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Corynebacterium Kroppenstedtii Breast Infections: Report of Four Cases

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Abstract: Corynebacterium kroppenstedtii is an immobile, non-sporulated, glucose-fermenting and lipophilic gram-positive bacillus of the skin microbiota. In recent years, numerous isolates of this species have been reported mainly in breast infections, such as abscesses and granulomatous mastitis. We present here four cases of C. kroppenstedtii infections isolated from breast aspiration samples in women. C. kroppenstedtii was identified by conventional methods and mass spectrometry (MALDI-TOF MS). Using the epsilometric method, these isolates showed susceptibility to penicillin, ceftriaxone, minocycline, ciprofloxacin, and vancomycin, and variable susceptibility to clindamycin and trimethoprim sulfamethoxazole. Due to the association of C. kroppenstedtii with mammary infections, the identification at the species level of those corynebacteria isolated from this location is highly advisable in order to reach the final diagnosis and to test the antimicrobial susceptibility in order to apply the appropriate antibiotic treatment.

Key words: corynebacterium kroppenstedtii; breast infections; granulomatous mastitis; lipophilic species

1. Introduction

Corynebacterium kroppenstedtii is a gram-positive, non-spore-forming, nonmotile, glucose-fermenting bacillus that was first identified and characterized in 1998 by Collins et al. [4], who isolated it from the sputum culture of an 82-year-old woman with lung disease. Most species of the genus Corynebacterium have fatty acids called mycolic acids in their cell wall. However, corynebacterium kroppenstedtii lacks them due to the loss of some genes necessary for their biosynthesis [14], which gives it the characteristic of lipophilicity. It is generally found on the skin as part of its usual microbiota. Molecular studies have also reported its location, with greater abundance, in the skin of patients with rosacea [13]. Since Paviour et al. [11] described C. kroppenstedtii in 2002 as the Corynebacterium species most frequently isolated in breast samples from patients with mastitis, numerous isolations of this species have been reported in different breast conditions, such as abscesses and granulomatous mastitis (MG) [9, 12].

The aim of this work was to evaluate the microbiological and clinical aspects of C. kroppenstedtii isolated from human clinical samples during 2019.

2. Clinical Cases

In a one-year period (2019), 4 isolates of C. kroppenstedtii were studied in the Bacteriology Service of the Hospital de Clínicas of the City of Buenos Aires (2 from patients treated at that hospital and another 2 from patients treated in private centers) recovered from samples obtained by breast puncture-aspiration from female patients, aged between 26 and 36 years. These women presented nodular lesions, compatible with an acute inflammatory process (in 2 of the cases) or chronic in one of the breasts, which did not remit despite empirical antibiotic treatment. The 4 cases also required surgical drainage. In 3 of the 4 patients, the breast lesions were diagnosed as abscesses, one of them associated with breastfeeding, while in the fourth patient the pathological diagnosis was granulomatous mastitis (Table 1).

Table 1. Main clinical data of the cases studied

Item	Patient A	Patient B	Patient C	Patient D
Age (years)	26	27	33	36
Pregnancies	G3P3	w/d	G1P1	s/d
Breastfeeding	Yes	No	No	No
Breast prosthesis	No	No	No	No
Reason for admission	Erythematous nodule	Erythematous nodule	Recurrent mastitis	Abscess
Empirical antibiotic treatment	Amoxicillin	AMC	AMC	AMC and TMS
Definitive diagnosis	Abscess	Chronic granulomatous mastitis	Abscess	Abscess
Targeted antibiotic treatment	Clindamycin	Minocycline	Clindamycin then ciprofloxacin	s/d

Note: AMC: amoxicillin-clavulanate; G: pregnancies; P: deliveries; s/d: no data; TMS: trimethoprim/sulfamethoxazole.

