Escherichia coli phylogenetic groups are associated with site of infection and level of antibiotic resistance in community-acquired bacteraemia: a 10 year population-based study in Denmark

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Objectives: The aim of this study was to assess whether Escherichia coli phylogenetic groups were associated with the site of infection and the level of antibiotic resistance in community-acquired bacteraemia (CAB).

Methods: The population-based cohort study included 1533 unique isolates of E. coli from Danish patients with CAB during a 10 year period. Triplex PCR was used to classify the phylogenetic groups, and susceptibility testing was performed by disc diffusion. Data were analysed using contingency tables and logistic regression.

Results: Overall, 65.9% of the 1533 E. coli isolates belonged to phylogroup B2, 16.6% to D, 13.1% to A and 4.4% to B1. B2 was the most prevalent group for all sites of infection, ranging from 69.9% in cases with a urinary tract site of infection to 54.8% in cases with a hepatobiliary tract site of infection. Antibiotic resistance to one and more than three antibiotics, respectively, was most frequent in group D (11.4%/33.9%), followed by A (5.5%/26.9%), B1 (5.9%/19.1%) and B2 (6.7%/7.5%). Regression analysis, with group B2 as reference, confirmed that groups A and B1 were associated with a site of infection other than the urinary tract and that groups A and D were associated with resistance to antibiotics including ampicillin, sulphonamide, trimethoprim, gentamicin and quinolones.

Conclusions: Phylogenetic group B2 was predominant in E. coli CAB. This was the least resistant of the four groups. Phylogroups A and B1 were associated with sites of infection other than the urinary tract, and resistance to multiple antibiotics was most prevalent for groups A and D.

Keywords: epidemiology, bloodstream infections, antimicrobials, PCR

Introduction

Escherichia coli is the most frequent cause of community-acquired bacteraemia (CAB). In recent years, increasing attention has been given to the phylogenetic affiliation of E. coli. However, only a few studies have been directed primarily at blood culture isolates. Sannes et al. studied 63 blood culture isolates from a US veterans hospital and found a predominance of phylogroup B2 isolates, with phylogroup D as second (67% and 19%, respectively). A similar distribution was reported in a French study including 161 isolates from two university hospitals. In this study, multivariable analysis indicated that group B2 was distinctive with regard to occurrence of resistance, nosocomial origin and site of infection. A Spanish cohort study including 185 adult patients from one university hospital found that phylogroup D accounted for 52% of the incidences, whereas groups A, B1 and B2 accounted for 12%–18% each. These results, however, should be interpreted with caution because of the limited sample sizes, discrepant inclusion criteria and varying extent of clinical information.

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Furthermore, the nosocomial origin of infection may have confounded the reported distributions. To expand knowledge of CAB and limit the impact of nosocomial infections, a population-based cohort study of *E. coli* CAB was performed in Northern Jutland, Denmark. The aim was to assess the association between phylogenetic groups of *E. coli* and site of infection and antibiotic resistance, respectively.

### Materials and methods

#### Study subjects

The historical cohort study included isolates of *E. coli* from patients with CAB in North Jutland County, Denmark, during a 10 year period from 1 January 1995 to 31 December 2004. The county’s population was 488,303 in 1995 and 495,669 in 2004. All county residents had access to tax-paid healthcare free of charge provided by general practitioners and seven public hospitals. Six were district hospitals and one, Aalborg Hospital (600 beds), served as both a district hospital for the greater Aalborg area (~200,000 inhabitants) and as a referral hospital. Private clinics did not offer acute hospital care. The Department of Clinical Microbiology, Aalborg Hospital, provided diagnostic services, including blood cultures, to the county’s hospitals. The department maintains the North Jutland Bacteraemia Research Registry, which provided information on all cases of CAB caused by *E. coli*.

