Phylogenetic background and carriage of pathogenicity island-like domains in relation to antibiotic resistance profiles among \textit{Escherichia coli} urosepsis isolates

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We studied 100 well-characterized \textit{E. coli} blood isolates from patients with urosepsis for their susceptibility to nalidixic acid, ampicillin and trimethoprim–sulfamethoxazole, according to prevalence of virulence factors, phylogenetic groups and subgroups, PAI II\textsubscript{J96}-like domains (determined by physical linkage of \textit{cnf1}, \textit{hly} and \textit{hra}) and PAI ICFT073-like domains (determined by physical linkage of \textit{papGII} to the \textit{hly} locus). Nalidixic acid resistance was associated with a lower prevalence of \textit{sfa/foc}, K1 antigen, pathogenicity island II\textsubscript{J96}-like domains, subgroup B2/I and a shift towards group A.

Keywords: virulence factors, ribotyping, quinolones, PCR

Introduction

\textit{Escherichia coli} is a common cause of extraintestinal infections, such as neonatal meningitis, urinary tract infection and bacteraemia.\textsuperscript{1–3} Several virulence factors (VFs), gathered into pathogenicity islands (PAIs) such as PAI ICFT073 and PAI II\textsubscript{J96}, enhance the capacity of \textit{E. coli} to cause systemic infections.\textsuperscript{4,5} Most strains that cause extraintestinal infections belong to phylogenetic group B2 or D, whereas commensal strains belong mostly to group A or B1.\textsuperscript{5–8} Recent studies show an increase in the incidence of \textit{E. coli} sepsis\textsuperscript{9,10} and in the prevalence of antimicrobial resistance.\textsuperscript{11–14} Few authors have examined the possible relation between antibiotic resistance among invasive isolates and their phylogenetic subgroup distribution or carriage of VFs and PAIs.\textsuperscript{15–17}

Here we examined 100 well-characterized \textit{E. coli} blood isolates from patients with urosepsis for their susceptibility to nalidixic acid, ampicillin and trimethoprim/sulfamethoxazole, according to prevalence of VFs, phylogenetic groups and subgroups, PAI II\textsubscript{J96}-like domains (determined by physical linkage of \textit{cnf1}, \textit{hly} and \textit{hra}) and PAI ICFT073-like domains (determined by physical linkage of \textit{papGII} to the \textit{hly} locus).\textsuperscript{3}

Materials and methods

The 100 \textit{E. coli} isolates previously published were recovered by blood culture from 100 consecutive adults with both community-acquired pyelonephritis and bacteraemia.\textsuperscript{3,18} The isolates were characterized by means of PCR phylogenetic grouping, ribotyping and PCR detection of virulence-related genes.\textsuperscript{3,18} Susceptibility to ampicillin, trimethoprim/sulfamethoxazole, nalidixic acid and ciprofloxacin was determined by the disc diffusion method (Bio-Rad),\textsuperscript{19} using CLSI interpretative criteria.\textsuperscript{20} \textit{E. coli} 25922 (ATCC) was used as the reference strain.

Statistical methods

\textit{P} values of <0.05 in Fisher’s exact test were considered to denote significant relationships.

Results

The prevalence of antibiotic resistance was as follows: nalidixic acid, 15%; ampicillin, 58%; trimethoprim/sulfamethoxazole, 25%; and ciprofloxacin, 9%.

Table 1 shows the prevalence of individual VFs and phylogenetic groups according to antibiotic susceptibility status.
Pathogenicity islands and resistant *E. coli*

**Table 1.** Distribution of phylogenetic groups and subgroups, virulence factors and PAI-like domains according to resistance phenotype among 100 *E. coli* urosepsis isolates

