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BRIEF REPORTS

A *KLUYVERA CRYOCRESCENS* STRAIN FROM A GALL-BLADDER INFECTION

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The isolation and the identification of a pure-culture *Kluyvera cryocrescens* strain in a gall-bladder pus specimen from a 76-year-old woman with acute cholecystitis is described. This is the first reported recovery of a *K. cryocrescens* strain from such a sample.

The genus *Kluyvera*, of the family *Enterobacteriaceae*, has had a turbulent history until 1981, when Farmer et al. (6) redefined its taxonomy by including in the genus two species, *K. ascorbata* and *K. cryocrescens*, and a group of bacteria named « *Kluyvera* species 3 ».

The redefinition of the genus facilitated recognition of the strains, and reports of its isolation began to appear. At the moment, we know that *Kluyvera* strains are encountered, though infrequently, in clinical specimens from upper respiratory tract (mixed cultures) and in urine, blood, and stool samples (1, 4, 5).

This paper describes the isolation and identification of a *Kluyvera cryocrescens* strain from a sample of pus collected from the gall-bladder of a 76-year-old female, with a 15-year history of cholic, who had the sudden onset of an acute empyematous cholecystitis. There is only one report in the literature (3) of *Kluyvera* (*K. ascorbata*, which is more frequent than *K. cryocrescens* in clinical specimens) playing an etiological role in biliary tree infection.

The patient was admitted to the Ospedale Regionale Zonale D. Parodi Colleferro, Rome, on May, 27, 1986 with a diagnosis of acute hepatic cholic. Physical examination showed that temperature was 39.5°C. She complained of epigastric and periscapular pain, which radiated to the right and left upper quadrants. Laboratory findings included an hemoglobin level of 13.8 g%; 4,500,000 red cells; a leukocyte count of 5,700 (73% neutrophiles and 27% lymphocytes), Aspartate aminotransferase- and Alanine-aminotransferase- values of 39 U/l and 45 U/l, respectively (normal range 0-26); and a glutamyl-transpeptidase value of 52 U/l (normal range 5-30).

Microscopic examination of the urine indicated an high number of leukocytes and a few red cells; no bacterial growth was recorded from urine specimen. The patient was started on mezlocillin (1g Q 8 h) and, on hospital day 7, underwent a cholecystectomy; during which a pus sample was aseptically collected for culture. The surgical diagnosis was acute calculous empyematous cholecystitis, with a surrounding zone of pericholecystitis.

Microscopic and cultural microbiological analysis revealed the presence of a gram negative, motile, aerobic bacterium, which grew on Mac Conkey Agar and had a fermentative metabolic pathway of glucose, reduced nitrates to nitrite and lacked cytochrome-oxidase. The isolate was in pure culture and seemed to belong to the *Entero-*

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bacteriaceae family. The susceptibility pattern, obtained by means of the MS2 automated system (Abbott Laboratories, North Chicago, It., U.S.A.), was recorded as shown in Table 1. On hospital day 8 (4/6/86) the antibiotic was changed to gentamicin (80 mg Q 12 h) as symptoms of intolerance (i.e. pruritus) had appeared. The patient had an uneventful recovery and was discharged from the hospital 2 weeks after surgery.

As the isolate was not identifiable by the MS2 Abbott commercial computerized system available in the hospital, it was submitted for identification to the Microbiology Institute of the University « La Sapienza » in Rome. The isolate, maintained in Nutrient Agar (Difco Laboratories, Detroit, Mich., U.S.A.), was streaked on Mac Conkey agar (Difco) and after 24h of incubation at 37°C, a colony was inoculated into Api 20E and Api 50CH (Api System, S.A., La Balme Les Grottes-Montalieu Vercieu, France) strips following the manufacturer's instructions. The strips were read according to the guidelines of manufacturer and the results were recorded (Table 2). The Api 20E numerical profile (1344153) was not mentioned in the Api 20E analytical catalogue. A statistical analysis of the results was therefore performed, as suggested by Lapage et al. (7), using as a data base the positivity percentages reported by J.J. Farmer III et al. (5). The identification likelihood (%id.) was equal to 99.72% for the species *Kluyvera cryocrescens*. As the results for the unknown strain were not obtained with the same testing procedures used by Farmer and co-workers (5), and the mathematical identification was, therefore, less reliable, we performed the glucose fermentation test at 4°C (6), the ascorbate fermentation test (6), and the irgasan susceptibility test (2) to confirm the strain identification. The isolate was able to acidify the glucose broth at 4°C within 72 hours, but not to ferment ascorbate, and it was highly sensitive to irgasan (M.I.C. <0.25 mcg/ml). It was, therefore, confirmed that the organism belonged to the species *Kluyvera cryocrescens*.

We hope this report will stimulate others to isolate and identify strains of *Kluyvera* so that their ecological niches and role in human disease can be better defined.

TABLE 1.
Antibiotic susceptibility of the isolated strain.

Antibiotic	M.I.C. (mcg/ml)
Amikacin	< 8 (S)
Amoxicillin	> 24 (R)
Cefoxitin	< 6 (S)
Cephalothin	> 30 (R)
Chloramphenicol	< 9 (S)
Gentamicin	< 4 (S)
Sulfamethoxazole-trimethoprim	> 75 (R)
Tetracycline	< 5 (S)
Tobramycin	< 4 (S)
Cefuroxime	> 18 (R)
Cefamandole	< 9 (S)
Cefotaxime	< 14 (S)
Moxalactam	< 16 (S)
Mezlocillin	< 30 (S)
Piperacillin	< 30 (S)
Ceftriaxone	< 16 (S)
Ceftazidime	< 10 (S)

S = sensitive; R = resistant.

TABLE 2.
Results of the biochemical tests of the isolated strain employing Api 20E and Api 50 CH.

Tests yielding Positive results (a)	Tests yielding Negative results
ONPG (b)	Arginine dihydrolase
Ornithine decarboxylase	Lysine decarboxylase
Simmon's citrate	H_2S production
Indole production	Urea hydrolysis
fermentation of:	Triptophane deaminase
Glucose	Voges-Proskauer test
Lactose	Gelatin hydrolysis
Rhamnose	fermentation of:
Mannitol	Sorbitol
Sucrose (48 h)	Glycerol
Melibiose	Erythritol
Amygdalin	D-Arabinose
L-Arabinose	L-Xylose
Ribose	Adonitol
D-Xylose	β-methyl-xyloside
Galactose	L-Sorbose
D-Fructose	Dulcitol
D-Mannose	myo-Inositol
alpha-methyl-D-glucoside	alpha-methyl-D-mannoside
N-acetyl-glucosamine	Inuline
Arbutin	Melezitose
Esculin	Starch
Salicin	Glycogen
Cellobiose	Xylitol
Maltose	D-Turanose
Trehalose	D-Lyxose
D-Raffinose	D-Tagatose
β-Gentiobiose	D-Fucose
L-Fucose	D-Arabitol
Gluconate	L-Arabitol
2-Keto-gluconate	
5-Keto-gluconate	

(a) The tests were positive within 24 h when not otherwise indicated.

(b) o-nitro-phenyl-β-galactopyranoside.

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