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Case report

Acute cholangitis due to *Micrococcus lylae*: First case report

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ABSTRACT

Micrococcus lylae, a Gram-positive bacterium of the Micrococcaceae family, is considered an opportunistic microorganism with only a few reported cases of infection. In this report, we present a case of cholangitis caused by *Micrococcus lylae* in a 69-year-old woman with a medical history of type 2 diabetes and a cholecystectomy performed a decade ago. She was admitted to the gastroenterology department with symptoms indicative of acute cholangitis. Abdominal computed tomography and endoscopic ultrasound showed a consistent and symmetrical dilatation and thickening of the main bile duct, containing micro stones and a macro stone in the cystic duct stump. The patient received empirical antibiotic therapy based on ceftriaxone and metronidazole. She underwent ERCP with biliary endoscopic sphincterotomy and marginal biopsy, followed by balloon-assisted manipulation to facilitate bile release and collection of an intraoperative bile fluid sample for microbiological examination to identify the pathogen and guide the treatment adjustments. The microbiological examination demonstrated the exclusive presence of *Micrococcus lylae*. The patient's condition notably improved, marked by the normalization of inflammatory indicators. After three days, the patient was discharged in a stable condition, continuing the antibiotic regimen with the oral administration of ciprofloxacin and metronidazole. Jaundice resolved after one week, and liver function tests were completely normalized on follow-up at one month.

Introduction

Micrococcus lylae, described by Kloos et al. in 1974, is a Gram-positive bacterium classified within the family Micrococcaceae. They are typical inhabitants of freshwater and soil environments and are commonly found on both human and animal skin. While being considered an opportunistic microorganism, documented cases of infection involving *M. lylae* remain scarce, limited to a small number of case reports [1,2]. To the best of our knowledge, there are no documented cases in the literature of cholangitis attributed to *M. lylae*. Herein, we present a rare case of cholangitis caused by *M. lylae*.

Case report

A 69-year-old woman with a medical history of type 2 diabetes and a cholecystectomy by laparotomy for acute cholecystitis a decade ago, was admitted to the gastroenterology department with obstructive jaundice and biliary colic.

One month before admission, the patient experienced sudden, sharp pain in the right upper quadrant that radiates to the right shoulder, associated with postprandial vomiting and anorexia. Three days before her admission, her condition deteriorated noticeably, marked by the appearance of cholestatic jaundice with pruritus. Throughout this progression, she maintained a consistent absence of fever, but exhibited an overall decline in her general well-being.

Upon admission, a general examination revealed high blood pressure. The patient was overweight, with a body mass index (BMI) of 27.2 kg/m2. Frank Jaundice was observed with pain in the right hypochondriac region on abdominal examination. No abnormalities were identified following the clinical examination.

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The complete blood count (CBC) upon admission revealed signs of neutrophilic leukocytosis, indicating an infection, with a white blood cell count of 14,770 WBC/ mL, of which 11,730 WBC/ mL were neutrophils. Additionally, the C-reactive protein (CRP) level was elevated, measuring at 124 mg/L. Renal tests indicated normal function, and electrolyte levels were within the normal range, Albumin was at 37 g/L. However, liver function tests showed disruptions, evident in a total bilirubin level of 28.8 mg/dl (direct bilirubin at 19 mg/dL). Cholestasis with Gama Glutamyl Transferase (GGT) at 685 IU/L (i.e., 19 times normal) and alkaline phosphatase (ALP) at 201 IU/L (i.e., 2 times normal). Serum aminotransferase levels were 46 IU/L for AST and 201 IU/L (i.e., 4 times normal) for ALT. Notably, the prothrombin time (PT) and international normalized ratio (INR) test values were within normal limits, and viral serologies were negative.

Following the suspected diagnosis of cholangitis, the patient received empiric intravenous antibiotic therapy based on ceftriaxone (2 g/24 h) and metronidazole (500 mg/8 h). Additionally, a regimen of 10 mg/24 h of vitamin K was administered alongside a calcium channel blocker to manage her hypertension, complemented by rehydration measures.

An abdominal CT scan revealed dilatation of the intrahepatic bile ducts and the common bile duct measuring 12 mm. Cystic duct stump lithiasis was also found [Fig. 1].

Endoscopic ultrasound (EUS) revealed regular and symmetrical thickening of the common bile duct measuring 7 mm and containing micro-calculi, as well as a remnant calculus of the cystic duct stump measuring 15 mm in its longest diameter [Fig. 2].