<table>
<thead>
<tr>
<th></th>
<th>All isolates (n = 100)</th>
<th>Nalidixic acid</th>
<th>Ampicillin</th>
<th>Trimethoprim/sulfamethoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>resistant (n = 15)</td>
<td>susceptible (n = 85)</td>
<td>resistant (n = 58)</td>
</tr>
<tr>
<td><strong>Group B2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subgroup B2/I^a^</td>
<td>20</td>
<td>0</td>
<td>20 (23)</td>
<td>0.036</td>
</tr>
<tr>
<td>subgroup B2/II</td>
<td>19</td>
<td>0</td>
<td>19 (22)</td>
<td>0.06</td>
</tr>
<tr>
<td>subgroup B2/III</td>
<td>12</td>
<td>1 (6)</td>
<td>11 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>subgroup B2/IX</td>
<td>4</td>
<td>0</td>
<td>4 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>subgroup B2/X</td>
<td>4</td>
<td>2 (13)</td>
<td>2 (2)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Group D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subgroup D/IV</td>
<td>12</td>
<td>0</td>
<td>12 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>subgroup D/V</td>
<td>7</td>
<td>1 (6)</td>
<td>6 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>subgroup D/VIII</td>
<td>4</td>
<td>4 (26)</td>
<td>0</td>
<td>0.0003</td>
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<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subgroup A/VI</td>
<td>6</td>
<td>2 (13)</td>
<td>4 (4)</td>
<td>0.2</td>
</tr>
<tr>
<td>subgroup A/VII</td>
<td>4</td>
<td>2 (13)</td>
<td>2 (2)</td>
<td>0.038</td>
</tr>
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<td><strong>Group B1</strong></td>
<td>1</td>
<td>0</td>
<td>1 (01)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>papC</strong></td>
<td>78</td>
<td>9 (60)</td>
<td>69 (81)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>papGII</strong></td>
<td>68</td>
<td>8 (53)</td>
<td>60 (70)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>papGIII</strong></td>
<td>14</td>
<td>0</td>
<td>14 (16)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>sfa/foc</strong></td>
<td>28</td>
<td>0</td>
<td>28 (32)</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>aer</strong></td>
<td>79</td>
<td>12 (80)</td>
<td>67 (78)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>fyuA</strong></td>
<td>92</td>
<td>13 (86)</td>
<td>79 (92)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>hly</strong></td>
<td>34</td>
<td>3 (20)</td>
<td>31 (36)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>K1</strong></td>
<td>20</td>
<td>0</td>
<td>20 (23)</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>PAI II_{p96}-like domain</strong>^b^</td>
<td>21</td>
<td>0</td>
<td>21 (24)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>PAI I_{CFT073}-like domain</strong>^b^</td>
<td>16</td>
<td>1 (6)</td>
<td>15 (17)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

*Phylogenetic subgroups are designated according to the phylogenetic group and the ribotype: i.e. subgroup B2/I corresponds to phylogenetic group B2/ribotype I.

Defined by physical linkage of hra, cnf1 and hly (3).

Defined by physical linkage of papGII and hly (3).

Nalidixic acid susceptibility was associated with phylogenetic group B2 (68% versus 20% of resistant isolates; P = 0.0004), the virulence genes sfa/foc (32% versus 0%; P = 0.009), K1 antigen (23% versus 0%; P = 0.03) and the PAI II_{p96}-like domain (24% versus 0%; P = 0.035), whereas nalidixic acid resistance was associated with group A (33% versus 7% of susceptible isolates; P = 0.003). Ampicillin and trimethoprim/sulfamethoxazole susceptibility status did not influence the prevalence of the studied VFs. Ciprofloxacin-resistant isolates were too rare to determine the relationship between fluoroquinolone susceptibility status and VFs. Trimethoprim/sulfamethoxazole susceptibility was associated with group B2 (68% versus 40%, P = 0.01), whereas trimethoprim/sulfamethoxazole resistance was associated with group D (48% versus 20%, P = 0.013).

Among the 15 ribotypes (I–XV) identified in our collection of 100 urosepsis isolates, nalidixic acid susceptibility (15 isolates) was associated with subgroup B2/I (P = 0.0036) and showed a borderline relationship with subgroup B2/II (P = 0.06). Nalidixic-acid-resistant isolates and trimethoprim/sulfamethoxazole-resistant isolates were significantly associated with subgroup D/VIII, while ampicillin-resistant isolates were significantly associated with subgroup A/VI.

Subgroup B2/III strains included three nalidixic-acid-susceptible isolates carrying a PAI II_{p96}-like domain (isolates P76, P52 and P77) and also one nalidixic-acid-resistant isolate lacking this PAI (isolate P66). No difference in terms of antimicrobial susceptibility and/or VF carriage was found between compromised and non-compromised hosts.

