Escherichia coli clonal group A causing bacteraemia of urinary tract origin

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Abstract

Escherichia coli clonal group A (CgA) causes disease in humans. This is the first study investigating the prevalence of CgA among *E. coli* from non-urine, extraintestinal infections in a northern European country. *E. coli* blood (n = 196) and paired urine (n = 195) isolates from the same patients with bacteraemia of urinary tract origin were analysed. The isolates were collected from January 2003 through May 2005 at four hospitals in Copenhagen, Denmark. Pulsed-field gel electrophoresis (PFGE) patterns, antimicrobial resistance and patient characteristics were determined for all CgA isolates; presence of virulence-associated genes (VAGs) and serotypes were determined for the blood CgA isolates. Thirty blood isolates (15%) belonged to CgA. CgA blood isolates were associated with female patients and sulfamethoxazole-trimethoprim resistance and they harboured a distinctive VAG profile. The blood and urine isolates from each pair were found to be related in 26 of 27 CgA blood/urine pairs, confirming a urinary tract origin of infection. Furthermore, a relationship between the PFGE patterns of CgA blood/urine isolates and CgA isolates from UTI patients in general practice and a CgA isolate from a community-dwelling human reported previously, was found, suggesting a community origin of CgA. The finding of CgA strains in 15% of the *E. coli* bloodstream infections with a urinary tract origin in Denmark suggests that CgA constitutes an important clonal lineage among extraintestinal pathogenic *E. coli*. A reservoir of this pathogenic *E. coli* group in the community causing not only UTI but also more severe infections such as bacteraemia has implications for public health.

Keywords: Antimicrobial resistance, bacteraemia, clonal group A, *Escherichia coli*, PFGE typing, serotyping, urinary tract infection, virulence

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Introduction

Evidence is increasing that *Escherichia coli* causing urinary tract infections (UTIs) and other extraintestinal infections may be responsible for community epidemics [1,2]. A multidrug-resistant clonal group, termed clonal group A (CgA), was identified as a cause of community-acquired UTI outbreaks in North America in 2001 [3,4]. CgA isolates belong to *E. coli* phylogenetic group D [5] and multilocus sequence typing (MLST) clonal complex 69 (CC69) [6,7], they possess a conserved virulence gene profile [3–5,8], and they are often resistant to trimethoprim-sulphonamides [5,6].

CgA has a worldwide endemic distribution with a concentration in the western world [9]. However, the distribution of CgA in Europe has received little investigation [8–11]. CgA isolates cause extraintestinal infections and especially its distribution among uropathogenic *E. coli* (UPEC) has been investigated [5,6,8,10,12]. Thus, assessment of the distribution of CgA isolates in Europe and especially its prevalence among complicated, extraintestinal infections such as bloodstream infections is limited.

The aim of the study was to investigate the prevalence of CgA among *E. coli* isolates from cases of bacteraemia with a urinary tract origin in a Northern European country. Also, the study of paired blood and urine isolates from the same patients could provide further insight into the pathogenesis of *E. coli* bacteraemia with a urinary tract origin.

Methods

Bacterial isolates and patients

E. coli blood (n = 196) and urine (n = 195) isolates collected from January 2003 through May 2005 from 195 adult patients with both bacteraemia and bacteriuria admitted to four hospitals in Copenhagen, as reported elsewhere [13], were studied. From one patient, two *E. coli* blood isolates were cultured. Epidemiological, clinical and laboratory data for each infection episode were extracted from the patients' medical records and the hospital administrative database. Bacteraemia episodes were considered to be community acquired when they were diagnosed within the first 48 h of hospitalization and were considered hospital acquired after this period. Sampling of the isolates was approved by the Scientific Ethics Committee for the Capital Region of Denmark [(KF) 01 2006-6371] [13].

Phylogenetic analysis

The phylogenetic background (A, B1, B2 and D) of the blood isolates [13] and the corresponding urine isolates was determined by triplex PCR [14]. Classification of the isolates into the four major *E. coli* phylogenetic lineages or non-typeable (NT) isolates was undertaken according to Gordon *et al.* [15].

Clonal group A

The phylogenetic group D isolates were screened by PCR for CgA-associated single-nucleotide polymorphisms (SNPs) in *fumC* and *gyrB* [12]. The presence of these SNPs identified strains belonging to CC69 and excluded strains belonging to the closely related CC394.

Virulence genotyping

The blood isolates were previously screened for the presence of 29 VAGs with known or suspected relevance to ExPEC pathogenesis [13].

Antimicrobial resistance

Minimum inhibitory concentrations (MICs) of ciprofloxacin, nalidixic acid, sulfamethoxazole and trimethoprim for the blood isolates and the corresponding urine isolates were determined by a micro broth dilution method as described in Skjøt-Rasmussen *et al.*, 2012 [13].

Pulsed-field gel electrophoresis

The CgA blood and urine isolates and the CgA reference strain ATCC BAA-457 were typed by pulsed-field gel electrophoresis (PFGE) with XbaI using the protocol made available by the CDC's PulseNet database [16,17]. The PFGE patterns were compared using BioNumerics 6.6 (Applied Maths, Kortrijk, Belgium). Isolates were considered to be related if their Dice similarity index was ≥85%. Also included in the analysis was *E. coli* from animals (Danish broiler chickens, n = 5), meat (Danish broiler chicken meat, n = 4) and humans (Danish community-dwelling humans, n = 5; UTI patients from general practice, n = 11) that were PFGE typed as described earlier by Jakobsen *et al.* [10].

Serotyping

O, K and H antigens of the CgA blood isolates were detected using standard methods [18].

Statistical analysis

Comparisons of proportions were based on the chi-square test or, when expected numbers were <5, Fisher's exact test (two-tailed). In order to reduce the risk of type-I error with multiple comparisons, a p-value <0.01 was considered statistically significant.

Results

Among the 196 blood and 195 urine isolates, 44 phylogroup D blood isolates and 42 phylogroup D urine isolates were found. Of the investigated phylogroup D isolates, 30 blood isolates and 28 urine isolates belonged to CgA. Of these, 27 blood and urine CgA isolates, respectively, came from the same 27 patients. Thus, CgA was found to be responsible for 15% (30/196) of the bloodstream infections.

The median age of the 30 patients experiencing CgA bacteraemia was 78 years (range, 24–94 years). Of the CgA blood isolates, 97% were from women whereas only 63% of the non-CgA blood isolates were from women (p <0.001) (Table 1).

Among the 196 blood isolates, 21 isolates were from hospital-acquired infections and 175 isolates were from community-acquired infections. CgA was responsible for 17% (29/175) of the community-acquired and 5% (1/21) of the hospital-acquired bloodstream infections with a urinary tract origin (Table 1).

Total 30-day mortality did not differ among the CgA bacteraemia episodes and the non-CgA episodes (Table 1).