Kluyvera ascorbata infection in Cystic Fibrosis airway disease

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Infezione da *Kluyvera ascorbata* in Fibrosi Cistica

SUMMARY

Bacilli belonged to genus *Kluyvera* are Gram negative non-fermentative microorganisms described in the *Enterobacteriaceae* family, generally considered as saprophytes of the digestive tract. Their role in pathogenesis of infectious disease is unclear, but recently, they are emerged as a cause of clinically significant disease in no-CF-population and several anatomic sites are involved as urinary, respiratory and gastrointestinal tracts.

In the present note, we describe the identification of *Kluyvera ascorbata* isolates, obtained from sputum samples of one CF patient (male, 10 years old). The note concerns a patient suffering from chronic obstructive bronchopulmonary disease and chronic *Pseudomonas aeruginosa* infection. Isolates showed a good growth on MacConkey agar and BCSA, after incubation at 37°C for 24-48 h and they were sensitive to several antibiotics, as cephalosporins, carbapenems, quinolones and trimethoprim-sulfamethoxazole. Clinical observation and the spirometric parameters early before and after isolation of microrganism did not show any worsening status in our patient.

The selective pressure given by intensive use of antibiotics in CF patients increases susceptibility to infections by opportunistic microrganisms. Our report underlines the importance of careful bacterial identification and antimicrobial susceptibility study in defining the potential role of new pathogen bacteria in CF lung and to address possible therapeutic strategies that may help to guide antibiotic therapy regimes in CF patients.

INTRODUCTION

Bacteria belonged to genus *Kluyvera* were described in 1936 by Kluyver and van Niel (5). Successively, in 1956 Asai proposed the genus *Kluyvera* for a group of flagellated bacteria produced α-ketoglutaric acid by glucose fermentation (1). Currently, the genus consists of four species, *Kluyvera ascorbata*, *Kluyvera cryocrescens*, *Kluyvera georgiana* (formerly group 3) and *Kluyvera cochleae*. Paricularly, the name "*ascorbata*" derives from the positivity to ascorbate test (4, 7).

All *Kluyvera* species can be recovered in the soil and water environments and also have been described as normal bacterial component of the gastrointestinal tract. All these species, except *K. cochleae*, have been recovered from human clinical specimens, but, because of the small number of reported clinical infections, the pathogenic role of these bacteria is uncertain. In spite of few data, actually they can be considered a cause of infection in several anatomic sites, such as urinary, respiratory and gastrointestinal tracts (8).

These bacteria are Gram negative non-fermentative rods described in the *Enterobacteriaceae* family. They are motile for peritrichous flagella, catalase-positive, oxidase-negative and indole- positive

The purpose of the present note is to describe the identification of *Kluyvera ascorbata* isolates obtained from a unique CF patient, to analyze the antibiotic-resistance pattern, and to give some information about clinical outcome of this patient.

PATIENT AND METHODS

Patient M.A. (ten years old) is regularly attended the Regional Referral CF Centre of Naples. In our Centre, patients over 6 years of age in regular follow-up have at least one lung function evaluation as measured by forced expiratory volume in 1 sec (FEV1) during each year of observation at clinic visit and/or hospital discharge expressed of a mean value of three years survey; oxygen saturation measurement is noted for each visit. Regular measure of weight, height, use of pancreatic enzymes, number of CF related hospital admissions, number of courses of intravenous

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antibiotics are obtained. Genetic analysis is detected for every patient. Sputum or bronchoaspirate swabs are obtained for each patient at regular clinical attendances (patients are reviewed on an average 3 months), as well as twice during hospital admission. Chronic bacterial infection is defined as persistent presence of Gram-negative bacteria according to modified Leeds criteria as > 50% of the sputum samples positive, collected during the last 12 months (at least 4 sputum samples during this period) (6).

Co-infection is defined as the sputum culture positive for more than one microorganism.

Isolates are considered multi-drug resistant according with the definition given by CDC of 2006 (2).

Processing of sputum samples, culture of microorganisms and phenotypic analysis

Natural or induced sputum samples are mixed with equal volumes of 1% dithiothreitol (Merck, Germany) before incubation at 37°C for 30 min. All specimens are examined microscopically and cultured. All isolates obtained are identified by the Phoenix system; the API 20NE identification system (bioMérieux, Marcy l'Etoile, France) was used when the identification with the Phoenix system was inaccurate. Preparation of suspensions, inoculations, incubation times, temperatures and interpretation of reactions are processed according with the manufacturer's instructions for each system.

Antimicrobial susceptibility testing method

To determine sensitivity to antimicrobial agents, a microbroth dilution assay using an automated Phoenix microdilution system was utilized. This system is based on serial dilutions of antibiotics and the MIC value is obtained from interpretative criteria for susceptibility breakpoints. Criteria adopted were according to the National Committee for Clinical Laboratory Standards (CLSI) Interpretative Criteria, document M100-S16 (3).

RESULTS AND DISCUSSION

K. ascorbata was isolated from M.A. on January 2010. After three months, *K. ascorbata* was reisolated from sputum.

Patient, a male diagnosed at 5 months of age for suggestive symptoms and sweat chloride test > 60 mm/l, was chronically infected by *Pseudomonas aeruginosa* since two years of age. On the basis of the definition applied in this study, *K. ascorbata* was not considered multi-drug resistant.

It showed resistance to amoxicillin-clavulanic acid (MIC>16/8 µg/mL), ampicillin (MIC>16 µg/mL), cephazoline (MIC>16 µg/mL) and cephoxitin (MIC>16 µg/mL). Molecules as aztreonam, cefepime, ceftazidime, cefotaxime, carbapenems, aminoglycosides, quinolones, trimethoprim-sulfamethoxazole, piperacillin and piperacillin/tazobactam were active against this microorganism. *K. ascorbata* isolates showed a good growth on MacConkey agar and BCSA, after incubation at 37°C for 24-48 h.

Clinical observation and the spirometric parameters early before and after isolation of microrganism did not show any worsening status in our patient.

The selective pressure given by intensive use of antibiotics in CF patients may increase susceptibility to infections by opportunistic microrganisms. At now, no risk factors have been hypothesized as inducing colonization of *K. ascorbata* in our patient. However, the impact of early aggressive antibiotic eradication therapy for chronic colonization by *P. aeruginosa* on detection of this new pathogen could be hypothesized in this subject. Nevertheless, our report underlines the importance of careful bacterial identification and antimicrobial susceptibility study in defining the potential role of new pathogen bacteria in CF lung and to address possible therapeutic strategies that may help to guide antibiotic therapy regimes in CF patients.

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