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***Escherichia coli* causing meningitis in an adult: A case report and experimental characterization of its virulence**



Meningitis por *Escherichia coli* en un adulto: caso clínico y caracterización experimental de su virulencia

Escherichia coli is a very infrequent cause of spontaneous community-acquired meningitis in adults (incidence lower than 0.1 cases per 100,000 adults per year).^{1–3} This organism usually reaches the meninges from a distant source, being a rare complication of a bloodstream infection.³ In general, the mortality rate of patients with Gram-negative bacillary meningitis is higher than that caused by *Neisseria meningitidis*,³ which is probably due to infections caused by especially virulent strains. Virulence factors

including toxins, adhesins, lipopolysaccharides, different capsular types, and proteases are important for the invasion and dissemination of *E. coli* in the host.⁴ We report a case of meningitis in an adult caused by *E. coli*, and characterize the pathogenicity of the isolate *in vitro* and *in vivo*.

A 58-year-old man was admitted to the emergency department of our hospital with fever, dysuria and frequent urination. Therapy with amoxicillin-clavulanic acid was started, and after 24 h of improvement, the patient developed a severe occipital headache and emetic syndrome, followed by hemodynamic instability and altered mental status. With the suspicion of meningococcal meningitis, a lumbar puncture was performed obtaining a cloudy cerebrospinal fluid (CSF) with severe neutrophilic pleocytosis (>4000 cells/ μ L), elevated proteins (3.8 g/L) and low glucose (<0.02 g/L). Empirical treatment with ceftriaxone and ampicillin was started. The patient

