

**Association of *bla*<sub>OXA-1</sub>, and *aac*(6′)-*Ib-cr* with ST405 *K. pneumoniae* clone****Asociación de los genes *bla*<sub>OXA-1</sub> y *aac*(6′)-*Ib-cr* con el clon ST405 de *K. pneumoniae***

Dear Editor:

In recent years, various plasmid-mediated resistance mechanisms that confer low-level resistance to quinolones (PMQR) have been described. These include enzymatic modification by the acetyltransferase AAC(6′)-Ib-cr, QepA active efflux pumps, and *qnr* genes, which have been associated with genes *bla*<sub>OXA-1</sub>, *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM-1</sub> genes.<sup>1</sup> These determinants have frequently been found to be related to successful clones producing *bla*<sub>CTX-M-15</sub> and *bla*<sub>OXA-48</sub>, such as sequence type (ST) 405<sup>2,3</sup> or ST15 *K. pneumoniae*.<sup>4</sup> In unsuccessful clones, an association of these determinants can also be found, such as an 3 years-outbreak in a hospital in Barcelona caused by ST14 *K. pneumoniae*.<sup>5</sup> Nevertheless, the prevalence of these genes in non-extended-spectrum beta-lactamases (ESBL) or carbapenemases-producing isolates is unknown.

A previous study carried out by Rodríguez-Martínez et al.<sup>6</sup> described a phenotypic detection algorithm for PMQR, which was validated in a collection of 99 isolates of Enterobacteriales of clinical origin from the University Hospital Virgen Macarena. Fifty-one isolates (51%) had at least one PMQR mechanism: *qnrS*, the most prevalent, appeared in 25 (49%) isolates, *qnrB* in 20 (39%) isolates, *aac*(6′)-*Ib-cr* in 9 (18%) isolates, *oqxAB* in 6 (12%) isolates, *qnrA* in 2 (4%) isolates and *qnrD* in 2 (4%) isolates. The phenotype compatible with the production of *bla*<sub>OXA-1</sub> consists of an intermediate sensitivity or resistance to amoxicillin/clavulanic acid, decreased sensitivity to piperacillin/tazobactam according to CLSI breakpoints and synergy by disc diffusion between clavulanic and cefepime in the absence of ESBL enzymes. In order to estimate the frequency of the association between PMQR determinants and *bla*<sub>OXA-1</sub> and *bla*<sub>TEM-1</sub>, 37 (37.3%) isolates with MIC values >8/4 μg/ml to amoxicillin/clavulanic acid and >2 μg/ml to cefepime were selected from the same collection. Screening of OXA-1 was performed by examining the synergy between discs of the amoxicillin/clavulanic and cefepime, and also by PCR, using specific primers for the *bla*<sub>OXA-1</sub> gene.<sup>7</sup> Twelve (32.4%) isolates were positive for *bla*<sub>OXA-1</sub>, including 8 (66.7%) *K. pneumoniae* (6 of them *qnrB*), 3 (25%) *E. coli* (1 *qnrA* and 1 *qnrS*) and 1 (8.3%) *E. cloacae* (*qnrA*). *Xba*I PFGE was used to study the clonal relatedness of *K. pneumoniae* isolates was studied by. Six isolates (75%) formed a cluster (less than 6 difference bands, 86% similarity using the Dice index) and shared the following determinants of resistance: all of them possessed *aac*(6′)-*Ib-cr* and *bla*<sub>OXA-1</sub>; 5 (83%) also have *qnrB*, and 2 of them (33%) also carried *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM-1</sub>. Two isolates from this group were selected for MLST (one isolate carrying *bla*<sub>OXA-1</sub>, *qnrB1*, *aac*(6′)-*Ib-cr*, and *bla*<sub>TEM-1</sub>; and the other isolate carrying *bla*<sub>OXA-1</sub>, *qnrB1*, *aac*(6′)-*Ib-cr*, *bla*<sub>TEM-1</sub>, and *bla*<sub>CTX-M-15</sub>) were assigned ST405. When these were also compared with the genetic profiles held at the Andalusian Reference Laboratory for molecular typing, they were found to share 85% similarity with isolates belonging to the same clone from other areas of Spain.

This clone with the genetic profile *bla*<sub>TEM-1</sub>, *bla*<sub>CTX-M-15</sub> and *bla*<sub>OXA-1</sub> was described for the first time in Casablanca (Morocco) in isolates collected between February 2007 and March 2008.<sup>8</sup> The first description of ST405 with OXA-48 production was in Belgium (between January 2010 to April 2011) 4 isolates. These isolates also involved *bla*<sub>TEM-1</sub> and *bla*<sub>CTX-M-15</sub>, and 3 of them additionally carried *aac*(6′)-*Ib-cr* and *qnrB1*.<sup>9</sup> The largest single-centre outbreak to date,

in the Hospital La Paz, Madrid,<sup>10</sup> was caused by a ST405 producer of OXA-48 that also carried *qnrB1* and *aac*(6′)-*Ib-cr*.<sup>2</sup> The same clone was associated with two outbreaks in neonatology units in Andalusian hospitals (in the South of Spain) caused by isolated producers of CTX-M-15, but not of OXA-48.<sup>3</sup>

