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Pulsed-field gel electrophoresis for the analysis of *Listeria monocytogenes* infection clusters after kidney transplantation

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Abstract *Listeria monocytogenes* causes a rare, life-threatening infection in recipients of transplanted organs. We used cultures of blood and cerebrospinal fluid to characterize isolates and to distinguish cases in clusters from what might have been sporadic cases. From December 1994 to November 1995, six systemic *L. monocytogenes* infections occurred at our renal-transplantation unit. We confirmed the clinical diagnosis with blood and cerebrospinal fluid cultures and characterized the isolates retrospectively with pulsed-field gel electrophoresis (PFGE), phage-typing, and serotyping. We also performed an environmental investigation (food, drug, and stool). We took samples after the first two *L. monocytogenes* infections and then after cases three and four occurred. All patients recovered completely, and no graft was lost. Four patients had identical or genetically related *L. monocytogenes* isolates in PFGE (type A) and serotyping (type 1/2b). The other two had PFGE type B and G. *L. monocytogenes* was not detected in food or drug samples from patients on the renal- transplantation ward or in stool samples from the ward staff. It was concluded that PFGE allows sporadic cases and cluster cases of *L. monocytogenes* infection to be distinguished.

Key words Renal transplantation · *Listeria monocytogenes* · Nosocomial infection · Pulsed-field gel electrophoresis (PFGE)

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Introduction

Listeria monocytogenes (Lm) is a gram-positive, catalase-positive, beta-hemolytic, facultatively intracellular bacterium that is widely distributed in the environment. It has been isolated from soil, animal feed, poultry, meat, raw milk, cheese, and asymptomatic human carriers [15]. Foodborne infections are facilitated by the bacterium's ability to grow at low temperatures, which might lead to cold enrichment in refrigerators [5]. *L. monocytogenes* is known to cause primarily septicemia, meningitis and encephalitis in immunocompromised patients, such as recipients of transplanted organs and people with neoplastic diseases or AIDS; healthy adults are rarely infected. *L. monocytogenes* infections often occur in groups of patients. *L. monocytogenes* infections of the central nervous system are characterized by a mortality rate of 20–50% [15]. In countries that register *L. monocytogenes* infections, an annual incidence of 0.2–0.8 per 100,000 inhabitants is reported [15]. A cluster of *L. monocytogenes* infections was observed in six patients at our renal-transplantation unit from December 1994 to November 1995 (Fig. 1). We performed an environmental investigation and a retrospective analysis, using pulsed-field gel electrophoresis (PFGE), serotyping, and phage-typing to characterize the isolates to try to distinguish sporadic cases from cluster cases.

Patients and methods

Six cases of systemic *L. monocytogenes* infection with central nervous system symptoms occurred at the renal-transplantation unit of the Hamburg University Hospital. The patients were still hospitalized and under a maximal level of immunosuppression when the episodes of listeriosis were detected. The important clinical data are presented in Table 1. Antithymocyte globulin (ATG) (Fresenius at 7.5 mg/kg of body weight or Merieux at 1.5 mg/kg of body weight), methylprednisolone (at 200–500 mg/day), and azathioprine (at 1.5–2 mg/kg of body weight) were used as the initial immunosuppression therapy. The initial dose of methylprednisolone was reduced to 20 mg/day. Azathioprin was given as a dose of 1.5–2 mg/kg of body weight. Cyclosporine A (at 10 mg/kg of body

