Genetic Diversity and Virulence Profiles of *Escherichia coli* Isolates Causing Spontaneous Bacterial Peritonitis and Bacteremia in Patients with Cirrhosis

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Among patients with cirrhosis, infections caused by *Escherichia coli* organisms that translocate from the gut are a frequent and severe complication. One hundred ten *E. coli* isolates from 110 cirrhotic patients with spontaneous bacterial peritonitis and/or spontaneous bacteremia were characterized for their phylogenetic group and virulence genotype (34 extraintestinal virulence factor genes). Genetic relatedness was investigated by enterobacterial repetitive intergenic consensus sequence type 2 (ERIC-2) PCR typing and multilocus sequence typing. Phylogenetic groups A, B1, B2, and D accounted for 24%, 4%, 48%, and 24% of the population, respectively. Overall, 68 distinct ERIC-2 profiles were encountered. Eleven clonal groups, represented by multiple isolates (2 to 11) from the same sequence type (ST) or sequence type complex, were identified. These clonal groups accounted for 54 (49%) isolates overall. Membership in one of these clonal groups was more frequent among B2 isolates than non-B2 isolates (67% versus 32%, *P < 0.001*). The most frequent sequence types were ST95 (*n* = 13) and ST73 (*n* = 8), followed by the ST14 and ST10 complexes (*n* = 7). ST131 and ST69 were represented by three isolates each. Clonal group-associated isolates exhibited a greater prevalence of 11 virulence genes, including *pap* elements, than the other isolates. However, no association between clonal groups and host factors, type of infection, or mortality was observed. In conclusion, *E. coli* isolates causing spontaneous bacterial peritonitis and bacteremia in cirrhotic patients are genetically diverse. However, approximately half of the isolates belong to familiar clonal groups and exhibit extensive virulence profiles that may be associated with greater invasive potential.

Infections caused by intestinal bacteria are common complications in patients with cirrhosis (8, 30). Spontaneous bacterial peritonitis (SBP) and bacteremia are the most frequent and severe infections in such patients, with in-hospital mortality rates being 20 to 30% and the survival expectancy at 6 months being <50% (1, 29, 34). Increasing evidence indicates that these infections result from bacterial translocation (BT) from the intestinal lumen to the mesenteric lymph nodes, combined with failure of host defense mechanisms to clear the translocating organisms (8, 37). BT is also associated with activation of the immune system and hyperdynamic circulatory status in cirrhosis, thereby contributing to the development of ascites and hepatorenal syndrome (37). The presence of bacterial DNA in the blood or ascitic fluid of infected patients, considered a surrogate marker of BT, has been shown to predict decreased 1-year survival (38). Given the key role of BT in cirrhosis, it is not surprising that *Escherichia coli*, which within the intestinal microflora is both the predominant facultative organism and the most adept at translocating to mesenteric lymph nodes (33), is also the most frequent agent of bacteremia and SBP in cirrhotic patients.

Most extraintestinal *E. coli* strains that infect immunocompetent humans belong to phylogenetic group B2 and, to a lesser extent, group D and are characterized by the presence of virulence factors (VFs) that are necessary to overcome host defenses and invade host tissues (31). Recent studies have documented the existence of lineages of genetically related *E. coli* strains, designated clonal groups, which commonly cause urinary tract infections and other extraintestinal infections in various host populations (6, 9, 12, 16, 20–22, 35).

To date, the genetic structure and VF profiles of strains infecting cirrhotic patients remain undefined. It is unknown whether the phylogenetic background and VF content of *E. coli* strains influence the organisms’ capacity for translocation, invasiveness, and disease causation in such patients. Such a role may be suspected on the basis of *in vitro* and experimental findings that certain *E. coli* strains can translocate more efficiently than others across intestinal mucosa when it is exposed to metabolic and inflammatory stress (15, 18, 19). These studies suggest that translocating strains may represent a particular group of *E. coli* isolates with specific abilities for BT and invasion rather than being mere opportunistic commensals.
Knowledge of the genetic diversity and virulence content of isolates infecting cirrhotic patients is a prerequisite for identifying potential invasive clonal groups and new targets for preventive measures. Accordingly, we sought to define the clonal distribution and virulence profiles of \textit{E. coli} isolates from cirrhotic patients with SBP and/or bacteremia.

