

^a Área de Microbiología, Laboratori de Referència de Catalunya, El Prat de Llobregat, Barcelona, España

^b Laboratori de Referència de Catalunya, Mas Blau, El Prat de Llobregat, Barcelona, España

^c Unidad de Cuidados Intensivos, Hospital del Mar, Barcelona, España

* Autor para correspondencia.

Correo electrónico: eportillo@irc.es (M. Eugenia Portillo).

doi:10.1016/j.eimc.2011.06.006

Detection of phylogenetic group B1 *Escherichia coli* by multiplex PCR: Description of a new amplification pattern

Detección del grupo filogenético B1 en *E. coli* por PCR múltiple: descripción de un nuevo patrón de amplificación

Dear Editor:

Phylogenetic analysis of *Escherichia coli* isolates has enabled five major groups into this species to be identified, namely A, B1, B2, D and E. Human isolates of groups B2 and D are often involved in extra-intestinal infections, while those of groups A and B1 are frequently associated with commensal microbiota.² Characterisation of phylogenetic groups is of clinical interest. Typically, isolates of groups B2 and D had been reported to frequently have an increased number of virulence factors and decreased rates of antimicrobial resistance than those of group A or B1,^{1–4} although more recent studies indicate that some particular clones of phylogenetic group B2 (ST131) or D (ST405) express a multi-resistant phenotype.⁵ Phylogenetic groups were initially recognised by multilocus enzyme electrophoresis,⁶ a rather complex and time-consuming approach. In 2000, Clermont et al.⁷ described a simple approach based on a triplex PCR detecting the genes *chuA* and *yjaA* and the DNA fragment TSPE4.C2. This method assigns the phylogenetic group in approximately 80% of the cases. We have used this triplex PCR to define the phylogenetic group of clonally unrelated (as defined by Rep-PCR) multi-resistant clinical isolates of *E. coli* (manuscript in preparation). In all, 100 *E. coli* producing extended-spectrum beta-lactamases (ESBL) and 100 isolates lacking ESBL and resistant to at least three of the antimicrobial agents: amoxicillin, nalidixic acid, cotrimoxazole and either or both gentamicin and tobramycin were evaluated. PCR was performed using primers and conditions described previously⁷ using a 2720 Thermal Cycle (Applied Biosystems, Foster City, CA, USA).

Fifty out of the 200 (25%) isolates [38 (76%) ESBL producers and 12 (24%) ESBL non-producers] showed an amplification pattern compatible with group B1, but in 42 (84%) isolates [31 (74%) ESBL producers and 11 (26%) ESBL non-producers] an extra band of approximately 550 bp was also observed (Fig. 1A). This band was not present in the control strains EcoR32 (B1), EcoRS218

(B2), EcoR16 (A) and EcoR39 (D). The experiment was repeated on two different occasions in different days using two different methodologies: in the first one, we used a short program with an annealing temperature (T_m) of 59 °C, and in the second one, we used a longer program with a T_m of 55 °C (both of them described by Clermont et al.⁷). The same results were obtained in the two assays. We are not aware of the description of this finding in previous reports related to phylogenetic group characterisation on *E. coli*.

In order to characterise the nature of the observed extra band, single PCR assays were performed using primers TspE4C2.1 and TspE4C2.2 and the extra band was not observed. New single PCR assays with all other possible primer combinations were also tested in one representative isolate (Fig. 1B).

The extra ca. 550 bp band was only observed when the primer pair *chuA1* and TspE4C2.1 was used. Sequencing of the corresponding amplicon resulted in a 528 bp sequence 100% homologue with a lipase (acetyl-hydrolase) found in the pathogenic strains of *E. coli* belonging to phylogenetic group B1 [*E. coli* O111:H– (Genbank access number AP010960.1), *E. coli* O103:H2 (AP010958.1), *E. coli* O26:H11 (AP010953.1), *E. coli* IA11 (CU928160.2), *E. coli* 55989 (CU928160.2), *E. coli* SE11 (AP009240.1) and *E. coli* E24377A (CP000800.1)]. In conclusion, we have documented that most isolates of *E. coli* of the phylogenetic group B1, the conventional triplex PCR assay may result in the amplification of a fragment of a gene coding for an acetyl-hydrolase. We suggest that this extra band could be used to characterise a new subgroup of phylogenetic group B1 (B1a), but additional studies should be done in order to evaluate the correlation of the possible subgroups with other typing methodologies such as MLST or PFGE.