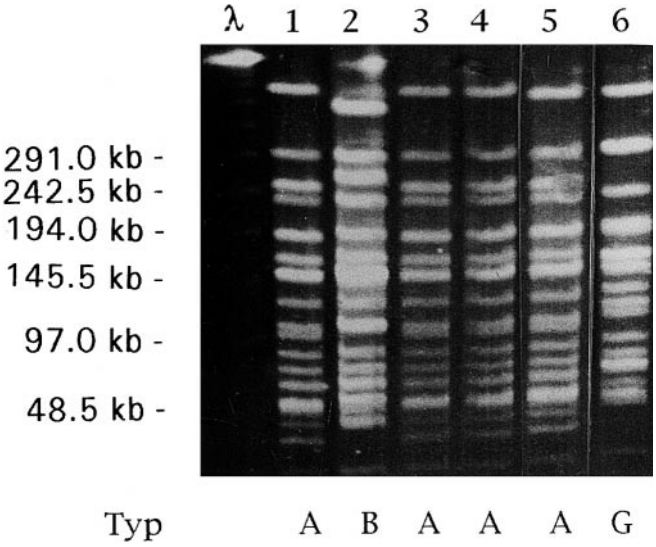

Fig. 1 Results of pulsed-field gel electrophoresis (PFGE) in six patients with *L. monocytogenes* infection after kidney transplantation. Restriction with Apa I; pulse time 1.0–30.0 s for 24 h

weight) was started after application of ATG, when a creatinine clearance of 20 ml/min was detected, or after the first postoperative week. Rejection was determined by core biopsy; steroid bolus therapy was initiated with intravenous methylprednisone at 500 mg/day for 3–5 days. Steroid-resistant rejection episodes were treated with polyclonal or monoclonal anti-T-cell antibodies (ATG or OKT 3).

When the patients' temperatures increased, blood specimens were drawn and stool samples were taken before antibiotic treatment with imipenem was started.

When *L. monocytogenes* infection was confirmed, antibiotic treatment was changed to ampicillin, which was applied intravenously for at least 2 weeks and then orally for 4 weeks.

In a retrospective analysis, the isolates were characterized with PFGE, phage-typing, and serotyping. We also performed an environmental investigation.

Identification of bacteria

The six isolates were identified as *L. monocytogenes* by Gram stain, positive motility test, detection of β-hemolysis, cAMP test, and a positive catalase reaction.

Serotyping was performed by C. Staak and A. Schönberg, of the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin, Germany.

Phage-typing of all isolates was performed by J. Rocourt, of the National Listeria Reference Center, Institut Pasteur, Paris, France.

Antibiotic susceptibilities of the isolates were determined with the agar-diffusion test on Mueller-Hinton agar.

PFGE was performed as a modification of the method described by Moore and Datta [2,3]. In brief, bacteria were grown overnight in 5 ml tryptic soy broth and harvested by centrifugation at 8 000 rpm for 10 min. The pellet was washed twice with 10 ml SE buffer. The bacterial suspension was adjusted to an OD 570 of 1. After 10-fold concentration, the suspension was mixed with the same volume of 2% low-melting-point agarose, which had been dissolved in the same buffer, at 45 °C. The solution was placed into a plug mold and each plug was incubated in 1 ml lysis solution (0.2% deoxycholic acid sodium salt, 1% *N*-lauroylsarcosine sodium salt, and 2.5 mg lysozyme per milliliter) for 24 h with gentle shaking at 37 °C. Thereafter, lysis solution was replaced with ESP solution (1% *N*-lauroylsarcosine sodium salt and 20 U proteinase K per milliliter) for 24 h at 56 °C. On the next step, plugs were washed four times with 10 ml TE buffer for 30 min at room temperature. Each plug was then washed for 1 h in 1 ml incubation buffer for restriction enzymes and then incubated overnight with 30 U restriction enzyme in 100 µl of buffer.

Restriction was performed with Sma I at 25 °C and with Apa I at 30 °C. PFGE of the restricted DNA was performed in a contour-clamped homogeneous electric field. The agarose plugs were placed into the wells and sealed with agarose. Lamda ladder DNA (Bio-Rad) was used as a size standard. The running buffer was 0.5x TBE, and electrophoresis was carried out at 200 V at 14 °C. Pulse times were 1.0–30.0 s for 24 h and 3.0–8.5 s for 26.6 h. Each DNA digest (Sma I and Apa I) was analyzed with each pulse time, so four runs were performed. The gels were stained in a solution of ethidium bromide, destained in distilled water, and then photographed.