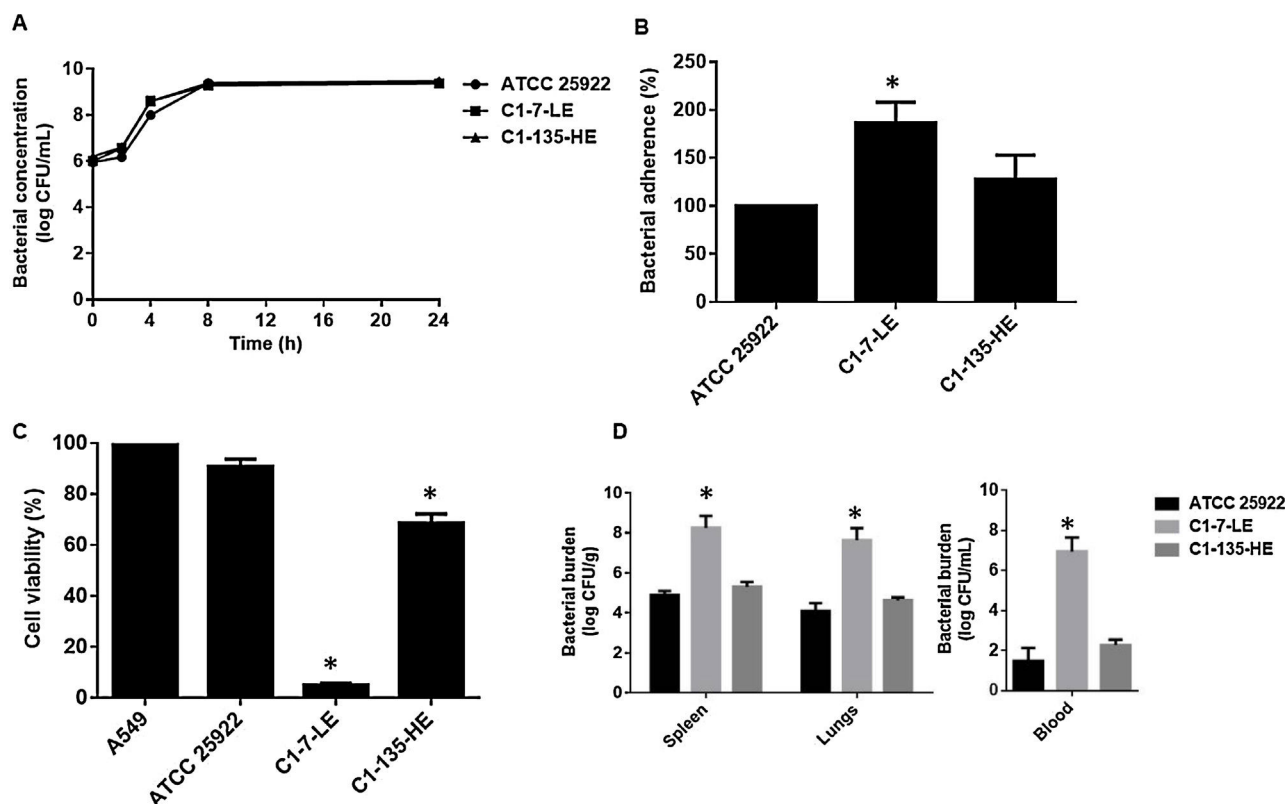


Fig. 1. (A) Growth curve of *E. coli* C1-7-LE strain producing meningitis, and control strains *E. coli* ATCC 25922 and *E. coli* C1-135-HE in Mueller-Hinton broth for 24 h. (B) Bacterial adherence assay in A549 cells of *E. coli* strains C1-7-LE, C1-135-HE, and ATCC 25922. Representative results of 3 independent experiments (mean $\pm$ SEM by unpaired Student *t*-test $P < 0.05$; *comparison between ATCC 25922 and C1-7-LE or C1-135-HE). (C) Viability of A549 cells after incubation with the 3 isolates for 6 h. Representative results of 3 independent experiments (mean $\pm$ SEM, by unpaired Student *t*-test $P < 0.05$; *comparison between A549 cells and other groups). (D) Bacterial burden in spleen, blood and lungs after 6 h of infection (inoculum of $7.3 \log_{10}$ CFU/mL) with the 3 strains (mean $\pm$ SEM, $P < 0.05$; *comparison between ATCC 25922 and C1-7-LE or C1-135-HE strains).

was admitted to the ICU and required mechanical ventilation for 9 days. *E. coli* C1-7-LE, only resistant to cotrimoxazole, was isolated from blood cultures, urine, and CSF. At day 7, the axial computerized tomography showed diffuse edema without other complications. At day 8, a second CSF analysis showed a biochemical improvement: proteins and glucose concentrations were 0.94 and 0.43 g/L, respectively, absence of bacteria in the Gram stain and absence of growth in the aerobic culture. At day 12, the patient was discharged to the infectious diseases ward. Due to persistent mutism and altered mental status, a magnetic resonance (MR) imaging was performed revealing the presence of multiple abscesses. Endovenous treatment with ceftriaxone was maintained, which improved the neurologic symptoms and the lesions in the MR. The patient was discharged from the hospital after 8 weeks of treatment.

We studied the pathogenicity of the *E. coli* C1-7-LE isolate causing the meningitis. MLST analysis showed that it belonged to the phylogroup B2.

Two control strains were used for comparison: (i) – the reference strain *E. coli* ATCC 25922, belonging to phylogroup B2, and (ii) – a clinical *E. coli* strain C1-135-HE belonging to phylogroup A, isolated from a patient without meningitis but with similar clinical parameters that our meningitis case patient. *In vitro* bacterial growth showed that C1-7-LE had similar growth rate compared to both control strains (Fig. 1A). A bacterial adhesion assay was performed as described previously.⁵ C1-7-LE presented (approximately) an 87% and 28% of adherence increase to A549 cells when compared with ATCC 25922 ($P=0.03$) and C1-135-HE ($P=0.29$), respectively (Fig. 1B).

In order to analyze the ability of the meningitis isolate to potentiate host cell death, we studied the viability of A549 cells in presence of both the C1-7-LE isolate and the control strains by the MTT assay as described previously.⁶ C1-7-LE was more cytotoxic than the control strains, reducing the A549 cell viability to $5.00 \pm 0.71\%$ ($P=0.006$) at 6 h, while the ATCC 25922 and C1-135-HE isolates reduced the cell viability to $90.75 \pm 3.00\%$ ($P=0.14$) and $68.54 \pm 3.67\%$ ($P=0.01$), respectively (Fig. 1C).

Finally, a previously characterized murine peritoneal sepsis model was used to assess the pathogenicity of the meningitis-producing *E. coli* in comparison with both control isolates.⁷ Spleen, blood and lungs showed higher bacterial concentrations in mice infected with the C1-7-LE isolate than those infected with ATCC 25922 and C1-135-HE as follows: 8.26 ± 0.59 , 6.95 ± 0.70 and 7.63 ± 0.61 log CFU/g (C1-7-LE) vs. 4.9 ± 0.21 , 1.48 ± 0.66 and 4.10 ± 0.40 log CFU/g, ($P<0.05$, ATCC 25922) and 5.21 ± 0.24 , 2.27 ± 0.28 and 4.64 ± 0.14 log CFU/g ($P<0.05$, C1-135-HE) (Fig. 1D).

In summary, the meningitis-producing *E. coli* isolate presented higher adherence to host cells, was more cytotoxic and showed increased pathogenicity in a murine experimental model, characteristics that could explain the high virulence of this *E. coli* isolate in our case. Experimental studies are useful in order to evaluate whether some of the virulence factors may be involved in the pathogenesis of isolates causing severe infections.

This study was approved by the Ethic Committee of University Hospital Virgen del Rocío, Seville (approval no. 0023-N-16).

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