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Isolation of *Escherichia fergusonii* from Four Different Sites in a Patient with Pancreatic Carcinoma and Cholangiosepsis

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***Escherichia fergusonii* was isolated from a 69-year-old male with pancreatic carcinoma and cholangiosepsis from gallbladder fluid, three blood cultures, feces, and a superficial wound of the abdomen. Biochemical reactions, antimicrobial susceptibility patterns, susceptibility to polyvalent phage 0-1, and rRNA gene restriction analysis suggested that the four strains were of clonal origin. Our data indicate that *E. fergusonii* possesses a pathogenic potential in humans.**

In 1985, Farmer et al. (3) described a new species of the family *Enterobacteriaceae*, formerly known as CDC enteric group 10, which is now referred to as *Escherichia fergusonii*. The species is phenotypically and genotypically closely related to *Escherichia coli*, but little is known about the natural habitat of the organism. Up to now, only three isolates of *E. fergusonii* derived from blood cultures have been described in the literature (3, 6). Thus, the pathogenic potential of *E. fergusonii* has remained unclear.

We present a case report revealing further evidence for the pathogenic potential of *E. fergusonii* in humans. A 69-year-old urban male with a history of weight loss, jaundice, and acholic stools presented on 13 July 1992 at the Cantonal Hospital of Winterthur with clinical signs of ascending cholangitis. Three blood cultures (BCB Release; Roche Diagnostics, Basel, Switzerland) were taken, a needle aspiration of the gallbladder fluid was performed, and antibiotic treatment was started empirically with amoxicillin-clavulanate (1.2 g intravenously three times daily) and tobramycin (80 mg intravenously once daily). After one day, *E. fergusonii* was grown as a single organism from the gallbladder fluid as well as from all three blood cultures. Because of resistance of *E. fergusonii* isolates to amoxicillin-clavulanate and elevated tobramycin levels in the patient's serum due to underlying impaired renal function, treatment with imipenem (500 mg intravenously twice daily) was initiated, and the patient became afebrile. Imipenem was chosen because of its activity against a broad spectrum of bacteria, including those growing anaerobically. From a stool sample taken on 20 July, *E. fergusonii* was also isolated by screening of lactose-negative colonies on MacConkey agar (2) for the presence of *E. fergusonii*. The patient underwent surgery in early August for a suspected pancreatic carcinoma, which was confirmed by histopathological examination. Even though the patient was still on antimicrobial therapy with imipenem, a postoperative abdominal wound infection developed from which *E. fergusonii* and *Klebsiella pneumoniae* were isolated on 9 September. In the further course of the illness, we were not able to isolate *E. fergusonii* again. The patient's general condition deteriorated, and he died on 11 December 1992 of acute heart failure.

Initial biochemical testing with the API 20E system (API Biomérieux SA, Marcy l'Etoile, France) yielded the numerical code 5144113 for all four isolates. According to the API data base (1), this represented a "very good identification" of *E. fergusonii*. The ability of our strains to ferment adonitol and the lack of yellow pigment made the diagnosis of *Escherichia hermannii*, the second choice for the numerical code, unlikely. With the biochemical reactions specified by Farmer and colleagues (3), further investigation showed no differences between the four strains obtained from different sites of the patient. As depicted in Table 1, biochemical reactions observed in our four strains corresponded very well with the data of Farmer et al. The only atypical reaction observed for our isolates was immotility at 36°C, whereas 93% of *E. fergusonii* strains tested by Farmer et al. (3) were motile. Our strains were sent to J. J. Farmer III at the Centers for Disease Control reference laboratory (Atlanta, Ga.) and were identified as *E. fergusonii*.

MICs generated by the broth microdilution method were equal for all four isolates when tested according to National Committee for Clinical Laboratory Standards guidelines (9), indicating susceptibility to ciprofloxacin (0.008 mg/liter); imipenem (0.25 mg/liter); gentamicin, netilmicin, and tobramycin (0.5 mg/liter); ceftriaxone (1 mg/liter); trimethoprim-sulfamethoxazole and ceftazidime (2 mg/liter); chloramphenicol (8 mg/liter); moderate susceptibility to piperacillin (64 mg/liter); and resistance to amoxicillin-clavulanate (16 mg/liter), penicillin G, and cephalothin (>256 mg/liter). With the Cefinase test (Becton Dickinson Microbiology Systems, Cockeysville, Md.), β-lactamase activity was detected in all of the four isolates (Table 2). Additionally, we noticed that all four isolates could be lysed with polyvalent phage 0-1 (Biokema AG, Lausanne, Switzerland), which lyses about 10% of all *E. coli* strains (4), whereas the *E. fergusonii* type strain (ATCC 35469) was phage resistant (Table 2).