* Definitions

We defined bacteraemia as a clinical episode with one or more positive blood cultures given significance by a medically trained microbiologist and the attending physicians. In patients with recurrent positive blood cultures, a new episode was recorded after an interval of 30 days, except for cases in which the second episode was associated with a site (focus) of infection different from the primary site. Determination of the most likely focus was based on available clinical and microbiological evidence. Nosocomial bacteraemia was classified according to CDC criteria. Patients with hospital contact within 30 days prior to the admission or regular hospital contact (e.g. for haemodialysis or chemotherapy) were considered a separate healthcare-related group. For this study, we excluded patients with nosocomial or healthcare-related bacteraemia.

#### Diagnostic bacteriology

The processing of blood cultures (BacT/Alert, bioMérieux, Marcy l’Étoile, France) is described in the Appendix [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Isolates were stored at −70°C until further study. Susceptibility testing was performed with disc diffusion (Oxoid, Basingstoke, UK) on Iso-Sensitest agar (SSI Diagnostika, Hillerød, Denmark) using semi-confluent growth. Bacteria were classified as either susceptible or resistant in accordance with the epidemiological breakpoint for resistance given by the Swedish Reference for Antibiotics (SRGA) (see Appendix).

#### Table 1. Gender and age of patients, site of infection and time period according to phylogenetic groups of 1533 *E. coli* isolated from bacteraemia patients in the North Jutland Country, Denmark, 1995–2004; data are displayed as percentages of the total number of isolates (*n*)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total</th>
<th>Phylogenetic group % (<em>n</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>60.6 (929)</td>
<td>55.2 (111)</td>
</tr>
<tr>
<td>male</td>
<td>39.4 (604)</td>
<td>44.8 (90)</td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–15</td>
<td>0.9 (14)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>16–65</td>
<td>25.0 (383)</td>
<td>18.4 (37)</td>
</tr>
<tr>
<td>66–80</td>
<td>40.1 (615)</td>
<td>47.8 (96)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>34.0 (521)</td>
<td>33.8 (68)</td>
</tr>
<tr>
<td>Site of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urinary tract</td>
<td>62.8 (963)</td>
<td>44.8 (90)</td>
</tr>
<tr>
<td>hepatobiliary tract</td>
<td>12.1 (186)</td>
<td>24.4 (49)</td>
</tr>
<tr>
<td>gastrointestinal tract</td>
<td>6.4 (98)</td>
<td>10.4 (21)</td>
</tr>
<tr>
<td>miscellaneous</td>
<td>2.2 (33)</td>
<td>1.5 (3)</td>
</tr>
<tr>
<td>unknown</td>
<td>16.5 (253)</td>
<td>18.9 (38)</td>
</tr>
<tr>
<td>Time period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995–1996</td>
<td>17.4 (267)</td>
<td>18.9 (38)</td>
</tr>
<tr>
<td>1997–1998</td>
<td>17.6 (270)</td>
<td>21.4 (43)</td>
</tr>
<tr>
<td>1999–2000</td>
<td>20.0 (306)</td>
<td>18.4 (37)</td>
</tr>
<tr>
<td>2001–2002</td>
<td>21.0 (322)</td>
<td>21.9 (44)</td>
</tr>
<tr>
<td>2003–2004</td>
<td>24.0 (368)</td>
<td>19.4 (39)</td>
</tr>
<tr>
<td>Total</td>
<td>100.0 (1533)</td>
<td>13.1 (201)</td>
</tr>
</tbody>
</table>
In addition, β-lactamase testing was carried out on selected isolates from each run. A total of 37 isolates either resistant to cefpodoxime or exhibiting the smallest zone size in the run was tested for extended-spectrum β-lactamase (ESBL) activity using both cefotaxime + clavulanic acid and ceftazidime + clavulanic acid (Etest, Biodisk, Solna, Sweden). A total of 48 isolates were tested for production of chromosomal β-lactamases (AmpC) by cefotetan and cefotetan/boronic acid discs (Oxoid) on Mueller-Hinton II agar.16 They included ESBL-negative isolates and random isolates which were both carbenicillin susceptible and resistant.