Environmental investigations

For all specimens, a cold enrichment of possible *L. monocytogenes* was performed for 14 days at 4 °C. The various environmental samples (food, drug, and stool) were cultured after the first two *L. monocytogenes* infections occurred, and environmental investigations were reintroduced after cases three and four appeared. From 11 fresh cheese specimens, about 10 g of the rind and the interior were transferred separately into 250 ml of *L. monocytogenes* enrichment broth and vortexed. From the other food, 23 samples (corny breakfast, cacao powder, fresh and melted cheese, powder of instant broth, jam, uncooked vegetables, pasteurized milk, cream, and bread) were transferred to 10 ml *L. monocytogenes* enrichement broth. After incubation for 48 h at 28 °C and 37 °C, 100 µl of the broth was plated on *L. monocytogenes*-selective agar and on sheep blood agar. Another incubation for 48 h at 28 °C and at 37 °C was performed. Six drug samples (ATG Fresenius, ATG Merieux, and Cyclosporine A) were processed as described above. Stool specimens from nine ward-staff members were also examined: one loopful of stool was dispensed on a sheep agar plate and incubated at 28 °C and 37 °C. In parallel, 0.5 g of stool was dispensed in 5 ml of *L. monocytogenes* enrichment broth. After incubation for 7 days, 100 µl of the broth were plated on *L. monocytogenes* -selective agar and incubated for another 48 h.

Serology

L. monocytogenes antibodies in six sera that had been collected before hospitalization were examined with *L. monocytogenes* suspensions type 1:0, 1:H, 4b:0 and 4b:H according to the Widal method.

Table 1 The pattern of PFGE, serotyping, and phage-typing found in transplant patients

Patient	Age (years)	Sex	Month of transplantation	Postoperative day of onset	T-cell antibody therapy (no. days)	PFGE type	Serotype	Phage type
1	31	f	December 1994	50	ATG Fresenius (6)	A	1/2b	Not typable
2	61	m	December 1994	17	ATG Fresenius (8)	B	4b	1
3	57	m	April 1995	14	ATG Merieux (13)	A	1/2b	Not typable
4	44	m	April 1995	34	ATG Merieux (6)	A	1/2b	Not typable
5	54	f	July 1995	27	ATG Merieux (5)	A	1/2b	Not typable
6	57	m	October 1995	14	ATG Merieux (3)	G	4b	Not typable

Results

The important clinical characteristics of *L. monocytogenes* infection in the transplantation patients and the pattern of PFGE, serotyping, and phage-typing are shown in Table 1. Clinical disease was apparent between days 14 and 50 after transplantation without prodromal symptoms. The patients had abdominal discomfort, tenesmus, and quickly rising temperature (38.5–39.5 °C). The white cell count revealed moderate leukopenia (2.200–5.700 granulocytes/μl) and an increase in serum c-reactive protein (16–260 μg/ml; reference is under10 μg/ml). Neurologic symptoms of brainstem effect were present in all patients, varying from moderate frontal headache to severe somnolence and indications of pathology on examination of eye-movement. The clinical diagnosis was confirmed on the basis of positive aerobic blood cultures in all patients and detection of *L. monocytogenes* in stool samples in patient six and in the liquor samples of patient three. Three weeks after initial treatment patient three, had an excellent recovery from *L. monocytogenes* infection and exhibited a steroid-sensitive rejection of the renal graft. After antirejection treatment with steroid boli of methylprednisone at 500 mg/day for 4 days, a severe relapse of *L. monocytogenes*-related symptoms was observed. Clinical diagnosis was confirmed on the basis of positive blood and cerebrospinal-fluid cultures. The patient developed a hydrocephalus internus, which had to be treated with drainage. All patients recovered completely, and no graft was lost.