After the latest investigations, Endoscopic retrograde cholangiopancreatography (ERCP) was performed, showing a dilatation of the common bile duct (CBD) measuring 14 mm upstream of lower bile duct stones [Fig. 3]. The cystic duct was low inserted with a big stone in the cystic duct stump. Consequently, common bile duct (CBD) stone extraction was performed by endoscopic sphincterotomy, followed by large balloon dilation (ESLBD). A bulging major duodenal papilla was endoscopically evident [Fig. 4], requiring a sphincterotomy margin biopsy with benign results. A bile aspiration sample was collected for microbiological examination to accurately identify the organism, thereby allowing personalized adjustments to the treatment regimen.

Culture on blood agar was performed, and the plate was incubated at 37 °C for 24 h. Simultaneously, an enrichment broth in an aerobic bottle (BD Aerobic, BACTEC; Becton Dickinson Microbiology Systems), described as an all-purpose fortified soy and casein digestion broth capable of supporting the growth of aerobic organisms and neutralizing

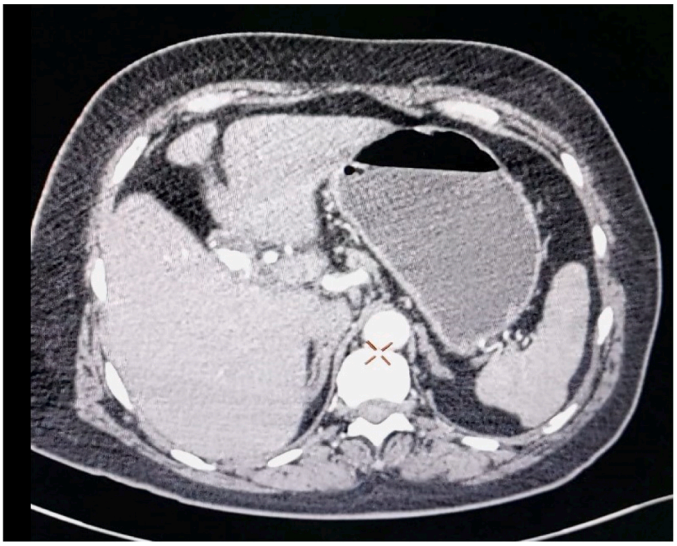

Fig. 1. CT scan image showing bile duct dilatation with lithiasis.

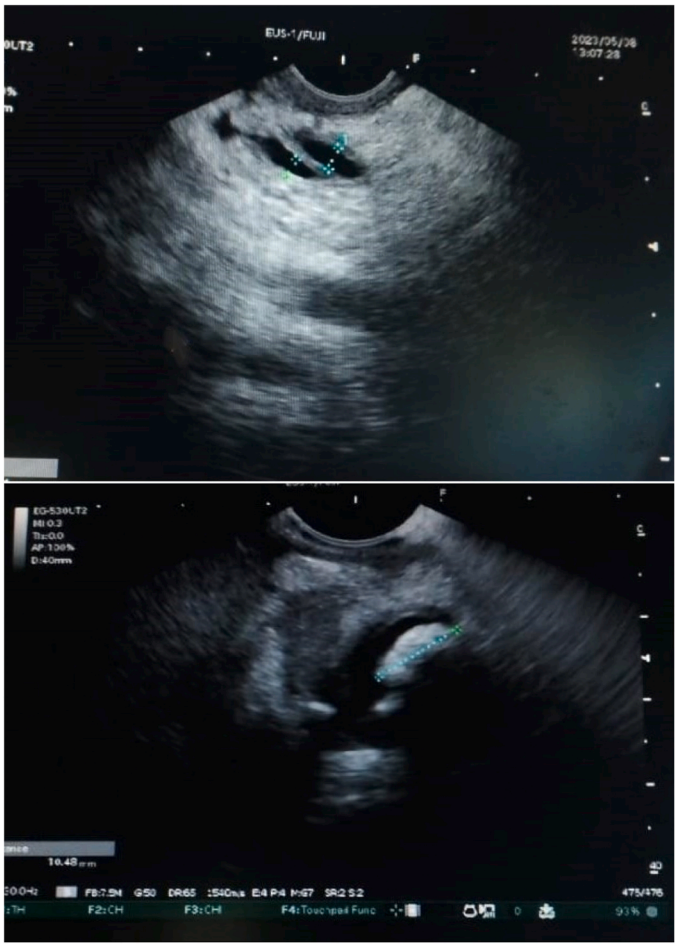

Fig. 2. Endoscopic findings showing common bile duct stones and cystic duct stump calculus.

