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Rapid identification of uropathogenic *Escherichia coli* of the O25:H4-ST131 clonal lineage using the DiversiLab repetitive sequence-based PCR system

S. H. Lau[1], J. Cheesborough[2], M. E. Kaufmann[3], N. Woodford[3], A. R. Dodgson[1,4], K. J. Dodgson[4], E. J. Bolton[5], A. J. Fox[5] and M. Upton[1]

1) Department of Medical Microbiology, School of Medicine, University of Manchester, Manchester Royal Infirmary, Manchester, UK, 2) Preston Microbiology Services, Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, Lancashire, 3) Health Protection Agency, Centre for Infections, London, 4) Central Manchester and Manchester Children's NHS Trust and 5) Health Protection Agency North West Laboratory, Clinical Science Building, Manchester Royal Infirmary, Manchester, UK

Abstract

Several recent studies have highlighted the emergence of a globally disseminated clone of uropathogenic and invasive *Escherichia coli* isolates of serotype O25:H4 and sequence type 131. The ability to characterize rapidly *E. coli* isolates of this lineage would facilitate enhanced surveillance for this pathogen. We have used the semi-automated DiversiLab repetitive PCR-based system to analyse a collection of 35 clinical isolates of uropathogenic *E. coli* from across the UK, with particular focus on the O25:H4-ST131 lineage. All isolates had been characterized using multilocus sequence typing (MLST), and 14 had previously been typed using pulsed-field gel electrophoresis (PFGE). The DiversiLab system allowed discrimination of O25:H4-ST131 isolates from those of other *E. coli* lineages. It was slightly more discriminatory than MLST, but was less discriminatory than PFGE. With an analysis time of <4 h between receipt of a cultured organism and provision of a typing result, the system offers information on a real-time basis, a major advantage over current practice. We suggest that introduction of the DiversiLab system would be useful for rapid exclusion of *E. coli* isolates during outbreak investigations, and that the approach could be employed for surveillance for pathogenic or antibiotic-resistant clones of this organism.

Keywords: DiversiLab, extended-spectrum β-lactamase, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), uropathogenic *Escherichia coli* (UPEC)
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Corresponding author and reprint requests: M. Upton, Department of Medical Microbiology, School of Medicine, University of Manchester, Clinical Sciences Building, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL, UK
E-mail: m.upton@manchester.ac.uk

Introduction

Strains of *Escherichia coli* producing the CTX-M-15 extended-spectrum β-lactamase (ESBL) enzyme have become the most important type of ESBL-producing organisms causing urinary tract infections in community and hospital settings in the UK [1,2]. Their emergence constitutes a serious public health concern that emphasizes the need for rapid and reliable epidemiological characterization of isolates and identification of outbreak/epidemic clones [1,3,4].

Numerous methods have been used previously to differentiate and characterize *E. coli*, and each method has its advantages and disadvantages [5], but pulsed-field gel electrophoresis (PFGE) is currently one of the most widely used methods in outbreak investigations, because of its high discriminatory power [2]. Several published studies have shown that multilocus sequence typing (MLST) is a useful tool for population biology and long-term or global epidemiological studies for a wide range of pathogens, including uropathogenic *E. coli* (UPEC) [3,6–10]. More recently, the use of MLST has revealed a globally disseminated clone of UPEC of sequence type (ST) 131, which is a significant cause of urinary tract infection and invasive infection in the UK, Europe, Asia, and Canada [6,7,11–13]. Members of this clone are of serotype O25:H4, are often resistant to fluoroquinolones, and frequently produce a CTX-M-15 β-lactamase, although a recent report documented the occurrence of this

strain in Europe with only four of 35 isolates expressing the CTX-M-15 enzyme [13]. Rarely, members of this lineage also produce a CMY-2-like AmpC enzyme [13,14]. CTX-M-15 ESBL-producing *E. coli* UK epidemic strains A–E[2] have recently been shown to belong to the ST131 lineage [15], emphasizing the importance of the clone in the UK.