All isolates were susceptible to ampicillin, gentamicin, netilmicin, clindamycin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole (TMP/SMX).

Comparison of *L. monocytogenes* isolates

The DNA-fragment patterns were interpreted according to the guidelines published by Tenover and others [16]. PFGE patterns of patients one, three, four, and five were identical (Table 1). Their serotype was 1/2b; none was phage-typable. Patients two and six were identified in the PFGE pattern as type B and G, respectively; both were identified with serotyping as type 4b (Table 1), but only patient two could be identified with phage-typing. In patient three, the DNA-fragment pattern of the isolates after the relapse was identical with the PFGE type and serotype in the previous identification.

Environmental investigations

L. monocytogenes was detected neither in the examined food samples nor in the stool samples of the ward staff.

Serology

No pretransplantation sera were positive for antibodies against *L. monocytogenes*. Even if serology was not standardized, this indicates that potentially no previous infections were due to *L. monocytogenes*.

Discussion

Outbreaks of *L. monocytogenes* infection due to contaminated food such as vegetables, fish, coleslaw, fresh cream, fruit, ice cream, salami, and pasteurized milk have been reported [7, 11].

In reports of nosocomial listeriosis in immunocompromised adults, there is no strong evidence of cross infection or of person-to-person transmission, so infection due to contaminated food is likely [11]. In one reported infection cluster, Simmons and others [13] suggested a person-to-person transmission among renal-transplantation recipients. In some nosocomial outbreaks of neonatal listeriosis, thermometers have been postulated to be the vehicle for transmission; another report found contaminated mineral oil as the common source of the *L. monocytogenes* cluster [12].

Environmental investigations that were undertaken to identify the source responsible for our cluster of six *L. monocytogenes* infections revealed no contamination of the hospital food and no *L. monocytogenes* carrier among the ward staff. The prehospitalization sera from all patients were negative for antibodies to *L. monocytogenes*. Thus, recently acquired infections were unlikely, although *L. monocytogenes* carrier status cannot be excluded. Despite negative results of food and and environmental investigations, a common source of *L. monocytogenes* in four of these patients has to be assumed. PFGE and serotyping revealed that the isolates obtained from patients one, three, four, and five were identical or genetically related.

In a previous study, Nguyen and others [10] demonstrated with PFGE analysis that recurrent *L. monocytogenes* infection in a heart-transplantation patient was due to reinfection, rather than relapse of the formerly isolated strain. In this case, conventional epidemiologic typing had not been predictive.

Intact T-cell-mediated immunity is an important mechanism in primary host defense against *L. monocytogenes* infection in adults [14]. All patients in our series were treated with T-cell antibodies, and three patients received steroid bolus therapy in addition to their basic immunosuppressive regimen. MacGowan and others [9] reported a fecal carriage rate of 5.6% (10/177) in renal-transplantation patients, and none developed *L. monocytogenes* infection. All their patients received prednisolone at less than 15 mg/day, a dosage that was described as associated with a low risk of *L. monocytogenes* infection in a previous study of renal-transplantation patients. In our patients, immunosuppression was much more pronounced. It might be hypothesized that in the recent cluster only a small amount of *L. monocytogenes* was present in a common source, such as food, and therefore clinical disease developed only in heavily immunosuppressed patients,

whereas other patients were capable of fighting off the small inoculate. Methodologic difficulties and variable results of investigations of food and environmental specimens are well known and might have caused the negative results of the investigations in our study [9].

The high mortality, 20–50%, reported by several authors [4, 11, 17] requires rapid and safe diagnostic procedures when organ recipients present with fever, septicemia, and neurologic complaints. Complete recovery was observed in all our patients, but one patient suffered an intermittent relapse of infection, probably owing to inadequate serum ampicillin when he was switched to an oral formulation because of a steroid-sensitive rejection of the renal graft. After high-dose parenteral ampicillin for 2 weeks, the patient fully recovered. That observation supports the general recommendation to extend the duration of treatment for *L. monocytogenes* infections from 2 weeks in uncomplicated cases to 3–6 weeks in immunocompromised patients [1].