**MATERIALS AND METHODS**

**Bacterial strains and patients.** A collection of 110 \textit{E. coli} isolates from the blood and/or ascitic fluid of patients with cirrhosis was studied. Isolates were collected between January 1997 and December 2006 at Beaujon Hospital (Clichy, France) and represented all consecutive episodes of SBP and spontaneous bacteremia caused by \textit{E. coli} (one isolate per patient) that occurred in the Hepatology Department during this period. SBP was defined by a positive ascitic fluid culture associated with a polymorphonuclear leukocyte cell count of \(\geq 250/\text{mm}^3\) in the absence of a documented intra-abdominal source of infection (29). Spontaneous bacteremia was defined by the isolation of \textit{E. coli} from at least one blood culture in the absence of a documented primary source of infection. Patients with secondary peritonitis and those with a documented source of bacteremia (e.g., urinary tract infection and cholangitis) were excluded from the study. All patients were epidemiologically unrelated; i.e., they belonged to distinct households and were not hospitalized at the same time. Thirty-six of the infection episodes occurred from 1997 to 2000, 36 from 2001 to 2003, and 38 from 2004 to 2006. Episodes were considered community acquired when they were diagnosed within the first 48 h of hospitalization and were considered nosocomial after this period.

\textit{E. coli} was identified by the API 20E system (bioMérieux, Marcy-l’Etoile, France). Antimicrobial susceptibility was tested by disk diffusion. All isolates were screened for the presence of extended-spectrum \(\beta\)-lactamase (ESBL) by the double-disk synergy test (2). Isolates were stored in 5% glycerol–Trypticase soy broth at \(-70^\circ\text{C}\) until use.

**Clinical data.** The clinical and laboratory data collected included age, sex, etiology of cirrhosis, prothrombin ratio, serum bilirubin level, serum creatinine level, and in-hospital mortality. The model for end-stage liver disease (MELD) score was calculated as described elsewhere (3).


**ERIC-2 PCR typing.** Isolates were screened for the presence of clonal groups by the enterobacterial repetitive intergenic consensus sequence type 2 (ERIC-2) PCR fingerprinting method (25). Electrophoretic banding patterns were compared by visual inspection.

**MLST.** Isolates with visually indistinguishable ERIC-2 profiles were further studied by multilocus sequence typing (MLST) to confirm their clonal relatedness and to identify their sequence types (STs) (13). Internal fragments of seven housekeeping genes (\textit{adh}, \textit{fun}C, \textit{gor}B, \textit{icl}, \textit{mdh}, \textit{parc}, \textit{rec}A) were amplified by PCR and sequenced for both strands. Alleles were assigned by comparing the sequence at each locus to all known alleles at that locus. A unique ST was assigned to each distinct combination of alleles at the seven loci. Allele and ST assignments were made via the \textit{E. coli} MLST website (available at: http://mlst.ucc.ie).

**Clonal group definition.** A clonal group was defined as two or more \textit{E. coli} isolates showing indistinguishable or highly similar ERIC-2 patterns and belonging to the same ST or ST complex (i.e., exhibiting different alleles at two loci or less).

**Statistical analysis.** Comparisons were based on the chi-square test or, for small numbers, Fisher’s exact test (two-tailed) for categorical variables and the Mann-Whitney \(U\) test for continuous variables. A \(P\) value of \(\leq 0.05\) was considered significant.

**RESULTS**

**Characteristics of patients.** The mean age of the 110 patients was 55 years (range, 32 to 73 years), and 85 (77.2%) were male. The most frequent origins of cirrhosis were alcohol (\(n = 62\)) and viral hepatitis (\(n = 33\)). The mean MELD score was 26. Of the 110 patients, 51 (46.4%) had SBP, 32 (29.1%) had bacteremia, and 27 (24.5%) had both SBP and bacteremia. Seventy-five (68.2%) of the infection episodes were community acquired. Of the 106 patients for whom data on outcome were available, 51 (48%) died during hospitalization.