ST405 shares a series of determinants in every location where it has been detected: *bla*<sub>OXA-1</sub>, *bla*<sub>TEM-1</sub> and *aac*(6′)-*Ib-cr*, even in non-ESBL or non carbapenemase-producing isolates, as well as having the characteristics of a successful clone. The dissemination of this clone seems to be associated with acquisition of determinants of low resistance to amoxicillin/clavulanic acid and quinolones, with acquisition of ESBL or carbapenemase genes occurring later. This clone could be more widely disseminated than initially thought, since there are few data available on cephalosporin- and carbapenems-susceptible isolates. In a recent study in our country, this was one of the 5 most important clones in the dissemination of carbapenemases in Spain, accounting for 13.5% of carbapenemases producers,<sup>11</sup> and only 2 of the five non-carbapenemases-producers ST405 were susceptible to third-generation cephalosporins. It would be of interest to find out the real prevalence of this clone in resistant and wild type isolates in order to study the selective forces that drive its dissemination.

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

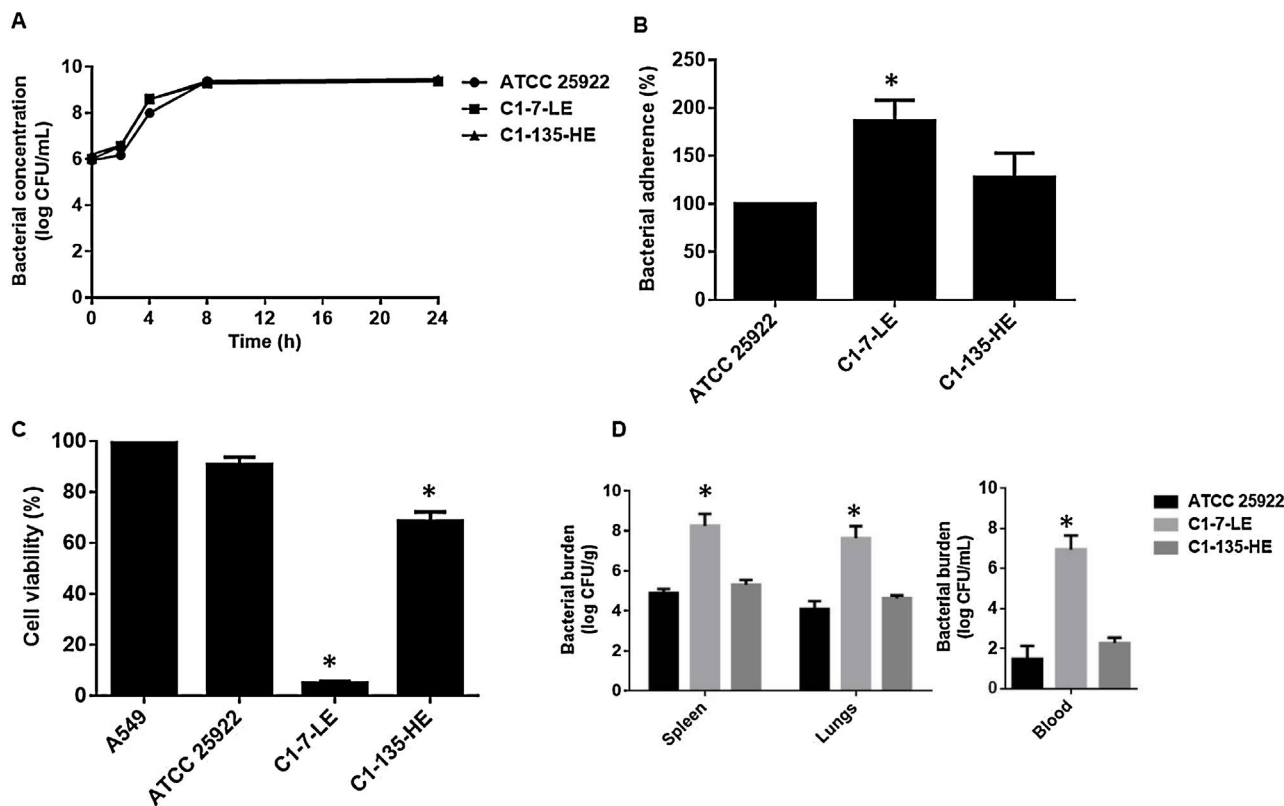


### **Meningitis por Escherichia coli en un adulto: caso clínico y caracterización experimental de su virulencia**

*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).<sup>1–3</sup> This organism usually reaches the meninges from a distant source, being a rare complication of a bloodstream infection.<sup>3</sup> In general, the mortality rate of patients with Gram-negative bacillary meningitis is higher than that caused by *Neisseria meningitidis*,<sup>3</sup> 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.<sup>4</sup> 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/ $\mu$ 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).