### Determination of E. coli phylogenetic groups

For each isolate included, genomic DNA was isolated from one colony.17 One hundred randomly selected isolates (10 from each year) were tested by PCR to confirm the species diagnosis by amplifying a region of the 16S rRNA gene.18 We determined four phylogenetic groups of E. coli (A, B1, B2 and D) by use of triplex PCR as described by Clermont et al.19 We included 72 isolates from the international Escherichia coli Reference Collection (ECOR) as reference strains.20 The methods are described in detail in the Appendix.

### Statistics

Study variables included phylogenetic group, resistance to main representatives of antibiotic groups (ampicillin, mecillinam, carbenicillin, cefuroxime, gentamicin, sulphonamide, trimethoprim and nalidixic acid), calendar year, gender and age group (0–15, 16–65, 66–80 and >80 years). Data were primarily analysed by contingency tables. Proportions were compared using the χ² test (P<0.05 being considered statistically significant). We used multinomial logistic regression to assess the association between phylogenetic groups and site of infection, and logistic regression was used to assess the association between phylogenetic groups and resistance to one antibiotic (STATA 9.2; StataCorp LP, College Station, TX, USA). In both models, phylogroup B2 was used as reference. We calculated odds ratios (ORs) with 95% confidence intervals (CIs), and calendar year, gender, age group and site of infection were included as potential confounders.

The study was conducted according to the guidelines of the regional scientific ethics committee for use of clinical and laboratory data.

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**Figure 1.** Antimicrobial resistance (%) among 1533 E. coli blood culture isolates from patients with CAB in North Jutland County, Denmark (1995–2004). (a) Trimethoprim (TMP), sulfamethoxazole (SUL), carbenicillin (CAR) and ampicillin (AMP). (b) Gentamicin (GEN), ciprofloxacin (CIP) and nalidixic acid (NAL). (c) Mecillinam (MEC), cefuroxime (CXM), cefadroxil (CFR), cefpodoxime (CPD) and amoxicillin/clavulanic acid (AMC).

**Figure 2.** Antimicrobial resistance (%) among 1533 E. coli blood culture isolates from patients with CAB in North Jutland County, Denmark (1995–2004). The total number of isolates within each phylogenetic group was set to 100%. AMP, ampicillin; MEC, mecillinam; CAR, carbenicillin; AMC, amoxicillin/clavulanic acid; CEF, cefalotin; CXM, cefuroxime; CFR, cefadroxil; CPD, cefpodoxime; NAL, nalidixic acid; CIP, ciprofloxacin; SUL, sulfamethoxazole; TMP, trimethoprim; GEN, gentamicin.
Table 2. Association between antibiotic resistance and *E. coli* phylogenetic groups

<table>
<thead>
<tr>
<th>Phylogroups</th>
<th>Antibiotic resistance, OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ampicillin</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>201</td>
</tr>
<tr>
<td><strong>B1</strong></td>
<td>68</td>
</tr>
<tr>
<td><strong>B2</strong></td>
<td>1010</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>254</td>
</tr>
</tbody>
</table>

For each antibiotic, the association between the resistant phenotype and phylogenetic groups was determined by logistic regression. Odds ratios (ORs) with a 95% confidence interval (CI) were determined using group B2 as reference, and gender and age groups were included as potential confounders.

*aResistance to ciprofloxacin and gentamicin was not detected among group B1 isolates.

Results

A total of 1542 cases of *E. coli* CAB were recorded from 1995 to 2004. The incidence rose from 27.3 per 100000 inhabitants per year in 1995–96 to 37.1 in 2003–04. Nine isolates were not available (1–3 isolates per year), leaving 1533 isolates for further study. The male/female distribution was 39.4%/60.6% and median age was 76 years (range 0–98 years).

A primary site of infection was determined in 1280 cases: the urinary tract in 963 (62.8%), the hepatobiliary tract in 186 (12.1%) and the gastrointestinal tract (including the pancreas) in 98 (6.4%). Miscellaneous sites accounted for 33 cases (2.2%), and the site remained unknown in 253 (16.5%).