Because rRNA gene restriction pattern analysis has been successfully applied in taxonomic and epidemiological investigations (8, 10), we examined our strains and the type strains of *E. fergusonii* and *E. hermannii* (ATCC 33650) by this means. As described before (7), total DNA was isolated, digested with restriction endonucleases *Sma*I, *Sph*I, and *Pvu*II (Boehringer, Mannheim, Germany) as recommended by the manufacturer, separated by electrophoresis through 0.8% agarose gels, stained with ethidium bromide, transferred to a nylon membrane (BiodyneA; Pall Biosupport,

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TABLE 1. Biochemical reactions of *E. fergusonii*

Test or characteristic	% Positive[a]	Reaction of our strains[b]
Indole production	98	+
Voges-Proskauer	0	−
Citrate (Simmons)	17	−
Hydrogen sulfide (triple sugar iron)	0	−
Urea hydrolysis	0	−
D-Phenylalanine deaminase	0	−
Lysine decarboxylase	95	+
Arginine dihydrolase	5	−
Ornithine decarboxylase	100	+
Motility at 36°C	93	−
Gelatin hydrolysis at 22°C	0	−
Malonate utilization	34	−
D-Glucose, acid production	100	+
D-Glucose, gas production	95	+
Acid production from:		
D-Adonitol	98	+
L-Arabinose	98	+
D-Arabitol	100	+
Cellobiose	98	+
Dulcitol	50	−
Erythritol	0	−
D-Galactose	100	+
Glycerol	13	−
myo-Inositol	0	−
Lactose	0	−
Maltose	98	+
D-Mannitol	100	+
D-Mannose	100	+
Melibiose	0	−
α-Methyl-D-glucoside	0	−
Raffinose	0	−
L-Rhamnose	93	+
Salicin	63	+[c]
D-Sorbitol	0	−
Sucrose	0	−
Trehalose	98	+
D-Xylose	98	+
Esculin hydrolysis	41	+[c]
Nitrate→nitrite	100	+
Oxidase	0	−
DNase at 25°C	0	−
o-Nitrophenyl-β-D-galactopyranoside	87	+
Yellow pigment at 25°C	0	−
Tyrosine clearing	0	−

[a] Percentage of positive reactions after 2 days of incubation at 36°C for 41 strains tested (3).
[b] For determination of acid production from carbohydrates, the API 50 CHE system was used.
[c] Weak reaction after 2 days of incubation.

East Hills, N.Y.), and probed with biotinylated plasmid pKK3535 carrying the *rrnB* rRNA operon from *E. coli*. All three restriction enzymes yielded identical ribotypes for the four isolates from the patient, whereas the type strains of *E. fergusonii* and *E. hermannii* showed different rRNA gene restriction patterns (Fig. 1 and Table 2). However, the fact that our strains had 10 bands in common with the type strain of *E. fergusonii* but only 2 with *E. hermannii* is compatible with the interpretation that our four strains represent a single clone within the species *E. fergusonii*.

To our knowledge, no reports of isolation of *E. fergusonii* from the gallbladder or of multiple isolations in one patient exist so far. Despite the fact that *E. fergusonii* was isolated from a stool sample taken 1 week after the initial septicemia, it is suggested that the intestinal flora could have been the source of a retrograde colonization of the patient's gallbladder, leading to cholangiosepsis (5). Because 9 of 41 strains of *E. fergusonii* described were of nonhuman origin (3), we considered that the patient had had animal or environmental exposure to *E. fergusonii*, but we were not able to explore this possibility. Furthermore, the patient's history did not reveal any kind of impairment of his gastric barrier (e.g., achlorhydria or use of H2 blockers or antacids). Antibiotic susceptibility testing revealed the presence of a β-lactamase in our strains, so far not shown in other studies, whereas data for the other antibiotics tested corresponded with observations published before (3, 6). Furthermore, previous reports (3, 6) did not mention phage susceptibility of *E. fergusonii*. This may be attributed to the fact that phage susceptibility has probably not been examined in *E. fergusonii* strains up to now. Despite adequate antimicrobial chemotherapy, *E. fergusonii* persisted in our patient, most likely in the intestinal flora, and was detected in a superficial wound of the abdomen up to 2 months after the primary isolation.

TABLE 2. Phenotypic and genotypic characteristics of *E. fergusonii* type strain and the isolates derived from the patient

Type strain or isolates from patient	Susceptibility to phage 0-1	β-Lactamase activity	Ribotyping pattern with[a]:		
			*Sma*I	*Sph*I	*Pvu*II
Type strain (ATCC 35469)	−	−	A	A	A
Isolates obtained from patient (n = 4)	+	+	B	B	B

[a] Designations for ribotyping patterns were chosen arbitrarily.

The results obtained from biotyping, resistotyping, phage typing, and rRNA gene restriction patterns suggested that

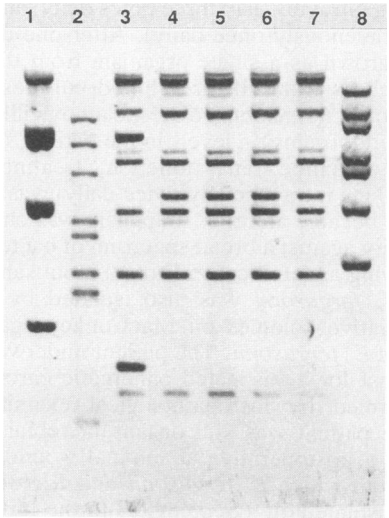

FIG. 1. rRNA gene restriction patterns of *E. fergusonii* obtained after *Sma*I digestion. Lanes: 1, biotinylated *Hin*dIII fragments of phage lambda (fragments are 23.1, 9.4, 6.6, and 4.3 kb); 2, *E. hermannii* (ATCC 33650, type strain); 3, *E. fergusonii* (ATCC 35469, type strain); 4 to 7, *E. fergusonii* isolates derived from gallbladder fluid, blood culture, feces, and superficial wound, respectively; 8, *Serratia fonticola* DNA digested with *Hin*dIII (fragments are 14.3, 11.9, 10.6, 8.5, 7.9, 6.6, and 5.4 kb).

the four strains were a single clone. This report adds further evidence on the clinical significance of *E. fergusonii*.

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