

## Infection with *Lawsonella clevelandensis* is Easily Overlooked using Routine Microbiology Methods: A Case of a Surgical Mesh-Associated Abscess

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### ABSTRACT

A patient with an abscess caused by *Lawsonella clevelandensis* is presented. *L. clevelandensis* was isolated only after prolonged incubation which was done because the Gram-stain was positive for actinomyces-like bacteria. *L. clevelandensis* was recently discovered and is part of the commensal flora of the skin. Infections have been rarely described but are probably more common since diagnosis using routine microbiology methods is hampered by poor staining and slow growth. The study describes the difficult microbiological diagnosis of an infection with *L. clevelandensis*.

**Keywords:** Abscess; Surgical mesh; Diagnosis; PCR; Microscopy; Culture

### CASE REPORT

A 70-year old female patient was admitted to the hospital for surgical drainage of an abscess in the left lower abdomen. Her medical history showed an abdominal hysterectomy and rheumatoid arthritis for which she used no medication. She had a laparoscopic total extra-peritoneal repair of an inguinal hernia on the left side 4 years ago using a non-absorbable polyester mesh, and a Lichtenstein open hernia repair using a partially absorbable polypropylene mesh 2 years ago because of recurrence.

Two weeks earlier she presented to the surgical outpatient department with a progressive painful mass in the left groin since 3 days. Both ultrasonography and CT scan showed a fluid collection adjacent to the pre-peritoneal mesh. In the absence of fever this was considered to be a seroma. Ilio-inguinal neuralgia or an uninfected seroma were considered to cause the pain. Ultrasound-guided infiltration of the ilio-inguinal nerve with a local analgesic (bupivacaine) and steroids (triamcinolone) was performed.

Since then the pain had worsened and she developed a fever (39.2°C). Bowel sounds were normal and the overlying skin was without defects. WBC count was  $21 \times 10^9/L$  and CRP was 312 mg/L. A new CT scan again showed the fluid collection but was

now interpreted as an abscess associated with the pre-peritoneal polyester mesh.

A laparotomy was performed to drain the pre-peritoneal located abscess and to remove the mesh. Ceftriaxone 1000 mg qd and metronidazole 500 mg tid were started intravenously after collection of cultures. Treatment was changed empirically after 10 days to oral clindamycin 600 mg tid. After 22 days she developed a rash and treatment was continued with oral doxycycline 100 mg bid based on the culture findings and susceptibility testing. After 6 weeks of treatment she was without complaints, leucocytes and CRP were normalized, and antimicrobials were discontinued.

Intra-operatively collected pus was collected for routine microbiology analysis including microscopy of a Gram-stained smear, and aerobic and anaerobic cultures using the following media. Aerobic culture consisted of CBA and CHOC in 5% CO<sub>2</sub> as well as McC and CNA in ambient air. Anaerobic culture consisted of Columbia blood agar 5% sheep blood, a chocolate agar, NV blood, NAT. In addition a Thioglycolate broth was inoculated. All media were incubated at 37°C. Incubation was continued until visible growth.

Visible growth was subcultured for identification using Matrix Assisted Laser Desorption-Ionization-Time of Flight (MALDI-TOF) mass spectrometry according manufacturer's instructions

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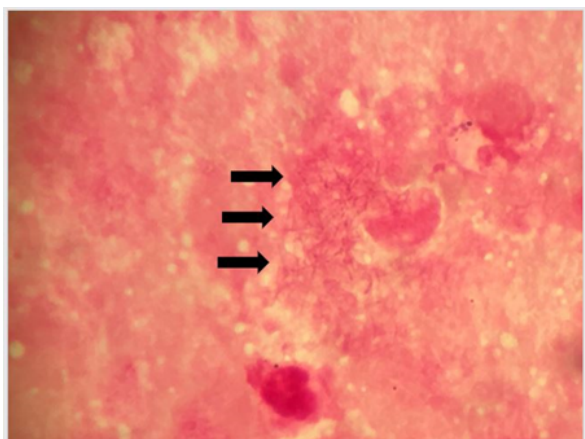
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(MALDI Biotyper CA System (Bruker Daltonics Inc.)) but did not result in identification. The isolate was subsequently identified using Whole Genome Sequencing (WGS) as well as 16sRNA DNA Sanger sequencing. In short, DNA of the isolate was prepared using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The WGS was executed with the Nextera DNA Flex Library prep (Illumina, Inc., San Diego, CA). The whole genome was amplified and after running in the MiniSeq (Illumina, Inc., San Diego, CA) the species identification was done using BLAST® and Basespace (Illumina, Inc., San Diego, CA). 16sRNA DNA Sanger sequencing was performed using the highly conserved regions of the eubacterial primers 8F and 575R to amplify a 500-bp to 600-bp 16sRNA gene sequence (lit 8F and 575R) [1,2].