**Phylogenetic groups and virulence genotype.** Phylogenetic groups A, B1, B2, and D were found in 24%, 4%, 48%, and 24% of the isolates, respectively. The most frequent VF genes were \textit{fim}H (type 1 fimbriae), \textit{fyu}A (yersiniabactin system), \textit{omp}T (outer membrane protease), and \textit{kps}M II (group 2 capsule), each of which was observed in \(\geq 60\%\) of the isolates (Table 1). In contrast, nine genes, including \textit{sfa}S (S fimbriae), \textit{afa}I/\textit{dra} (Dr-binding adhesins), \textit{afa}E8 (variant afimbrial adhesin), \textit{bma}E (M fimbriae), \textit{cdt}B (cytolethal distending Toxin B), \textit{tsh} (temperature-sensitive hemagglutinin), \textit{asta} (enteroaggregative \textit{E. coli} toxin), \textit{kps}MT III (group 3 capsule), and \textit{rfc} (O4 lipopolysaccharide), were each detected in less than 10% of isolates. The median virulence score was 9 (range, 0 to 14). Eighteen of the 34 VF genes studied were significantly associated with group B2 isolates in comparison with the gene association of non-B2 isolates, whereas none were significantly associated with non-B2 isolates (Table 1).

**Antimicrobial resistance.** The overall prevalence rates of resistance to individual antimicrobial agents were as follows: amoxicillin, 60%; co-trimoxazole, 34%; nalidixic acid, 22%; ciprofloxacin, 17%; and ceftoxime, 4%. No isolate produced an ESBL.

**Identification of clonal groups.** Overall, 68 distinct ERIC-2 banding patterns were observed among the 110 isolates. Twelve of these patterns (which collectively accounted for a total of 54 isolates) were observed in two or more isolates each, whereas the remaining 56 profiles were observed in a single isolate each. MLST showed that isolates with the same ERIC-2 pattern were from the same ST or ST complex (i.e., they differed at two loci or less). The reverse relationship also held, except that two distinct (albeit closely related) ERIC-2 patterns were observed among isolates from ST95. Overall, 11 clonal groups were identified, accounting for 54 (49%) of the 110 isolates. Membership in one of these clonal groups was more frequent among B2 isolates than non-B2 isolates (67% [36/53] versus 32% [18/57]; \(P < 0.001\)). The most prevalent STs were ST95 and ST73, with 13 and 8 isolates, respectively, followed by the ST14, ST10, and ST23 complexes (Table 2). A novel ST belonging to the ST14 complex (ST1604) was identified in two isolates. The emerging, antimicrobial resistance-associated clonal groups ST131 and ST69 were found in three isolates each.

The proportion of isolates belonging to one of the identified clonal groups was stable over the study period, i.e., 47.5% (17/36) of isolates collected from 1997 to 2000, 50% (18/36) of isolates collected from 2001 to 2003, and 50% (19/38) of isolates collected from 2004 to 2007. Furthermore, no significant association between specific clonal groups and time period was observed.

**Association of clonal groups with host factors and mortality.** Patients harboring clonal group-associated isolates did not differ from other patients regarding age, gender, etiology of cirrhosis, MELD score, type of infection, or nosocomial versus
The community origin of infection (data not shown). The in-hospital mortality rate was 45.1% among patients harboring a clonal group-associated isolate, whereas it was 52.8% among other patients (the difference was nonsignificant). There also was no significant association between specific clonal groups and the patients’ characteristics, type of infection, or outcome.

**Virulence genotype of clonal groups.** Of the 34 VF genes tested, 11 were significantly associated with clonal group membership, whereas none exhibited the opposite association (Table 3). Consequently, virulence scores were higher for clonal group-associated isolates than for other isolates (median scores, 12 and 8, respectively; \( P < 0.01 \)). This relationship also held specifically among group B2 isolates but did not among non-group B2 isolates. That is, compared with other B2 isolates, clonal group-associated B2 isolates exhibited a greater prevalence of \( \text{pap}AH \) (75% and 35%, respectively; \( P = 0.005 \)), \( \text{pap}C \) and \( \text{pap}EF \) (78% and 35%, respectively; \( P = 0.003 \)), \( \text{pap}G \) (81% and 29%, respectively; \( P < 0.001 \)), and \( \text{sat} \) (42% and 12%, respectively; \( P = 0.03 \)), whereas among non-B2 isolates, virulence gene prevalence values did not vary significantly according to clonal group versus nonclonal group status (data not shown).