The fresh examination of the samples showed the presence of an abundant inflammatory reaction, and in 2 of the 4 cases, gram-positive diphtheroid bacilli were observed in the gram stain. The samples were cultured on Columbia base agar supplemented with 5% blood and on chocolate agar (bioMèrieux), with incubation in an enriched atmosphere with 5% CO_2 at 35°C and on Brucella agar incubated in an anaerobic atmosphere; thioglycollate broth (Britania, Argentina) was used as enrichment medium. The isolates were obtained from the primary culture in the indicated media after 72 hours of incubation, as very small colonies, less than 0.5 mm in diameter, grayish, opaque, with entire edges. The development was favored by the addition of 1% Tween 80 to the culture medium. No development of strict anaerobic bacteria was obtained. Mycobacterial culture and mycological culture were negative. Isolates were identified by conventional methodology according to the Funke and Bernard scheme [7], and by MALDI-TOF MS mass spectrometry (MS) (BrukerDaltonik®, Bremen, Germany). Differential tests between CK and other species of the same genus that hydrolyse esculin are shown in Table 2. The combination of the positive esculin hydrolysis test, the absence of beta-glucuronidase activity, and the lipophilic nature of the microorganism (i.e. the finding that bacterial growth was favoured by the presence of lipids in the culture medium) were key to differentiating C. kroppenstedtii from the other species.

Table 2. Corynebacterium species that hydrolyze esculin

Item	C. kroppenstedtii	C. pyruviciproducens	C. glucuronolyticum	C. durum	C. matruchotii	C. phoceense
Metabolism	F	F	F	F	F	F
Lipophilicity	+	v	v	−	−	−
Beta-glucuronidase	−	+	+	−	−	ND
CAMP	−	v	+	−	−	ND
Urea	−	v	v	v	−	−
FAL	−	+	v	−	−	+

Note: F: fermentative; ALP: alkaline phosphatase; ND: not determined.

Antibiotic susceptibility was determined by the epsilometric method (Etest, bioMèrieux, Solna, Sweden) on Mueller Hinton agar with 5% sheep blood (bioMèrieux), with incubation at 35°C in a 5% CO_2 atmosphere for the following antibiotics: penicillin, ceftriaxone, ciprofloxacin, minocycline, clindamycin, trimethoprim/sulfamethoxazole and vancomycin; the breakpoints established by CLSI for corynebacterium spp. and related coryneform genera were used [3]. The reading was taken after 48 hours of incubation. According to these breakpoints, all isolates were susceptible to penicillin, ceftriaxone, ciprofloxacin, minocycline and vancomycin, while susceptibility to clindamycin and trimethoprim/sulfamethoxazole (TMS) was variable (Table 3).

Table 3. Antibiotic sensitivity of the isolates tested

Antimicrobial drugs	Patient A		Patient B		Patient C		Patient D	
	CIM (µg/ml)	Category[a]	CIM (µg/ml)	Category[a]	CIM (µg/ml)	Category[a]	CIM (µg/ml)	Category[a]
Penicillin	0.125	S	0.064	S	0.19	I	0.25	I
Ceftriaxone	0.25	S	0.064	S	0.5	S	0.5	S
Ciprofloxacin	0.094	S	0.094	S	0.094	S	0.064	S
Clindamycin	0.064	S	> 256	R	0.064	S	> 256	R
Trimethoprim Sulfamethoxazole	0.5	S	8	R	0.19	S	> 32	R
Vancomycin	0.75	S	0.5	S	0.5	S	0.25	S
Minocycline	0.25	S	0.5	S	0.19	S	0.25	S

R: resistant; S: susceptible.
[a]: According to the breakpoints established by the CLSI.

The association of CK with MG was first described in 2002 in Polynesian women, mostly young, with histologically proven granulomatous lobar mastitis. [11] Since then, numerous studies have supported the association of CK with acute and chronic inflammatory or infectious breast processes in women of childbearing age. [6, 9, 10, 11, 15] One of the 4 cases described corresponded to a 27-year-old patient who presented an erythematous plaque on the left breast that had lasted 7 months, which progressed to an abscess despite empirical antibiotic treatment with amoxicillin-clavulanate. The definitive diagnosis based on pathology was chronic granulomatous mastitis. Differential diagnosis of MG with other granulomatous processes such as tuberculosis, Wegener's granulomatosis and sarcoidosis, and even with other conditions such as breast cancer, is often difficult.

