TITLE: TWO RARE CASES OF WOUND INFECTIONS CAUSED BY TRUPEPERELLA BERNARDIAE

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RUNNING HEAD: INFECTIONS DUE TO TRUEPERELLA BERNARDIAE

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SUMMARY

*Trueperella bernardiae* is a Gram-positive curved rod that is considered an uncommon pathogen involved in few infections. The true incidence of infections with this bacterium as well as the clinical implications are still unknown. We report two cases of wound infections in two patients that underwent different surgical procedures, although in the second case the microorganism was isolated in mixed culture. Culture of wound secretion resulted in *T. bernardiae* isolation. Treatment was performed and resolution of these infections were documented.

*T. bernardiae* was originally classified within CDC fermentative coryneform group 2 (1), transferred to the genus *Arcanobacterium* (2) and finally reclassified to the genus *Trueperella* (3). *T. bernardiae* was firstly isolated from a variety of clinical sources (1), but the first isolation in pure culture was reported by Ieven et al. in 1996 (4). To our knowledge, only eight more cases of human infection in pure culture have been reported in the medical literature, which consist of urinary tract infections (5), joint infections (6-8), skin and wound infections (9, 10), blood (11) and both kidney and pleura (12). We were recently confronted with two cases of wound infections caused by *T. bernardiae* in two patients after different surgical procedures, one of them in pure culture.

A 69-year-old women underwent an anterior rectal resection due to a rectum carcinoma in 2000. In 2001 the patient was treated with an abdomino-perineal amputation and an eventroplasty, and in 2006 a terminal colostomy was finally performed. Otherwise, her clinical history was unremarkable. Since then, the patient has suffered several episodes of peri-colostomy eventration, and in April 2017 a medial laparotomy was performed in order to relocate the colostomy.

On the 7th day post-operatively the patient had fever (38.0°C), pain and drainage of purulent material through the surgical wound. A complete blood count, chemical profile, and urinalysis were all normal except C-reactive protein (CRP) level of 218 mg/L (normal value 0.02-5 mg/L) and white cell blood count (WBC) of 12,270/mm³ (70% neutrophils). Treatment with metronidazole (500 mg/8 h/7 days) and ciprofloxacin (500 mg/12 h/7 days) was started. An aspirate of purulent exudate was sent to the microbiology laboratory for culture. The sample was inoculated in blood agar (either aerobic or anaerobic) (BD Columbia Agar 5% Sheepblood®, Becton Dickinson), chocolate agar (BD Choco Agar, Becton Dickinson) and thioglycolate broth (BD™ Fluid Thioglycollate Medium, Becton Dickinson). All media were incubated at
37°C. Gram staining of the exudate exhibited no microorganisms, and on the third day of incubation the growth of Gram-positive bacilli in pure culture was observed in all plates. The colonies were observed as non-hemolytic, rounded, creamy and whitish (Fig. 1). A mass spectrometry method (Bruker Biotyper, Billerica, MA, USA) was employed to identify the strain as *T. bernardiae* (score 2.13), and was confirmed by 16S rRNA gene sequencing. The MIC of the bacteria to different antibiotics was carried out by the E-test method. As no specific clinical breakpoints have been established for *T. bernardiae*, we used the EUCAST PK/PD (Non-species related) clinical breakpoints. The isolate was susceptible to penicillin G (MIC 0.094 µg/ml), linezolid (MIC 0.38 µg/ml), amoxicillin/clavulanate (MIC 0.047 µg/ml), and resistant to ciprofloxacin (MIC 1.5 µg/ml). Vancomycin, clindamycin, daptomycin, erythromycin and gentamicin were not interpreted because no PK/PD clinical breakpoints have been defined for them.

Blood cultures taken on admission were negative. Treatment was changed and amoxicillin-clavulanate (500 mg/8 h) was administered for 7 days and at 1 month of follow-up the patient remained well.

A 70-year-old women who came to the Emergency Department in April 2017 due to fever, pain and infection signs in an inguinal granuloma. The patient underwent a major abdominal surgery in 2012 due to an unilateral ovarian cancer, and she was also treated with coadjuvant chemotherapy based on Carbop Taxol (Paclitaxel and carboplatin). In May 2014 the patient suffered an inguinal ganglionar metastasis treated with Caelyx, Carbo Taxol and Topotecan. In January 2016 a removal of the inguinal adenopathies was performed. Since then, the patient presents with an ulcerated inguinal granuloma with chronic purulent secretion. A complete blood count, chemical profile, and urinalysis were all normal except C-reactive protein (CRP) level of 18 mg/L (normal value 0.02-5 mg/L). Treatment with metronidazole (500 mg/8 h/7 days) was started without improvement. An aspirate of purulent exudate was sent to the microbiology laboratory for culture, and the sample was processed like in the case 1. Gram staining of the exudate exhibited Gram-positive bacilli and Gram-negative bacilli. On the first day of incubation the growth of a predominant Gram-positive bacilli along with a Gram-negative bacilli was observed in all plates. The aspect of the colonies of *T. bernardiae* were similar to case 1. Using the same mass spectrometry method, both bacteria were identified as *T. bernardiae* (score 2.08) and *Escherichia coli* (score 2.26),
respectively. *Trueperella* was also confirmed by 16S rRNA gene sequencing. Following the same procedure as in case 1 for antimicrobial susceptibility, the isolate was susceptible to penicillin G (MIC 0.064 µg/ml), linezolid (MIC 0.75 µg/ml), amoxicillin/clavulanate (MIC 0.047 µg/ml) and resistant to ciprofloxacin (MIC 2 µg/ml). Treatment with amoxicillin-clavulanate (500 mg/8 h) was administered for 7 days and improvement was observed.

*T. bernardiae* is a Gram-positive bacilli that is a member of the normal biota of human skin and oropharynx. Few cases of infection due to this microorganism in pure culture have been reported until now (4-12), and five more cases have been published as causing mixed infections (Table 1). The true incidence of infections with this bacterium is unknown, because when isolated in clinical samples these coryneforms are not identified. For that reason, today its occurrence is uncommon and its pathogenicity and clinical implications are not still clearly established, especially when infections are polymicrobial and also contain other kind of microorganisms.

The diagnosis of *T. bernardiae* infection is mainly based on culture of an adequate sample obtained from the site of infection, although the final identification may be difficult, because it could be confused with other microorganisms such as other coryneform, Gram-positive bacilli or streptococci (13). Regarding the identification, the recent introduction of mass spectrometry technology may strongly help in the final identification, as in our case. The use of MALDI-TOF has represented a breakthrough in the diagnosis of these kind of microorganisms due to its speed and accuracy (14).

The treatment of choice for *T. bernardiae* infections has not yet been established due to both the scarcity of data and the absence of clinical breakpoints for this bacterium. Some studies show that all antimicrobial drugs tested for *T. bernardiae* were susceptible, except to ciprofloxacin (4, 15). However, other studies show resistance to clindamycin and erythromycin (9), norfloxacin and fosfomycin (13), metronidazole (6), pefloxacin (5), and penicillin G (7). However, lack of standardization and the absence of clinical breakpoints for antimicrobial studies to *T. bernardiae* prevents any conclusion being drawn on antimicrobial susceptibility and make it necessary further studies. Meanwhile, monitoring through susceptibility testing is advisable.

Conflict of interest: None to declare
REFERENCES


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<td>2013</td>
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**M:** male; **F:** female; **NR:** not reported; **CRP:** C-reactive protein; **IoS:** intraoperative specimens;