*E. coli* phylogenetic group

Initially, we analysed 72 ECOR isolates to evaluate the triplex PCR method and found a phylogenetic affiliation that was in accordance with previous reports. For the 1533 bacteraemia isolates, Table 1 shows the distribution for each phylogenetic group according to gender, age group, site of infection and time period. The preponderance of women was most notable for groups D and B1 \((\chi^2, \text{df}=3, P=0.001)\). Compared with the other groups, group B1 isolates were most prevalent in patients of >80 years \((\chi^2, \text{df}=9, P=0.003)\). We observed more bacteraemias in the most recent years, but the phylogenetic distribution did not vary significantly over the years \((\chi^2, \text{df}=12, P=0.75)\).

Group B2 predominated in all sites of infection [Figure S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Groups B2 and D accounted for 87.5% of cases with a urinary tract focus, whereas group A and B1 isolates were more frequently associated with the hepatobiliary tract \((\chi^2, \text{df}=12, P<0.001)\).

Groups A and B1 were associated with sites of infection other than the urinary tract, whereas group D was comparable to the reference group B2 [Table S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. Adjusting for age and gender only marginally changed the risk estimates.

Antimicrobial resistance

In 1995, the annual prevalence of resistance was 28.1% for ampicillin, 26.4% for carbenicillin, 26.4% for sulphonamides and 12.3% for trimethoprim (Figure 1a). In 2004, the resistance had increased to 33.1% for both ampicillin and carbenicillin, 35.5% for sulphonamides and 20.1% for trimethoprim (Figure 1a). Annual prevalence was lower (≤8%) for nalidixic acid, ciprofloxacin and gentamicin (Figure 1b), and even lower for amoxicillin/ clavulanic acid, mexitilmin and cephalosporins (Figure 1c).

The frequency of resistance among phylogenetic groups is shown in Figure 2. Overall, group B2 isolates were less resistant compared with group A, B1 and D isolates. Resistance to ampicillin, carbenicillin, sulfamethoxazole and trimethoprim was especially prevalent among group D isolates and to a lesser extent among group A isolates. We mainly detected cephalosporin resistance among group A isolates, and this group accounted for 50% of AmpC-positive isolates (8 of 16). Conversely, isolates resistant to quinolones and aminoglycoside were mainly found in group D. Among 37 isolates, 28 of which were cefpodoxime resistant, we detected one phylogroup B2 isolate from 2002 with a CTX-M (an ESBL) profile.

Combined resistance to ampicillin, sulfamethoxazole and trimethoprim was most prevalent among isolates classified into groups D (21.7%), A (16.4%) and B1 (13.2%), but infrequent among group B2 isolates (4.9%). Only 2.9% of group B1 isolates were resistant to both ampicillin and sulfamethoxazole compared with ~12% of isolates in the other groups. Multiple resistance was more prevalent among group A and D isolates compared with group B1 and B2 isolates \((\chi^2, \text{df}=12, P<0.001)\) [Table S2, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

We further studied the association between phylogenetic groups and antibiotic resistance (Table 2). Adjusting for age and gender did little impact on the risk estimates (data not shown). We confirmed strong associations between groups A and D, respectively, and resistance to each of the six antibiotics in Table 2. The only notable association found for group B1 was trimethoprim resistance (OR 4.0, 95% CI 2.1–7.5).

Discussion

This study showed that two-thirds of 1533 *E. coli* isolates in Danish patients with CAB were classified into phylogenetic group B2 using the triplex PCR method of Clermont et al. Groups A and D were comparable in size, whereas B1 was the...
E. coli phylogenetic groups

least abundant. Interestingly, our B2 isolates were by far the least resistant of the four phylogroups examined. Compared with B2, our groups A and B1 were associated with sites of infection distinct from the urinary tract, and groups A and D were associated both with resistance to individual antibiotics and with multiple resistance (more than three antibiotics).