Acknowledgements

This study was supported by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III-FEDER [Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008) and FIS (PI05-0690)]. B. Ruiz del Castillo is supported by a grant from Instituto de Formación e Investigación Marqués de Valdecilla (IFIMAV). We acknowledge Dr. J.R. Johnson (Minneapolis, MN, USA) for helpful discussion.

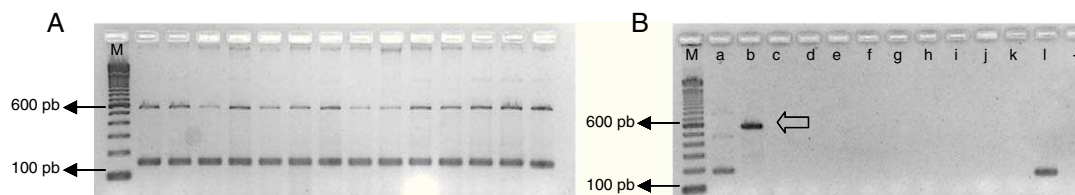


Fig. 1. (A) Results of the triplex PCR for phylogenetic group B1 of *E. coli* showing (arrow) the extra 550 bp band. (B) PCR assays with all other possible primer combinations for representative isolate belonging to phylogenetic group B1; (a) *chuA1*/*yjaA1*; (b) *chuA1*/tspE4C.1; (c) *chuA1*/*chuA2*; (d) *chuA1*/*yjaA2*; (e) *chuA1*/tspE4C.2; (f) *yjaA1*/tspE4C.1; (g) *yjaA1*/*chuA2*; (h) *yjaA1*/*yjaA2*; (i) *yjaA1*/tspE4C.2; (j) tspE4C.1/*chuA2*; (k) tspE4C.1/*yjaA2*; (l) tspE4C.1/tspE4C.2. M: 100 bp DNA Ladder (Invitrogen, Barcelona, Spain).

Bibliografía

1. Johnson JR, Johnston B, Kuskowski MA, Colodner R, Raz R. Spontaneous conversion to quinolone and fluoroquinolone resistance among wild-type *Escherichia coli* isolates in relation to phylogenetic background and virulence genotype. *Antimicrob Agents Chemother*. 2005;49:4739-44.
2. Johnson JR, Murray AC, Kuskowski MA, Schubert S, Prère MF, Picard B, et al. Distribution and characteristics of *Escherichia coli* clonal group A. *Emerg Infect Dis*. 2005;11:141-5.
3. Moreno E, Prats G, Sabaté M, Pérez T, Johnson JR, Andreu A. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. *J Antimicrob Chemother*. 2006;57:204-11.
4. Moreno E, Planells I, Prats G, Planes AM, Moreno G, Andreu A. Comparative study of *Escherichia coli* virulence determinants in strains causing urinary tract bacteremia versus strains causing pyelonephritis and other sources of bacteremia. *Diagn Microbiol Infect Dis*. 2005;53:93-9.
5. Leflon-Guibout V, Blanco J, Amaqdouf K, Mora A, Guize L, Nicolas-Chanoine MH. Absence of CTX-M enzymes but high prevalence of clones, including clone ST131, among fecal *Escherichia coli* isolates from healthy subjects living in the area of Paris, France. *J Clin Microbiol*. 2008;46 December:3900-5.
6. Ochman H, Selander RK. Standard reference strains of *Escherichia coli* from natural populations. *J Bacteriol*. 1984;157:690-3.
7. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol*. 2000;66:4555-8.

Belén Ruiz del Castillo, Alain A. Ocampo-Sosa,
Luis Martínez-Martínez*

Service of Microbiology, University Hospital Marqués
de Valdecilla-IFIMAV, Santander, Spain

* Corresponding author.

E-mail address: lmartinez@humv.es (L. Martínez-Martínez).

doi:10.1016/j.eimc.2011.07.004