Title: Two Cases of Granulomatous Mastitis Caused by Corynebacterium kroppenstedtii Infection in Nulliparous Young Women with Hyperprolactinemia

Abstract

Recently, an association between granulomatous mastitis and local infection with Corynebacterium (C.) kroppenstedtii has been suggested. We herein report two cases of granulomatous mastitis resulting from C. kroppenstedtii infection in nulliparous young women with hyperprolactinemia. Both cases involved nulliparous patients with drug-induced hyperprolactinemia, and both individuals received incision and drainage, after which the pus was sent to our laboratory. Corynebacterium spp. grew on blood agar, and 16S rRNA gene sequencing identified the pathogen as C. kroppenstedtii. In conclusion, lactational changes caused by drug-induced hyperprolactinemia may increase the risk of granulomatous mastitis after C. kroppenstedtii infection.

Key words: granulomatous mastitis, Corynebacterium kroppenstedtii, 16S rRNA gene sequencing, hyperprolactinemia


Introduction

Granulomatous mastitis, an inflammatory disease of unknown etiology, is an uncommon benign disorder of the breast (1). Recently, an association of this condition with local infection with Corynebacterium (C.) kroppenstedtii has been suggested. In 2002, Paviour et al. reported 24 patients with mastitis (2), among whom Corynebacterium spp. were isolated from breast tissue, pus and/or deep wound swabs, with C. kroppenstedtii being the most commonly identified pathogen. In this report, we describe two cases of granulomatous mastitis due to C. kroppenstedtii infection identified on 16S rRNA gene sequencing.

Case Reports

Case 1

A 20-year-old nulliparous Japanese woman presented to our hospital with recurrent mastitis in October 2013. Prior to this consultation, she had experienced two episodes of mastitis in March and October 2012, after which she was diagnosed with granulomatous mastitis and treated with ultrasound-guided aspiration drainage. She had also been diagnosed with mixed connective tissue disease at 15 years of age and had been taking prednisolone (5 mg/day), azathioprine (50 mg/day), alfacalcidol and sulpiride since that time. She was not allergic to any medications and did not smoke or drink alcohol. She was nulliparous and not pregnant. The findings of a physical examination were normal, except for...
bacteria to be sequent 16S rRNA gene sequencing revealed the causative as the pathogen, with a probability of 87.1%, whereas sub-
white colony of and a few Gram-positive bacilli (Fig. 1). A small creamy-
drainage was performed, and the pus was sent to our labora-
periareolar mass. Therefore, ultrasound-guided aspiration
and mammary ultrasonography showed a right-sided external
her right breast. The serum prolactin level was 108.0 ng/mL,
France) identified
the API Coryne System (bioMérieux, Marcy-l’Etoile,
chemical identification of the corynebacterial strain using
was supplemented with 1% Tween 80. Initial routine bio-
tion. The colony grew at an increased rate when the agar
agar with capneic incubation and thioglycolate me-
formed, and the pus was sent to our laboratory. Gram stain-
dritically expanded mass. Incision and drainage were per-
sonography showed a right-sided external periareolar den-
vealed redness and swelling of the right breast (Fig. 2), with
not pregnant. The findings of a physical examination re-
though she was a social drinker. She was nulliparous and
currently taking fluphenazine, zolpidem and duloxetine. She
sultation. She had a history of schizophrenia and was cur-
cable sensation in her right breast two weeks before the con-
cember 2013. She had begun to experience an uncomfort-
tal with redness, pain and swelling of the right breast in De-

![Figure 1](image1.jpg)  Figure 1. Gram staining of the pus with a number of neutrophils and a few Gram-positive bacilli (Case 1).

![Figure 2](image2.jpg)  Figure 2. Right breast of the patient exhibiting redness and swelling (Case 2).

an elevated body temperature of 37.8°C and two masses in her right breast. The serum prolactin level was 108.0 ng/mL, and mammary ultrasonography showed a right-sided external periareolar mass. Therefore, ultrasound-guided aspiration drainage was performed, and the pus was sent to our laboratory. Gram staining of the pus revealed several neutrophils and a few Gram-positive bacilli (Fig. 1). A small creamy-white colony of Corynebacterium spp. grew on 5% sheep blood agar with capneic incubation and thioglycolate medium with enrichment approximately 72 hours after inoculation. The colony grew at an increased rate when the agar was supplemented with 1% Tween 80. Initial routine biochemical identification of the corynebacterial strain using the API Coryne System (bioMérieux, Marcy-l’Etoile, France) identified C. argentoratense (profile No. 2040104) as the pathogen, with a probability of 87.1%, whereas subsequent 16S rRNA gene sequencing revealed the causative bacteria to be C. kroppenstedtii (sequence similarity to the C. kroppenstedtii DSM 44385 strain of 100%). The isolate was sensitive to gentamicin, vancomycin, and linezolid (Table). Based on the findings of a previous study (2), doxycycline (100 mg BID) was administered, although it was discontinued shortly thereafter due to the development of gastrointestinal side effects. Levoﬂoxacin was then administered for eight weeks as a second-line drug, and the patient’s symptoms gradually resolved. The drain was removed, and no relapse occurred during an observation period of two years. After withdrawing the dose of sulpiride, the serum prolactin level normalized.