L. monocytogenes infections can be prevented effectively with low-dose TMP/SMX [6, 8].

Conclusion

By using serotyping and PFGE as tools of molecular epidemiology a distinction can be made between sporadic cases and cluster cases of *L. monocytogenes* infection. That is important for making decisions about preventive measures, especially when no common source of infection can be identified through environmental investigation.

References

1. Amstrong D (1995) *Listeria monocytogenes*. In: Mandell ED (ed) Principles and practice of infectious diseases, 4th edn. pp 1888
2. Boerlin P, Boerlin-Petzold F, Bannerman E, Bille J, Jemmi T (1997) Typing *Listeria monocytognes* isolates from fish products and human listeriosis cases. Microbiology 63: 1338
3. Datta AR, Moore MA, Wentz BA, Lane J (1993) Identification and Enumeration of Listeria monocytogenes by Nonradioactive DNA probe colony hybridization. Microbiology 69: 144
4. Donelly JP (1995) Bacterial complications of transplantation: diagnosis and treatment. J Antimicrob Chemother 36: Suppl B 59
5. Farber JM, Peterkin PI (1991) *Listeria monocytogenes*, a foodborn pathogen. Microbiol Rev 55: 476
6. Fishmann JA, Rubin RH (1998) Infection in organ-transplant recipients. New Engl J Med 24: 1741–1751
7. Fleming DW, Cochi SL, McDonald KL, Brondum J, Plikaitis BD, Holmes MB, Audurier A, Broome CV, Reingold A (1985) Pasteurized milk as a vehicle of infections in an outbreak of listeriosis. New Engl J Med 312: 404
8. Haralambie E, Mahmou HK, Linzeneier G, Wendt F (1983) The "clostridial effect" of selective decontamination of the human gut with trimethoprim/sulfamethoxazole in neutropenic patients. Infection 11: 201
9. MacGowan AP, Marshall RJ, MacKay IM, Reeves DS (1991) Listeria faecal carriage by renal transplant recipients, hemodialysis patients and patients in general practice: its relation to season, drug therapy, foreign travel, animal exposure and diet. Epidemiol Infect 106: 157
10. Nguyen van JC, Nguyen L, Guillemain R, Amrein C, Buu-Hoi A, Gutmann L (1994) Relapse of infection or reinfection by Listeria monocytogenes in a patient with heart transplant: usefulness of pulsed field gel electrophoresis for diagnosis. Clin Infect Dis 19: 200
11. Schlech WF (1991) Lowbury lecture: Listeriosis: Epidemiology, virulence and the significance of contaminated foodstuffs. J Hosp Infect 19: 211
12. Schuchat A, Lizano C, Broome CV, Swaminathan B, Kim C, Winn K (1985) Outbreak of neonatal listeriosis associated with mineral oil. Ped Infect Dis 10: 183
13. Simmons BT, Dixon RE, Bennet JV (1980) Listeria monocytogenes infection in a renal transplant unit in an Alabama hospital. CDC EPI 80: 16–2
14. Southwick FS, Purich DL (1996) Intracellular pathogenesis of listeriosis New Engl J Med 334: 770
15. Swaminathan B, Rocourt J, Bille J: Listeria (1995) In: Murray PR, Baron EJ, Phaller MA, Tenover FR, Yolke RH (eds) Manual of Clinical Microbiology, 6th edn. American Society for Microbiology, Washington DC, p 341
16. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 33: 2233
17. Tolkoff-Rubin NE, Rubin RH (1992) Opportunistic fungal and bacterial infection in the renal transplant recipients. J Am Soc Nephrol Suppl 12: 264