The associations between specific clonal groups and virulence gene content are shown in Table 2. All ST95 isolates harbored the four \( \text{pap} \) genes but lacked several other VF genes (\( \text{sfa} / \text{foc} \), \( \text{hly}A \), \( \text{cva} \), \( \text{cnf}1 \), \( \text{pic} \)) that were present in most ST73 isolates. Most ST95 isolates harbored two genes, \( \text{cva} \) and \( \text{iss} \), that occurred in none of the other B2 clonal groups. ST131 isolates were associated with a lesser virulence gene content than other clonal group-associated B2 isolates, in particular, the absence of \( \text{pap} \) genes. A number of VF genes (\( \text{fim}H \), \( \text{fyu}A \), \( \text{kps}M \) II, \( \text{omp}T \), \( \text{usp} \)) were common to all the group B2-associated clonal groups.

**DISCUSSION**

To our knowledge, this is the first study to describe the genetic structure and virulence-associated genes of \( E. \) *coli* isolates from cirrhotic patients with SBP and/or spontaneous bacteremia. By definition, all episodes occurred in the absence of a discernible primary source of infection and so were considered to result from BT. Our hypothesis was that particular clonal groups would exhibit specific virulence profiles that may be clinically relevant because they confer on these isolates a greater ability to translocate and to cause invasive disease. To test this hypothesis, we determined the prevalence of 34 genes...
associated with extraintestinal virulence, assessed clonal diversity by using ERIC-2 PCR and MLST, and compared these bacterial traits with clinical variables.

Our isolates belonged, in decreasing order of frequency, to phylogenetic groups B2, A, D, and B1. As expected from the immunocompromised status of our patients, group B2 was less prevalent than previously appreciated (6, 12, 17). Similarly, the susceptibility to extended-spectrum cephalosporins may be more prevalent than previously appreciated (5, 11, 28, 32). However, as expected, group B2 was strongly associated with the presence of VF genes. ERIC-2 PCR typing showed that our isolates were genetically diverse, with 68 distinct patterns being observed among the 110 isolates. However, 49% of the isolates belonged to clonal groups which comprised from 2 to 13 genetically related isolates. Compared to other isolates, clonal group-associated isolates were significantly more likely to harbor a number of virulence genes that are associated with extraintestinal infections, which mostly originated from noncompromised patients with a urinary source of infection (5, 11, 28, 32). However, as expected, group B2 was strongly associated with the presence of VF genes.

TABLE 3. Comparative virulence gene profiles of clonal group-associated isolates versus other Escherichia coli clinical isolates from patients with cirrhosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Virulence gene</th>
<th>Gene prevalence (no. [column %] of isolates)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clonal group isolates&lt;sup&gt;c&lt;/sup&gt; (n = 54)</td>
<td>Other isolates (n = 56)</td>
</tr>
<tr>
<td>Adhesins</td>
<td>papAH</td>
<td>33 (61)</td>
<td>17 (30)</td>
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<tr>
<td></td>
<td>papC</td>
<td>34 (63)</td>
<td>16 (29)</td>
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<tr>
<td></td>
<td>papEF</td>
<td>29 (54)</td>
<td>16 (29)</td>
</tr>
<tr>
<td>Toxins</td>
<td>cnf1</td>
<td>14 (26)</td>
<td>6 (11)</td>
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<tr>
<td></td>
<td>vat</td>
<td>35 (65)</td>
<td>15 (27)</td>
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<tr>
<td>Siderophores</td>
<td>ironN</td>
<td>30 (56)</td>
<td>18 (32)</td>
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<tr>
<td></td>
<td>fyuA</td>
<td>45 (83)</td>
<td>33 (59)</td>
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<tr>
<td></td>
<td>ire</td>
<td>22 (41)</td>
<td>11 (20)</td>
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<tr>
<td>Miscellaneous</td>
<td>usp</td>
<td>36 (67)</td>
<td>19 (34)</td>
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<tr>
<td></td>
<td>ompT</td>
<td>44 (81)</td>
<td>33 (59)</td>
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</table>

<sup>a</sup> The 11 genes listed are those that yielded P values of ≤0.05. Definitions: papAH, P fimbria structural subunit; papC, P fimbria assembly; papEF, P fimbria tip pilins; papG, P fimbria adhesin; cnf1, cytotoxic necrotizing factor; vat, vacuolating autotransporter toxin; ironN, salmochelin receptor; fyuA, yersiniabactin system; ire, siderophore receptor; usp, uropathogenic specific protein; ompT, outer membrane protease.