**Discussion**

The present study shows that nalidixic-acid-resistant urosepsis strains isolated in France are significantly less likely than their susceptible counterparts to harbour sfa/foc (in accordance with Moreno et al.21), K1 antigen and PAI II_{p96}-like domains. Previous
studies have shown that quinolone-resistant compared with quinolone-susceptible *E. coli* UTI isolates have a lower prevalence of *cnf1* and haemolysis,\(^2,22\) \(\beta\)-haemolysin and *papEF,\(^2,23\) and *hly* and *cnf1.\(^1,24,25\) *hly* and *cnf1* are specific markers of PAI II,\(^2,26\) All these studies suggest that nalidixic-acid-resistant strains are significantly less likely than their susceptible counterparts to harbour PAI II-like domains. PAI II-like domains, such as *sfa/foc*, are restricted to group B,\(^2\) and differences in phylogenetic distribution can therefore largely account for the observed differences in the prevalence of VFs between resistant and susceptible urosepsis isolates.\(^2,27,28\) We found a lower prevalence of group B2 (specifically subgroup B2/1) among quinolone-resistant isolates, in accordance with Moreno et al.\(^21\) and Johnson et al.\(^27\) but we also found a higher prevalence of group A strains, in agreement with Horcajada et al.\(^24\) and Moreno et al.\(^21\) Such a relationship could arise from a greater exposure to antibiotics of group A strains belonging to the faecal flora. We found that our subgroup B2/III strains included both nalidixic-acid-susceptible isolates carrying a PAI II-like domain and one nalidixic-acid-resistant isolate lacking PAI II.\(^26\) These results suggest that nalidixic-acid-resistant isolates may be mutants of nalidixic-acid-susceptible strains. The strain lacking PAI II may have lost this PAI in exchange for resistance. Indeed, by activating the SOS response to inhibition of DNA replication, quinolones may contribute to the excision of bacteriophages or PAIs from the bacterial chromosome.\(^2,29,30\) Recently, Soto et al.\(^31\) found that subinhibitory concentrations of quinolones induced the loss of a PAI containing *hly* and *cnf1*. However, recent studies suggest that the loss of virulence genes rarely occurs concomitantly with the acquisition of quinolone resistance and that this phenomenon is therefore unlikely to explain the paucity of VFs in resistant UTI isolates.\(^28\) Indeed, spontaneous ‘en bloc’ deletion of PAIs can occur in the absence of quinolone exposure, generating mutants with markedly reduced virulence.\(^5\)

Another possible explanation is the clonal spread of nalidixic-acid-resistant *E. coli* strains lacking VFs and/or PAIs during antibiotic exposure. However, ribotyping and VF profiling showed marked genetic heterogeneity in our panel: among the 15 nalidixic-acid-resistant isolates, 11 had unique profiles whereas the other four isolates had an identical profile. Thus nalidixic acid resistance in the population examined is not likely due to the spread of a single resistant clone but rather from several independent events.

Trimethoprim/sulfamethoxazole resistance was associated with a lower prevalence of highly virulent group B2 strains and with a higher prevalence of moderately virulent group D strains. This latter shift may help to explain why trimethoprim/sulfamethoxazole resistance was not associated with a marked reduction in infected virulence, in keeping with Vila\(^22\) but not with Moreno et al.\(^21\)

It is unclear how strains with apparently low virulence can cause sepsis not only in compromised hosts but also in non-compromised hosts. It is conceivable that these strains possess unrecognized VFs or that only certain VFs facilitate bacteremia. Epidemiological and experimental studies suggested that *papC*, *fyuA*/*irp-2* (encoding the HPI iron-uptake system) and *aer* were the minimal prerequisite for bacterial passage from a renal focus of infection into the bloodstream.\(^2,3,12,23\) As we tested only urosepsis isolates, all these VFs were present in almost all the strains, and no difference in the prevalence of these latter genes was found between resistant and susceptible isolates.

**Transparency declarations**

We do not have any commercial or other associations that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy).

**References**

17. Johnson JR, Moseley SL, Roberts PL et al. Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing


