Diagnostic Microbiology and Infectious Disease 88 (2017) 58–61

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Multidrug-resistant *Achromobacter animicus* causing wound infection in a street child in Mwanza, Tanzania

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ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form 3 February 2017
Accepted 4 February 2017
Available online 10 February 2017

Keywords:
Achromobacter animicus
Street child
Wound infection
Tanzania

ABSTRACT

Achromobacter animicus (*A. animicus*) is an aerobic, motile, gram-negative, non-fermenting small bacillus that can also grow anaerobically with potassium nitrate. It has been isolated from sputum of humans suffering from respiratory infections. Literature regarding the role of *A. animicus* in wound infections is limited. We report a first case of a chronic post-traumatic wound infection caused by a multidrug-resistant *A. animicus* in a street child from Africa and accompanied diagnostic challenges.

1. Introduction

Achromobacter species, previously described as aquatic environmental bacteria have recently been frequently isolated from cystic fibrosis patients (Dupont et al., 2015). There are few reports of *Achromobacter xylosoxidans* (*A. xylosoxidans*) causing skin and soft tissue infections, bacteremia and osteomyelitis (Shinha and Oguagha, 2015; Spear et al., 1988; Tena et al., 2014). Efflux pumps and production of class D beta-lactamase enzymes have been associated with intrinsic resistance of *A. xylosoxidans* to antimicrobials (Doi et al., 2008). In addition, other acquired aminoglycosides and beta-lactam resistance genes have recently been detected in *A. xylosoxidans* (Hu et al., 2015). *A. animicus* is an aerobic, motile, gram-negative, non-fermenting rod which was first isolated in 2006 from a cystic fibrosis patient (Vandamme et al., 2013). Up to the present time, there is no report of *A. animicus* causing wound infection and the mechanisms of resistance and genes involved in resistance are not well understood. In this report, we describe a case of wound infection caused by *A. animicus* and the involved mechanisms of resistance.

2. Case report

A 14-year-old male from Igoma ward in urban Mwanza was enrolled in a study via the founded Wound Care Project on 16th April, 2015. He was a street child type one i.e. those who spend the day on streets and at night they either sleep at home or in street children institutions (Ayaya and Esamai, 2001). His survival relied on selling scrap metals. His main complaint was a chronic post-traumatic wound of unspecified duration on the left foot as a result of being knocked by a car. He denied any history of being admitted to a hospital and of antibiotic use. He reported to putting sand-like local herbs on the wound which were given to him by a samaritan. He reported to smoking cigarettes but did not drink alcohol. He had a history of swimming in Lake Victoria, drinking pipe water, and toileting in streets and bushes.

On physical examination, he appeared unkempt, fairly nourished with a Body Mass Index of 22.2 kg/m^2 (height = 1.47 m, weight = 48 kg). The axillary temperature and Random Blood Glucose (RBG) were 36.6 °C and 3.2 mmol/l respectively. Local examination revealed 8 cm (length) × 3 cm (width) non-smelly wound with irregular margins, some necrotic tissues and little pus (Fig. 1A). Wound debridement was performed following an infiltration of 2% lignocaine as a local anesthesia. Following the debridement, two wound swabs were taken for gram stain and culture (Smith et al., 2014). A sterile swab moistened in a normal saline (0.9%) was firmly placed at the centre of the wound, rolled outward towards the wound edge to cover the wound area (Bowler et al., 2001). The swab was dipped in Amies transport medium (Mast Group Ltd., United Kingdom) and promptly transported to the Catholic University of Health and Allied Sciences (CUHAS) laboratory for processing and analysis. Wound dressing was done with normal saline (0.9%) and covered with sterile gauze.

The gram stain of the wound swab revealed gram-negative rods with few polymorphonuclear cells. Swab culture after 24 hours of aerobic incubation showed a massive growth of pure culture of small,

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http://dx.doi.org/10.1016/j.diagmicrobio.2017.02.002
0732-8893/

Fig. 1. A: Wound presentation on day 1. B: Wound status after 1 week of treatment and dressing.

whitish, mucoid-slimy colonies on blood agar and colorless colonies on MacConkey agar (BD Difco, USA). In addition, the isolate grew on MacConkey agar supplemented with 2 μg of cefotaxime (Medochemie Ltd., Cyprus, EU) and also on ESBL CHROMagar (Mast Group Ltd.). Using in-house biochemical tests the colonies were oxidase positive, catalase positive, citrate negative, urease negative and tested positive for motility. The definitive identification was not reached based on in-house biochemical tests results. The VITEK-MS (bioMérieux, France) identified the isolate (TV80) as either *Ochrobactrum anthropi (O. anthropi)* or *Achromobacter denitrificans* (*A. denitrificans*) or *A. xylosoxidans*, each with 33.3% confidence level. Consecutive identification and susceptibility testing with VITEK-2 system (bioMérieux, France) reported the isolate (TV80) as *O. anthropi* (97% confidence level) without susceptibility results. Epsilometer test (Etest) was then performed to establish the susceptibility profile of the isolate. The isolate (TV80) was resistant to cefotaxime (≥32 μg/ml), ceftazidime (≥32 μg/ml), chloramphenicol (≥256 μg/ml), ampicillin-sulbactam (≥32 μg/ml), piperacillin-tazobactam (≥128 μg/ml), aztreonam (≥256 μg/ml), tetracycline (≥6 μg/ml), and trimethoprim-sulfamethoxazole (≥32 μg/ml). It was susceptible to ciprofloxacin (0.38 μg/ml), colistin (0.5 μg/ml), gentamicin (≤1 μg/ml), amikacin (4 μg/ml), imipenem (≤1.5 μg/ml), meropenem (0.5 μg/ml), ertapenem (0.094 μg/ml), and tigecycline (0.5 μg/ml) as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