An accurate and timely diagnosis of MG together with the isolation and identification of C. kroppenstedtii is of utmost importance, since empirical antibiotic treatment regimens are often ineffective, either because antibiotics that are hydrophilic and poorly distribute in lipid-rich tissues, such as the breast bed, are used [5], or because the strain involved may be resistant to the administered antibiotics [6]. However, targeted antibiotic treatment is often not sufficient and surgical intervention is required for resolution of the condition [5, 11].

Due to the lipophilic nature of C. kroppenstedtii, which has been shown to be present within lipid vacuoles in breast biopsies of patients with MG [5], treatment with antibiotics that have a high volume of distribution and penetrate well into tissues, such as ciprofloxacin, doxycycline, linezolid, clindamycin and TMS [5], would be advisable. It should be noted that 2 of the 4 CK isolates that we present (Table 3) were resistant to these last 2 antibiotics. Fernandez et al. [7] have reported that resistance to macrolides and lincosamides could be due to the presence of the ermX gene. The 2 isolates that presented resistance to erythromycin were also resistant to clindamycin, with a complete absence of inhibition halo around both disks, so it could be inferred that both isolates presented a constitutive MLSb phenotype. According to bibliographic data, resistance to sulfonamides could be attributed to the presence of the sul gene [6].

Several studies have reported that CK isolates are sensitive to most antibiotics. However, resistance to penicillin, imipenem, erythromycin, clindamycin and TMS has been described. [10, 15]

Despite its lipophilic nature, which often makes its isolation and identification difficult, C. kroppenstedtii is the Corynebacterium species that has been most frequently reported in breast conditions such as abscesses and MG. We therefore recommend prolonged incubation of the primary isolation media (blood agar and chocolate agar for at least 7 days and, if diphtheroid gram-positive bacilli are observed in the gram stain, adding a blood agar plate with 0.1-1% Tween 80 to stimulate their development). Other species, including some lipophilic ones such as C. tuberculostearicum and C. accolens, and others that are non-lipophilic such as C. amycolatum, C. striatum and C. minutissium, [12, 15] have also been described in breast infections. Regarding identification by conventional biochemical tests, esculin hydrolysis allowed us to differentiate C. kroppenstedtii from these other Corynebacterium species, none of which hydrolyze esculin [11, 12]. In this context, we emphasize that this test must be supplemented with Tween 80 to visualize the growth and change of the medium in the case of lipophilic species.

Although identification was possible using traditional biochemical tests, MS had a clear advantage over this methodology and allowed rapid identification to be established at the species level. According to the results previously obtained by our working group [2], and those reported by other authors, identification by MS allows the identification of the most commonly isolated Gram-positive bacilli in the microbiology laboratory, and constitutes a rapid, simple and effective alternative to 16S rRNA gene sequencing [1, 2, 5, 14]. In this regard, Barberis et al. have shown that obtaining a score ≥ 1.7 with MALDI-TOF MS is sufficient to identify the Corynebacterium genus and other related genera at the species level [2].

The use of this and other new methodologies, such as direct detection in clinical samples by PCR amplification and then sequencing of a region of 16S rRNA [8], have probably influenced the increase in cases of infections by this species published in the literature [14] in recent years.

Due to the association of C. kroppenstedtii with MG and the formation of breast abscesses, we consider that microbiologists should be on the alert for breast tissue or puncture samples in which gram-positive bacilli are observed in the gram stain, or even those with an inflammatory reaction and negative direct examination, and should proceed to incubate the primary isolation media for up to 7 days and, ideally, supplement them with Tween 80 to promote the development of this microorganism, given its lipophilic nature. In this way, it is possible to approach the definitive diagnosis and then test the sensitivity to antimicrobials, in order to establish the most appropriate antibiotic treatment.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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