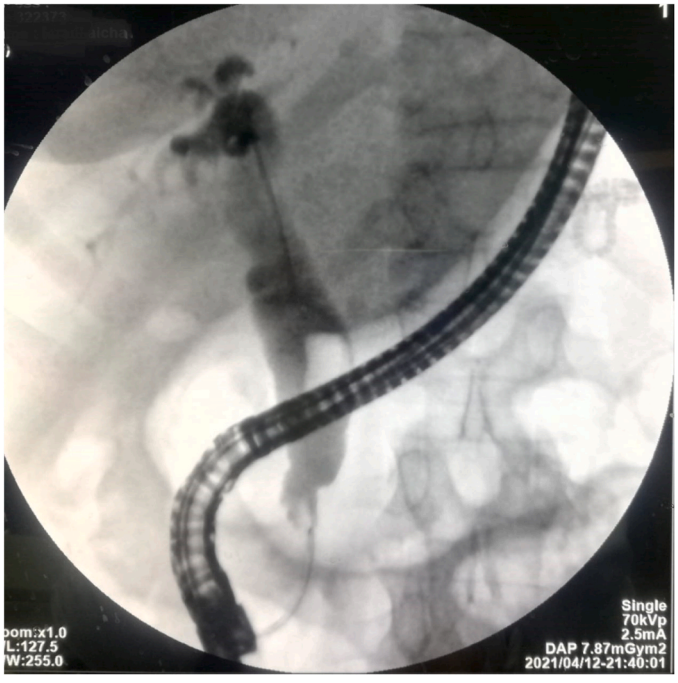

Fig. 3. ERCP findings showing common bile duct (CBD) dilatation with CBD stones.

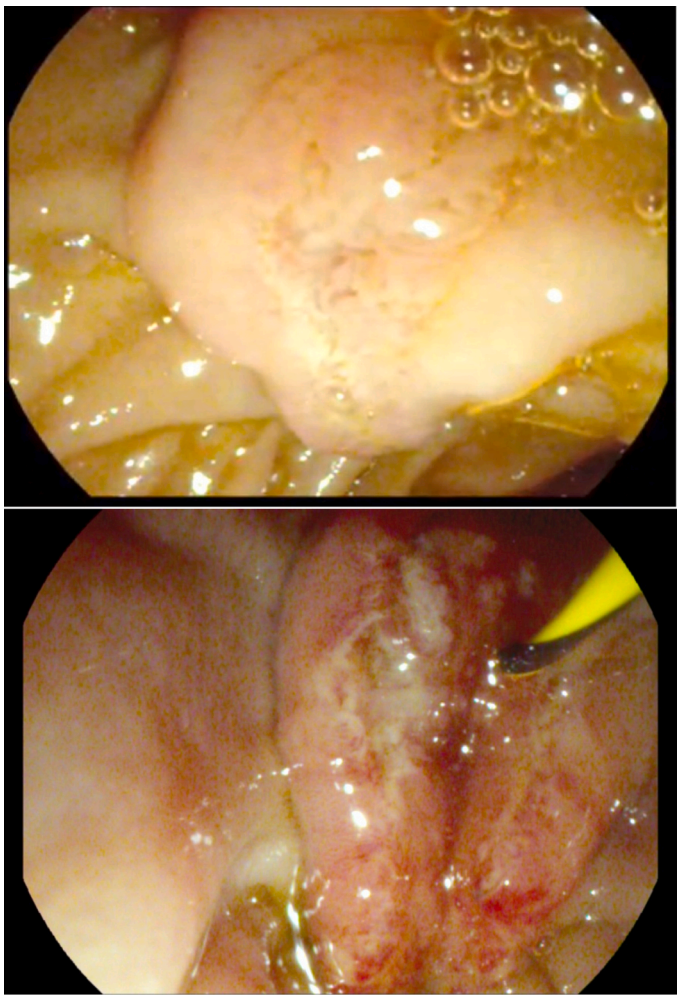

Fig. 4. Endoscopic findings of a bulging major duodenal papilla suspicious for intra-ampullary papillary malignancy.

antibiotics using resins, and on an anaerobic bottle (BD Anaerobic, BACTEC; Becton Dickinson Microbiology Systems), designed to increase the detection and recovery of anaerobes by also using a detergent to lyse red and white blood cells present in the sample to release any intracellular organisms, were incubated for 15 days in the BD BACTEC™ FX-400 automated blood culture instrument.

