Listeriosis Due to Infection with a Catalase-Negative Strain of *Listeria monocytogenes*

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A strain of *Listeria monocytogenes* recovered from blood and cerebrospinal fluid had no detectable catalase activity, a characteristic used for primary identification. The sporadic occurrence of pathogenic catalase-negative strains highlights the need for a reconsideration of diagnostic criteria and questions the role of catalase in the pathogenesis of listeria infection.

CASE REPORT

A 28-year-old Caucasian male receiving immunosuppressive therapy with cyclosporine and prednisolone following renal transplantation developed a sudden onset of rigors, high fever (38.9°C), vomiting, and progressive confusion after routine hemodialysis. On arrival in the emergency room, he sustained a generalized tonic-clonic seizure requiring anticonvulsants. Physical examination revealed no obvious source of infection or skin rash, mild photophobia but no other signs of meningeal irritation, and generalized hyperreflexia consistent with a postictal state. A lumbar puncture was performed, and a full septic screen was obtained before the patient was started on intravenous ceftriaxone therapy (2 g every 12 h). Cerebrospinal fluid (CSF) revealed a lymphocyte count of 1,008 cells/mm³; therefore, intravenous acyclovir (10 mg/kg of body weight every 8 h) was commenced, and co-trimoxazole (10 mg/kg every 12 h) commenced. Subsequent susceptibility testing of both isolates by agar dilution at the Health Protection Agency antibiotic reference laboratory confirmed the organism’s susceptibility to ampicillin and co-trimoxazole, with MICs of 0.5 mg/liter and 0.064 mg/liter, respectively.

The patient made a slow but progressive recovery, regained consciousness, and experienced no further seizures. The antibiotic therapy with co-trimoxazole was continued for a total of 4 weeks.

The organism was identified as *L. monocytogenes* by PCR amplification and sequence analysis of 1,156 base pairs of the 16S rRNA genes and confirmed by the Food Safety Microbiology Laboratory at the Health Protection Agency (Colindale, London, United Kingdom) as *L. monocytogenes* serogroup 4.

The apparent lack of catalase activity was investigated further using crude whole-cell extracts as follows. The isolate was grown at 37°C in 5 ml of Luria-Bertani broth to an optical density at 600 nm of 0.6 to 0.8 (mid-log phase), and cells were harvested by centrifugation. Cell extracts were obtained by resuspending the bacteria in 2.5 ml of phosphate-buffered saline containing 2% (wt/vol) sodium dodecyl sulfate (SDS) and then incubating the suspension at 37°C for 30 min with shaking. Suspensions were centrifuged, and proteins present in the supernatants were quantified by Bradford’s method (2a).

Proteins were also extracted from a catalase-positive strain (Listeria monocytogenes NCTC 10357) following growth under similar conditions. Extracts (5-μl volumes; 2 mg protein/ml) were diluted in 1 ml of distilled water, and 0.5 ml of 59 mM H₂O₂ diluted in 50 mM K₂HPO₄ was added. The absorbance at 240 nm was measured every 15 s for 1 min with a DU 800 spectrophotometer (Beckman Coulter, High Wycombe, United Kingdom), and the specific catalase activity was calculated using the formula described by Beers and Sizer (2).

The isolate had a catalase-specific activity of 0 compared to an activity of 17.2 ± 0.56 for the catalase-positive control. Catalase activity was also investigated by zymography. Crude cell extracts (25 μl) and purified bovine catalase were mixed with 5 μl of 5× SDS-polyacrylamide gel electrophoresis buffer and electrophoresed on a 7.5% SDS-polyacrylamide gel. Gels...
FIG. 1. Catalase zymogram of 25 μl of purified bovine catalase (lane 1) and crude cell extracts from catalase-positive (lane 2) and catalase-negative (lane 3) strains of *L. monocytogenes*. Lane 4 is a negative control. Arrows within the zymogram field indicate zones of catalase activity. M, molecular mass markers.

*L. monocytogenes* is a well-recognized human pathogen, causing invasive disease in debilitated individuals, children, and pregnant women. Serotypes 1/2 and 4 are the most commonly reported (1) and may cause meningitis in immunocompromised patients or patients receiving immunosuppressive therapy (11). Identification of *L. monocytogenes* relies on a number of phenotypic criteria, including Gram stain appearance, colonial morphology, hemolysis, tumbling motility, and a number of biochemical reactions. The production of catalase is thought to be an important characteristic and is employed by a number of commercial identification systems (7). Misidentification using the API Coryne test but not the API Rapid ID Strep, which does not rely on a number of phenotypic criteria, has been previously reported (3). Strains of *L. monocytogenes* that do not produce catalase have only rarely been reported in sporadic cases of human infection (12) but can fairly readily be isolated from a number of foods (4).

**REFERENCES**