**Case 2**

A 34-year-old nulliparous woman presented to our hospital with redness, pain and swelling of the right breast in December 2013. She had begun to experience an uncomfortable sensation in her right breast two weeks before the consultation. She had a history of schizophrenia and was currently taking ﬂuphenazine, zolpidem and duloxetine. She was not allergic to any medications and did not smoke, although she was a social drinker. She was nulliparous and not pregnant. The findings of a physical examination revealed redness and swelling of the right breast (Fig. 2), with a serum prolactin level of 40.5 ng/mL. Mammary ultrasonography showed a right-sided external periareolar dendritically expanded mass. Incision and drainage were performed, and the pus was sent to our laboratory. Gram staining of the pus revealed several neutrophils, but no bacteria. However, Corynebacterium spp. grew on 5% sheep blood agar with capneic incubation 72 hours after inoculation. The colony grew at an increased rate when the agar was supplemented with 1% Tween 80. Routine biochemical identiﬁcation of the corynebacterial strain using the API Coryne System identiﬁed the pathogen as C. argentoratense (proﬁle No. 2040104), with a probability of 87.1%, whereas 16S rRNA gene sequencing revealed the causative bacteria to be C. kroppenstedtii (sequence similarity to the C. kroppenstedtii DSM 44385 strain of 99.71%). The isolate was sensitive to ampicillin, imipenem/cilastatin, gentamycin, erythromycin, clindamycin, vancomycin and linezolid (Table). Doxycycline (100 mg BID) was administered for eight weeks, and the discharge of pus gradually decreased. The incision was closed, and no relapse occurred during an observation period of 11 months. After withdrawing the dose of ﬂuphenazine, the serum prolactin level normalized.

**Table. Minimum Inhibitory Concentration of Various Antibiotics on Corynebacterium kroppenstedtii Isolated from Case1 and 2. Interpretation Results Were Based on the Criteria of Clinical and Laboratory Standards Institute (M45-A).**

<table>
<thead>
<tr>
<th>MIC (mg/L) interpretation result</th>
<th>MIC (mg/L) interpretation result</th>
</tr>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Imipenem/Cilastatin</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≥ 8</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≤ 0.25</td>
</tr>
</tbody>
</table>

MIC: minimum inhibitory concentration
S: susceptible
R: resistant
Discussion

We herein reported two cases of granulomatous mastitis caused by *C. kroppenstedtii* infection in nulliparous young women. In both cases, the patients were initially treated for granulomatous mastitis. Granulomatous mastitis, an inflammatory disease of unknown etiology, is an uncommon benign disorder of the breast (1). It is most commonly seen in parous young women, often within a few years of pregnancy. Although systemic glucocorticoid therapy is often used successfully to treat symptomatic patients, partial or total mastectomy has been performed in some refractory cases, as granulomatous mastitis is suspected to be an autoimmune disease (3).

*C. kroppenstedtii* was first isolated from human sputum in 1998 (4). Bernard et al. reported 72 strains of rare or recently described *Corynebacterium* species (5). In their study, four of 72 strains were identified as *C. kroppenstedtii*, and one *C. kroppenstedtii* strain was isolated from a breast abscess.

In 2002, Paviour et al. reported 24 patients with mastitis (2), among whom *Corynebacterium* spp. were isolated from breast tissue, pus or deep wound swabs, with *C. kroppenstedtii* infection being the most common finding. Unlike many other *Corynebacterium* spp., *C. kroppenstedtii* contains tuberculostearic instead of mycolic acid in the cell wall and requires lipids for its growth (4). As seen in our two cases, supplementation with fatty acids increases the growth rate of *C. kroppenstedtii*. This lipophilic characteristic explains why *C. kroppenstedtii* is often found in the lipid-rich mammary glands (6). We suggest that the incubation time be extended if infection with *C. kroppenstedtii* is suspected, as the rate of colony growth is slower without fatty acid supplementation.

In order to identify which *Corynebacterium* spp. was responsible for the infections in our two cases, we first used the API Coryne System; this test identified *C. argentoratense* (profile No. 2100104) as the pathogen in both cases. Sugahara et al. also reported a case in which *C. kroppenstedtii* was isolated from a breast abscess.

Based on these findings, we suggest that the identification was appropriate for Global Health and Medicine (25-114).

**References**