16sRNA DNA sequencing was also performed on the intra-operative pus that was stored at -80°C using the DNA isolation procedure as described above.

Light-microscopy of the pus showed very light staining, slender filamentous, possible branching, Gram-positive rods (figure 1). After the routine incubation of 5 days there was no growth. Subsequently, the anaerobic culture media were further incubated because of the positive Gram-stain findings. After 16 days of incubation at 37°C in anaerobic atmosphere, barely visible small rough adherent colonies, creamy white in colour with some black pigment were present on the nalidixic acid tween agar. No other growth was observed. The other anaerobic media including the liquid thioglycolate broth were without growth. Using E-test, the MICs were low for penicillin (<0.016 mg/L), clindamycin (<0.016 mg/L), and tetracycline (0.064 mg/L). No bacterial identification was possible with MALDI-TOF. Using WGS and 16sRNA DNA sequence analysis the isolate was identified as *Lawsonella clevelandensis*. *L. clevelandensis* was also identified from the frozen intra-operative pus using 16sRNA DNA sequencing. After the identification, smears from the frozen stored pus specimen were made for acid fast staining using the stain according to Ziehl-Neelsen and the modified Kinyoun stain using 1% H<sub>2</sub>SO<sub>4</sub> for decolourization.



**Figure 1:** Gram stain of pus showing slender filamentous Gram positive rods that hardly took up any stain. The bacteria are present in a large cluster suggestive of branching.

*L. clevelandensis* was first reported in 2013 and was described as a novel species within the suborder *Corynebacterineae* in 2015 [3].

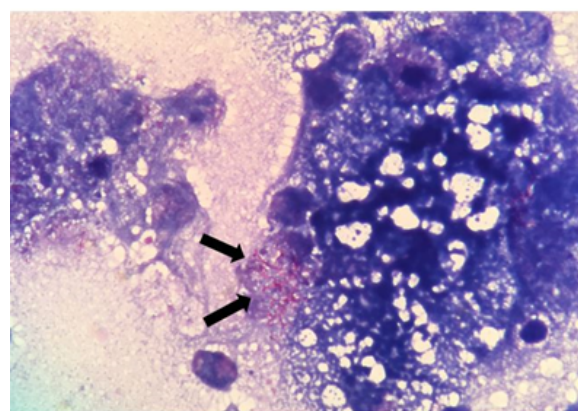
Since then 10 cases have been described including the current case [4-8]. All cases describe monobacterial abscesses with gross pus, mostly in association with the skin. Most patients had some kind of impaired immune system (diabetes, malignancy, steroid therapy).

Three recent studies showed *L. clevelandensis* to be among the most common species of the human skin, scalp, and nostrils. At this point it is unclear whether *L. clevelandensis* is also a commensal of the mucous membranes [5-11].

In our patient, *L. clevelandensis* may have been introduced during the initial hernia repair causing a low grade infection in the presence of the foreign body possibly exacerbated by the infiltration with steroids. Alternatively, introduction of bacteria during infiltration of the ilioinguinal nerve cannot be excluded despite sterile handling and the use of ultrasound guidance. Finally, recent seeding of the mesh and scar tissue via the bloodstream during occult bacteraemia causing the initial symptoms of pain and subsequent pus formation is also a possibility.

*L. clevelandensis* is a Gram-positive rod but has been described as refractory or poorly staining like in our case (Figure 1) [3]. The poor visualization with light-microscopy together with the very slow growth only visible beyond routine incubation periods, as well as the absence of growth in liquid media all contribute to the difficult isolation of this pathogen. The isolation will be even more difficult when there is a polymicrobial infection and this may have contributed to the description of monobacterial infections only. The identification was done with WGS. 16sRNA DNA sequencing was later performed directly on the pus and also showed *L. clevelandensis* as the single bacterium present. The latter result confirms that also in our patient the infection was monobacterial.

In most but not all cases *L. clevelandensis* has been described as partially acid-fast in clinical material using this stain. This property was lost on subculture [3]. In our case no acid-fastness was demonstrated using the Ziehl-Neelsen stain, but the branching bacteria were acid-fast when the modified Kinyoun stain was used (Figure 2).



**Figure 2:** Modified Kinyoun stain of pus showing partial acid-fast branching bacteria.

In conclusion, *L. clevelandensis* is a common commensal of the human skin. Infections are sporadic and with its abundance in

the human skin microbiome it is likely a bacterium of low virulence. However, the occurrence of infection with *L. clevelandensis* is probably underestimated by routine microbiology methods and it may well be a more frequent cause of purulent infections where routine microbiology investigations remain negative. An etiological diagnosis can be made by using a modified Kinyoun stain, extended incubation times, and molecular methods directly on clinical specimens and isolates.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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