Our study has a number of strengths. First, we included >1500 blood culture isolates leaving out only a few isolates for technical reasons. Secondly, antibiotic susceptibility tests were performed using a disc diffusion method, which has previously been shown to be useful for epidemiological studies of E. coli.22 Thirdly, the population-based design reduced the risk of selection bias. Finally, we restricted the study to patients with CAB and thereby reduced confounding by nosocomial origin of infection. Clinical data were collected prospectively in a bacteraemia research database of acknowledged validity.2,23,24

There are, however, a number of limitations. First, concern has been raised about basing phylogenetic affiliation solely on triplex PCR.25,26 Although more extensive taxonomic investigations may be warranted, the triplex method was still highly reproducible as indicated by our complete match with published results pertaining to the ECOR collection. Moreover, the use of this method enables comparison with a number of recent studies of E. coli infections in humans including the three bacteraemia studies.11–13 Secondly, we used phenotypic methods to determine antibiotic resistance. Genetic information on β-lactam, aminoglycoside and quinolone resistance as well as the presence of integrons would have been more definitive. At the inception of the study, we were aware of the rare but significant occurrence of up-regulated chromosomal β-lactamase in E. coli,27 but ESBLs were not yet recognized as a clinical problem in Denmark. Still, the inclusion of cefpodoxime enabled us to perform a secondary search for ESBL isolates. However, these limitations should not seriously affect our conclusions with regard to the E. coli phylogenetic groups.

Extraintestinal pathogenic E. coli are most frequently classified into phylogroups B2 and D.5,9,19 As for bacteraemic E. coli, our results corroborate the distribution of phylogenetic groups previously reported by Sannes et al.11 and Jauréguy et al.12, and deviate distinctly from the results of Martínez et al.13 In France, Jauréguy et al.12 also found B2 to be more susceptible to antibiotics than isolates belonging to the other three groups. On the other hand, in Spain, Martínez et al.13 observed phylogroup D isolates to be predominant (52%) as opposed to the 16.6% found in our study. Phylogroup B2 accounted for 18% of Spanish isolates compared with 65.9% in our study. The inclusion of a mixture of community-acquired and nosocomial cases from a single hospital makes confounding a likely explanation. In our study, group D had the highest prevalence of antibiotic resistance, and we speculate whether extensive use of broad-spectrum antibiotics could be another confounding factor in the Spanish study.

We find the association between groups A and B1 and a site of infection other than the urinary tract to be of clinical interest. Much attention has been devoted to uropathogenic E. coli that mostly belong to groups B2 and D.9,28 In comparison, little is known about the propensity of E. coli to infect sites other than the urinary tract, with the exception of the CNS.29 In our study, patients with hepatobiliary septicemia were relatively numerous, and the association with group A deserves further study.

Our results concerning phylogenetic groups and antibiotic resistance are in line with previous studies,11,12,30 including the association between group A and expression of chromosomal β-lactamase.30 Still, many studies lack detailed information on the origin of isolates or the precise contribution of nosocomial versus community-acquired infections. Based on data on phylogenetic groups, patterns of antibiotic resistance and molecular typing, poultry has been incriminated as a source of uropathogenic E. coli infections in humans.31 Also the human intestinal flora is considered an important reservoir for extraintestinal E. coli infections, but may potentially be linked to other reservoirs. Currently, these reservoirs have not been identified. Pigs and ruminants (e.g. sheep, goats and cattle), but also wildlife, have been shown to host various strains of pathogenic E. coli.32,33 Furthermore, drinking water and recreational waters in rural areas are frequently contaminated with diverse E. coli strains.34,35 As a result, environmental exposure should not be neglected as a potential source of extraintestinal E. coli infections. Our observation of different prevalence and patterns of antibiotic resistance among the four phylogenetic groups is compatible with the existence of several ecological niches in humans, animals or nature with different antibiotic exposure profiles.

Acknowledgements

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Transparency declarations

None to declare.

Supplementary data

The Appendix, Figure S1, Table S1 and Table S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

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