The patient was treated with ciprofloxacin 500 mg p.o. b.i.d. for 7 days and was scheduled to continue with daily wound dressing. Although there was significant improvement of his wound after 1 week of treatment (Fig. 1B), the final outcome was not confirmed because of lost to follow up.

3. Isolate characterization

Following the inconclusive isolate identification results further taxonomic examination was done. The 1511 bp complete 16S rRNA gene of TV80 was amplified using D88 and E94 primers and sequenced using F16 and F17 primers as per previous description (Paster et al., 2001). The BLAST analysis on the NCBI web-site of the complete 16S rRNA gene provided 99.7%, 99.6% and 99.6% match with *A. animicus* (NR_117615), *A. xylosoxidans* (CP014065) and *A. denitrificans* (CP013923) respectively. Further identification to confirm the species was by amplification of 765 bp of *nrdA*_765 gene, a single locus which has been previously established to distinguish among *Achromobacter* spp. (Spilker et al., 2013). The *nrdA*_765 gene sequence resulted into the closest match with *A. animicus nrdA*_765_75 with one nucleotide difference at position 637. The latter led to the identification of the new *nrdA*_765 allele which was assigned number 240 (Fig. 2). In addition, it confirmed the strain to belong to *A. animicus*. The multilocus sequence typing (MLST) scheme for *Achromobacter* spp. (Vandamme et al., 2013) unveiled 4 new alleles, i.e. *gltB*_82, *lepA*_114, *nrdA*_99 and *nuoL*_92, that resulted into the new sequence type ST-320 of *A. animicus*.

Complete genome sequencing of *A. animicus* TV80 confirmed the MLST. The raw sequencing data of *A. animicus* (TV80) have been made available at the European Nucleotide Archive (ENA) under the project number PRJEB18461. Sequence analysis with resfinder 2.1 (Zankari et al., 2012) and Resistance Gene Identifier (Jia et al., 2016) was performed to complement the phenotypic antibiotic resistance profile. It revealed efflux pumps and class D beta-lactamase from the OXA-50 family, OCH family class C beta-lactamase from *O. anthropi* and ampC-related *Pseudomonas*-derived cephalosporinase (PDC) from *Pseudomonas aeruginosa* explaining the beta-lactam resistance profile (see above). Furthermore, the detected sulfonamide (*sul*1 and *sul*2), aminoglycoside (*aph*(3″)-IIb), chloramphenicol (*cat*B7) and fosfomycin (*fos*A) resistance genes were also found to be associated with the phenotype.

4. Discussion

Although multidrug-resistant non-fermenting bacteria have been implicated in causing wound infections (Bessa et al., 2015), information on the role of *Achromobacter* spp. in causing wound infections is limited. *Achromobacter* spp. have been previously described as environmental pathogens (Yabuuchi and Ohyama, 1971). The recent increasing rate of isolating them from human clinical samples warrants clinicians awareness due to treatment challenges posed by their antimicrobial resistance profiles (Ridderberg et al., 2015).

The phenotypic biochemical characteristics of *Achromobacter* spp. have been reported to be easily confused with other non-fermenters such as *Pseudomonas* spp. (Igra-Siegman et al., 1980); a fact which highlights the failure or misidentification of these isolates by conventional phenotypic methods (Barrado et al., 2013) commonly used in clinical microbiology laboratories in low-income countries (LICs). In addition, it might contribute to the underestimation of the clinical significance of these strains in resource-limited countries.

Literature on the pathogenicity and adaptation of *A. animicus* to the human host is also limited. Our patient had a history of frequent lake water contact which might be a source of acquiring the bacterium as previously reported in *A. xylosoxidans* (Spear et al., 1988). Furthermore, the patient reported to put sand-like local herbs of unknown contents on the wound which might as well be a possible source of introducing the bacterium on the wound. Although wound contamination by dressing solutions has been reported to be another risk factor for *A. xylosoxidans* infection (Vu-Thien et al., 1998), this was not the case as our patient did not visit any health center. Other risk factors such as diabetes and vascular diseases have been reported to associate with *Achromobacter* spp. wound infection in older people (Lipsky, 2004; Shinha and Oguagha, 2015); however, they could not be related to our case as the patient was young with fairly RBG levels on examination.

In contrast to a previous report of 14 wound infection cases caused by *A. xylosoxidans* in which more than 90% of the isolates were susceptible to trimethoprim-sulfamethoxazole, ceftazidime and piperacillin-tazobactam and 20% were resistant to ciprofloxacin (Tena et al., 2014), the *A. animicus* isolate in this report showed a unique pattern of being