The organism exhibits strict aerobic growth. After 5 days of incubation, growth was detected in the aerobic broth bottle using BD BACTEC™ FX-400, while the anaerobic broth bottle showed no growth for 15 days. Microscopic examination of the positive enrichment in the aerobic broth revealed gram- positive cocci arranged in tetrads and irregular clusters [Fig. 5 (B)]. Subsequent subculture on blood agar, incubated for 24 h at 37 °C, resulted in the isolation of creamy white convex circular colonies [Fig. 5 (A)] that tested positive for catalase and oxidase.

The obtained isolate, identified using the BD Phoenix™ PID Panel, revealed the exclusive presence of *Micrococcus lylae* with 99 % confidence in an inoculum of 0.5 McFarland density [Table 1]; no other

Table 1
Biochemical properties of the Micrococcus strains tested using BD phoenix PID Panels.

Biochemical reaction		Instrumental results
4MU-BD-CELLOBIOSIDE	**M_BDCEL**	-
L-ALANINE-AMC	**A_LALT**	-
4MU-BD-GLUCOSIDE	**M_BDGLU**	-
L-PROLINE-AMC	**A_LPROB**	+
L-PYROGLUTAMIC ACID-AMC	**A_LPYR**	-
L-PHENYLALANINE-AMC	**A_LPHET**	+
L-TRYPTOPHAN-AMC	**A_LTRY**	+
4MU-PHOSPHATE	**M_PHOS**	+
METHIONINE-AMC	**A_META**	+
4MU-AD-GLUCOSIDE	**M_ADGLU**	-
ARGININE-ARGININE-AMC	**A_ARARR**	+
GLYCINE-PROLINE-AMC	**A_GLPRB**	-
4MU-BD-GLUCURONIDE	**M_BDGLC**	-
L-LEUCINE-AMC	**A_LLEUH**	+
4MU-N-ACETYL-BDGLUCOSAMINIDE	**M_NAG**	-
L-ARGININE-AMC	**A_LARGH**	+
4MU-PHOSPHATE (with Trehalose)	**M_PHOT**	+
L-HISTIDINE-AMC	**A_LHIST**	-
L-ISOLEUCINE-AMC	**A_LISO**	-
4MU-BD-GALACTOSIDE	**M_BDGAL**	-

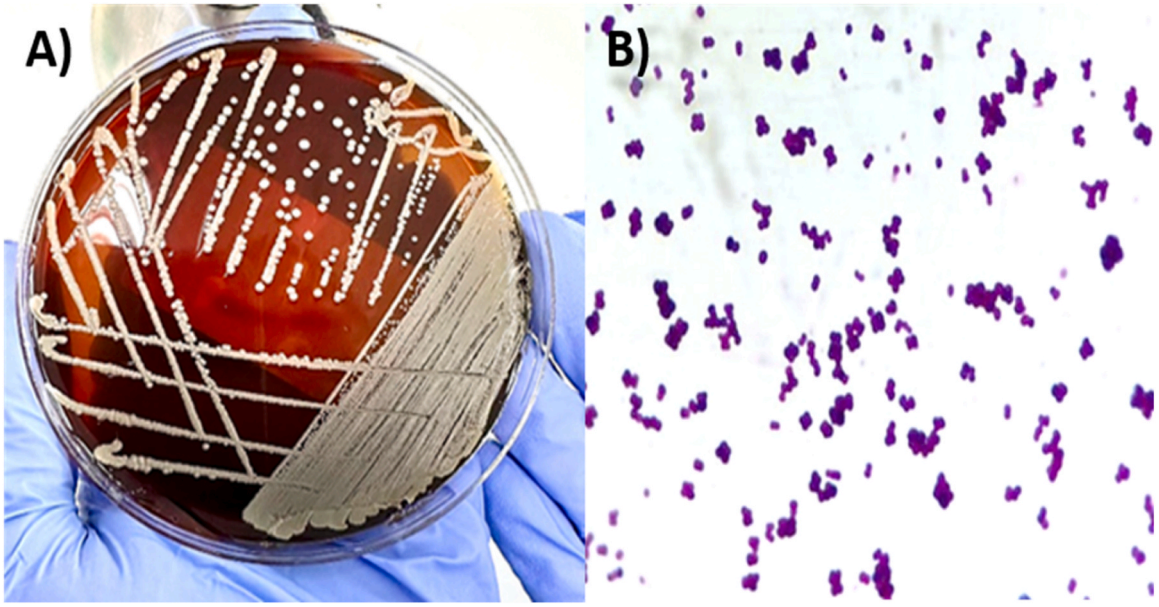

Fig. 5. Morphology of *Micrococcus lylae* on Blood agar culture (A) and in Gram staining under the microscope (B).

bacterial pathogens were isolated from the bile sample.