Repetitive sequence-based PCR (Rep-PCR) is based on amplification of non-coding repetitive DNA sequences interspersed throughout the genome [16]. During PCR, multiple DNA amplicons of different sizes are generated to produce a unique Rep-PCR profile for each bacterial strain. Differences in these Rep-PCR profiles can be used to distinguish among strains [16]. Several studies have used manual Rep-PCR methods for typing ESBL-producing *E. coli* [17] and verocytotoxin-producing *E. coli* O157 [18], although lack of standardization can lead to difficulties in data interpretation and interlaboratory comparisons.

A standardized, semi-automated Rep-PCR system, DiversiLab (bioMérieux, Basingstoke, UK), which uses microfluidic separation of PCR products, is now commercially available [19]. The approach has been successfully used to distinguish among strains of different organisms, such as *Acinetobacter* spp. [20], methicillin-resistant *Staphylococcus aureus* [21,22], *Staphylococcus epidermidis* [23], vancomycin-resistant *Enterococcus* spp. [24,25], *Clostridium difficile* [26], *Salmonella* [27], *Mycobacterium* spp. [28], and fungi [29]. These studies have shown that the DiversiLab system is a valuable tool for rapidly identifying the source of infection and tracking its spread, and suggest that the approach could be used to improve patient management and reduce the costs associated with treatment, contamination, and decontamination [19].

In the current study, a collection of well-characterized UPEC strains, including strains of the O25:H4-ST131 lineage with different antibiotic resistance phenotypes, were analysed using the DiversiLab system.

Materials and Methods

Bacterial isolates

Thirty-five UPEC isolates that had been previously typed using MLST [7], including a subcollection that had also been previously analysed by PFGE (N. Woodford, M.E. Kaufmann, A.J. Fox, M. Upton, unpublished data), were used to evaluate the DiversiLab system. The collection comprised 20 CTX-M ESBL producers, three isolates producing both CTX-M ESBLs and CMY-2-like AmpC enzymes, two that were ESBL-negative but CMY-2-like AmpC-positive, and ten cephalosporin-susceptible isolates. The isolates included representatives of five PFGE-defined UK epidemic strains (A–E) [2] and isolates from hospitals in the North West region of England.

The isolates were cultured on Columbia agar plates (Oxoid, Basingstoke, UK) by incubation for 18 h at 37°C.

DNA extraction

A loopful of colonial growth was used for DNA extraction with the UltraClean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The concentration of DNA was measured with an ND-1000 spectrophotometer (NanoDrop; Agilent Technologies Inc., Santa Clara, CA, USA) and was diluted to a working concentration of 25–50 ng/μL.

Rep-PCR amplification

The DiversiLab Escherichia DNA Fingerprinting Kit (bioMerieux, Inc., Basingstoke, UK) was used for Rep-PCR amplification of non-coding intergenic repetitive elements in the genomic DNA. The kit included Rep-PCR master mix 1, primer mix J, and kit-specific positive and negative controls.

Amplification reactions were carried out in 25-μL reaction volumes containing 2 μL of DNA (approximately 25–50 ng/μL of DNA), 18 μL of Rep-PCR master mix 1, 2 μL of primer mix J, 2.5 μL of GeneAmp 10$\times$ PCR buffer (ABI), and 0.5 μL of AmpliTaq DNA polymerase (Applied Biosystems, Warrington, UK).

The Rep-PCR reactions were carried out using an Eppendorf thermal cycler (Mastercycler; Helena Biosciences, Gateshead, UK), and the conditions included an initial denaturation at 94°C for 2 min and 35 cycles of the following: denaturation at 94°C for 30 s; annealing at 50°C for 30 s; extension at 72°C for 90 s; and a final extension at 72°C for 3 min. The kit-specific positive and negative controls were run together with each set of reactions for validation of amplification.

Separation of Rep-PCR amplification products

Rep-PCR products, mixed with a gel–dye matrix with intercalating fluorescent dye, were detected using microfluidic 'lab-on-chip' technology, in which DNA fragments are separated on the basis of their size. There were 13 sample wells on each chip. Each sample well was loaded with 5 μL of DNA marker and 1 μL of PCR product. The microfluidics chip was placed on an Agilent 2100 bioanalyzer (Agilent), and separation of the amplicon was carried out in <1 h.

Data analysis

The DNA profiles were automatically downloaded onto a secure DiversiLab website dedicated to our laboratory, where each chip went through a quality control step in which only patterns with a fluorescence intensity >100 units