one patient died due to secondary fungaemia and the remaining responded well to antibiotics.

In our case, as the second blood culture performed after starting empiric antibiotics remained sterile even after extended incubation, we assume *Oligella* bloodstream infection was cleared as the isolate was sensitive to imipenem and piperacillin/tazobactam. However, we could not ascertain this as the patient's clinical condition remained the same and she died due to additional comorbid conditions.

To the best of our knowledge, we believe this to be the first case report on *Oligella urethralis* bacteraemia in India. Recently, a case report on *Oligella ureolytica* bacteraemia was reported by Baruah *et al.* (2014).

Table 1 shows that *O. urethralis* is either misidentified or not identified by conventional phenotypic methods, except in a study by de Melo Oliveira *et al.* (2013) where two isolates were assigned to the species level.

Bosshard *et al.* (2006) concluded that VITEK 2 Compact GN ID card results showing excellent or very good identification of species other than *Achromobacter xylosoxidans*, *Acinetobacter* spp., *Burkholderia cepacia* group, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* should be subjected to 16S rRNA gene sequencing.

Zbinden *et al.* (2007) concluded that 16S rRNA gene sequencing is a more accurate and effective means for the identification of non-fermenting Gram-negative bacilli, as commercially available VITEK 2 Compact phenotypic test systems have a major drawback in that their available databases are limited (159 taxa).

de Melo Oliveira *et al.* (2013) proposed a cost-efficient algorithm based on molecular analysis by 16S rRNA for difficult to differentiate species of these groups.

In clinical laboratories, most bacteria are routinely identified by biochemical characteristics using a commercial kit or automated identification system, such as API and VITEK. This case illustrates the challenge for clinicians when suspecting this rare disease and the failure of automated systems to reliably identify *O. urethralis* – a rare and emerging pathogen. However, the results from automated systems should be analysed critically, taking into consideration the reliability of these systems for accurate identification of slow-growing, rare and biochemically inert organisms, such as *F. tularensis*, *Burkholderia pseudomallei*, *Brucella* spp. and *Yersinia pestis* (ASM, 2014). These isolates should be confirmed with a second method such as 16S sequencing and/or matrix-assisted laser desorption/ionization time-of-flight MS.

In conclusion, molecular methods should be considered to correctly identify such rare organisms and this will likely impact patient care in addition to microbiologic research. Hence, strict microbiological vigilance is required in the identification of these clinically relevant non-fermenting Gram-negative bacilli.

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