As for antimicrobial susceptibility testing, there are no established standardized methods validated specifically for *Micrococcus lylae* [3,4]. Consequently, we used the breakpoints established for *staphylococcus*, following the guidelines outlined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5]. Sensitivity diffusion assays were conducted on Mueller-Hinton agar plates, assessing inhibition diameters in line with staphylococcal antibiogram criteria.

The results of the susceptibility test revealed that the strain exhibited sensitivity to antibiotics in several classes: Beta-lactam (including penicillin G, ampicillin, and cefoxitin), macrolide (erythromycin), lincosamide (clindamycin), tetracycline (tetracyclin), fusidane (fucidin acid), streptogramin (pristinamycin), rifamycin (rifampicin), sulfonamide (trimethoprim/Sulfamethoxazole), and oxazolidinone (linezolid). Additionally, it showed sensitivity to higher doses of some quinolone (levofloxacin and ciprofloxacin) while demonstrating resistance to other quinolone (norfloxacin) and aminoglycoside (gentamicin).

A significant clinical improvement was noted, as well as a normalization of inflammatory indicators, as evidenced by a reduction in CRP levels to 3.31 mg/L. Therefore, the patient was discharged, continuing oral administration of the antibiotics: ciprofloxacin (750 mg/12 h) and metronidazole (500 mg/8 h) for seven days.

Weekly follow-up showed a disappearance of cholestatic jaundice and pruritus, while liver function tests were completely normalized on follow-up at one month. No complications were revealed during the eight-month follow-up.

Discussion

The genus *Micrococcus*, first described by Cohn in 1872, belongs to the Micrococcaceae family [6]. These bacteria are ubiquitous gram-positive organisms, strictly aerobic, non-sporulating, and possess catalase and cytochrome oxidase. They are rarely motile, appearing as spherical cocci measuring 0.5 to.

2.0 μm, usually found arranged in tetrads but occasionally seen in pairs or irregular clusters [7]. After approximately 4 days of incubation, colonies of this bacterium develop a distinctive appearance, notably more convex compared to staphylococcal colonies. Moreover, they exhibit a spectrum of pigmentation, ranging from pale yellow, cream white, pink, to pale orange [7].

In our case, we were able to identify strictly aerobic gram-positive cocci, arranged mostly in tetrads but also in irregular clusters. When incubated on blood agar culture at 37° for 24 h, we were able to obtain creamy white colonies that were circular, convex, and that tested positive for both catalase and oxidase.

They commonly inhabit fresh and marine water, sand, vegetation, and soil. They are frequently found on animal and human skin, serving as the main reservoir of micrococcal strains. Therefore, contamination of animal-derived foods is still a potential concern [8]. *Micrococcus lylae* does not take part in the intestinal microbiota [9].

The description of Micrococcus has undergone multiple modifications over time [6,8]. At the time of writing, a total of 10 *Micrococcus* species validly published and with correct names are listed in the LPSN database [10].

The Micrococcus genus is not considered pathogenic. However, Micrococcus species could adhere to medical devices and cause associated infections, particularly catheter-related blood stream infections in immunocompromised patients [11]. Different cases of infection due to Micrococcus strains have been reported and considered opportunistic [12]. Three cases of *Micrococcus spp.*- related peritonitis were documented in patients receiving peritoneal dialysis [13]. Several cases have reported Micrococcus luteus as the causative agent in cases of infective endocarditis, recurrent bacteremia, septic arthritis, and a rare case of brain abscess [12,14]. Six cases of endocarditis associated with *Micrococcus lylae* were reported in 1980 [15]. Moreover, this bacterium was found to be involved in a case of endocarditis on aortic prostheses [1]. The first case report of *Micrococcus lylae* isolation from a urinary catheter was reported in 2022 [2]. For all of that, some authors highlight the fact that *Micrococcus spp.* should no longer be considered a contaminant but rather an emerging pathogen requiring appropriate treatment [12]. In our report, we present the first documented case of cholangitis associated with *Micrococcus lylae*.

Antibiotic susceptibility data for *Micrococcus* spp. are limited. Clinical reports from the literature indicate the sensitivity of most strains to various antibiotics [16]. However, cases of resistance to penicillin and erythromycin have been documented [17,18]. Some strains require high minimum inhibitory concentrations for penicillin, erythromycin, fosfomycin, and aminoglycosides [16]. In a case of endocarditis on an aortic protheses, the antibiogram of the identified *Micrococcus lylae* strain revealed resistance to B-lactams while being sensitive to aminoglycosides, macrolides, tetracycline, rifampicin, chloramphenicol, sulfonamides, and trimethoprim/sulfamethoxazole [1]. Antimicrobial susceptibility tests of *Micrococcus lylae* stain isolated from a urinary catheter revealed susceptibility to aminoglycosides (streptomycin, erythromycin, neomycin, and kanamycin), cephalosporine (cefotaxime), glycopeptide (vancomycin), macrolide (azithromycin), chloramphenicol, and tetracycline, while showing resistance to aminoglycosides (gentamicin) and penicillin (oxacillin) [2]. In our case, the identified strain of *Micrococcus lylae* showed overall sensitivity to most antibiotics, with sensitivity to penicillin, contrasting with the resistance observed in another case [2]. Additionally, our strain, like a documented case [2], showed resistance to gentamicin, while another documented case showed sensitivity [1].

Some organisms are never or rarely found to be resistant to some antimicrobial agents. Therefore, when susceptibility to such an agent is tested, any result other than "susceptible" cannot be defined. If the organism's identification or the antimicrobial susceptibility test results suggest otherwise, they should be confirmed [3]. Regarding staphylococcus, quinolones are tested and reported to clinicians' requests when other classes are not optimal for use due to numerous factors [4]. Furthermore, it is noted that *Staphylococcus spp.* may develop resistance during prolonged therapy using quinolones [4]. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) The diameter of the inhibition zone of ciprofloxacin 5 μg and levofloxacin 5 μg disks should be equal to or greater than 50 mm to be defined as "susceptible." [5]. For all of that, given that we used the breakpoints established for Staphylococcus in our case to define antibiotic susceptibility, it is legitimate to wonder if this affected our results, especially since our strain was sensitive to higher doses of levofloxacin and ciprofloxacin while demonstrating resistance to Norfloxacin.

Micrococcus lylae is not normally found in the bile ducts or duodenum, and its exact source in our patient remained undetermined. There is one consistent predisposing factor for infection associated with *Micrococcus spp.*, and that is an immunocompromised state [18], which is the case with our patient, who is diagnosed with type 2 diabetes. The identification of the agent of cause is primordial for the management of acute biliary infections. When cholangitis is suspected, the common bile duct should be sent for microbiological examination in all cases [19]. According to numerous studies, the main pathogens associated with cholangitis are *Escherichia coli*, primarily, followed by *Klebsiella* spp., originating from the intestinal flora and causing infection through an endogenous mechanism [20,21]. Other involved pathogens have also been reported including *Enterobacter*, *Citrobacter*, *Enterococcus* spp., *Pseudomonas* spp., and *Clostridium* spp [22,23]. Regarding treatment, patients should be managed according to the Tokyo 2018 revised international Guidelines [19]. Considering our patient, initially she received empiric parenteral antibiotics using ceftriaxone and metronidazole during her hospitalization, and later, when discharged, was switched to oral ciprofloxacin and metronidazole. After obtaining culture results the patient remained on the same oral treatment.

Recently, there has been an emergence of antimicrobial resistance

among clinical isolates, leading to cases of presumedly less effective antibiotic therapy. Early biliary culture associated with continuous monitoring and updating of local antibiograms has its place in providing effective and well-targeted therapy [19].

Conclusion

Micrococcus Lylae was isolated in a bile aspiration sample collected during an endoscopic retrograde cholangiopancreatography in a 69-year-old female patient admitted to the gastroenterology department for cholestatic jaundice and pruritis. To the best of our knowledge, it is the first case of cholangitis due to *Micrococcus lylae*. Micrococcus are considered non-pathogenic, and only occasional cases of infection due to certain strains have been reported. Our case highlights the emerging significance of *Micrococcus lylae* as a potential pathogen, prompting further consideration and increased vigilance in the clinical setting.

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Ethical approval

Not applicable, case report.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Author contribution

All authors contributed to the work described in this report. OA, BA, CB, KG, YS, IA, HZ, SB, conducted the laboratory analyses, collected the clinical data and wrote the initial draft of the manuscript. OA, AS, SE and AM performed a review of the literature, AM, ZI, EB, YBL, ME, SE and AS supervised and edited the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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