<sup>b</sup> The prevalence of virulence genes among clonal group-associated isolates versus the prevalence among other isolates was compared by the chi-square test or Fisher’s exact test.

<sup>c</sup> The distribution of isolates by clonal group is shown in Table 2.
cause of trimethoprim-sulfamethoxazole-resistant urinary tract infection outbreaks in different communities (12, 22, 35). Although patients with a urinary source of infection were excluded from the present study, ST69 was identified in three isolates. Similarly, Manges et al. found ST69 isolates in 8% of non-urinary-source E. coli bloodstream isolates (21). Likewise, a CGA isolate has been reported to be a cause of community-acquired pneumonia in a renal transplant recipient (14). These findings indicate that although CGA isolates are primarily associated with urinary tract infections, they are not exclusively uropathogens.

Although animal studies have shown that E. coli translocation is strain dependent (18, 33), no specific bacterial VFs involved in translocation have been identified to date. Macutkiewicz et al. recently suggested the existence of a “translocating” phenotype that would result in little or no penetration across the gut barrier in healthy subjects but that under conditions of metabolic stress, such as those occur in cirrhosis, may promote interactions of these strains with enterocytes and increase their ability to translocate (19). In the present study, approximately half of the isolates infecting cirrhotic patients belonged to clonal groups with a high prevalence of VFs, which suggests that the extensive virulence profile of these clones may be a significant invasive determinant. However, it is noteworthy that the isolates expressing the translocating phenotype in the study of Macutkiewicz et al. belonged to phylogenetic group A or D and had sparse virulence profiles that differed from those that typify extraintestinal pathogenic E. coli (19). In particular, they lacked the pap genes that were associated with clonal groups in our study. Thus, it is possible that the present clonal group-associated isolates do exhibit an enhanced virulence potential, but at the level of increased survival within the bloodstream or lymphatic system rather than during the initial step of epithelial translocation.

Alternatively, the high prevalence of clonal group-associated isolates in SBP and primary bacteraemia could merely reflect the prevalence of these isolates in the intestinal flora of cirrhotic patients rather than increased invasiveness. Indeed, several of these clonal groups, including ST131, ST95, ST10, and ST117, were recently identified in fecal E. coli isolates from healthy subjects living in the same area as our patients (the Paris, France, area) (17). As clonal group-associated B2 isolates exhibited a higher prevalence of P fimbriae, which may contribute to enhanced fitness in the gut (24, 26), they may be more likely to persist within the intestinal flora of cirrhotic patients and therefore to cause SBP or primary bacteraemia, regardless of their invasive potential, as liver dysfunction advances and host defenses weaken. A higher prevalence of particular clonal groups in the gut may also result from a steady input from environmental sources such as food reservoirs (36).

Therefore, further studies are needed to compare the prevalence of these clonal groups in patients with invasive diseases and in the fecal flora of cirrhotic patients. An overrepresentation of particular clones among disease isolates compared with the level of representation of intestinal carriage isolates would suggest a greater invasive potential and could provide insight into the pathogenesis of E. coli infection during cirrhosis. Moreover, longitudinal studies are required to investigate whether the E. coli intestinal clonal structure is stable or varies over time for a given patient. The persistence of particular clonal group-associated isolates in the gut compared with the occurrence of nonclonal isolates would suggest a role of VFs in successful intestinal colonization.

Traditionally, O serotyping has been used to identify virulent clones of extraintestinal E. coli. However, Sannes et al. have shown that virulence genes from bacteremic isolates are more closely associated with extraintestinal virulence than the O serotype (32). Thus, O serotyping was not performed for the present isolates.

One strength of the present study is that it included all consecutive episodes of SBP and spontaneous bacteraemia in cirrhotic patients, whether they were nosocomial or community acquired, in a single hospital over a 10-year period. It is therefore likely to provide a good estimate of the genetic diversity and virulence profile of E. coli isolates infecting cirrhotic patients, at least those living in this geographical area. The patients belonged to distinct households and were not hospitalized at the same time, indicating the absence of intrafamilial or hospital transmission between patients. Moreover, the clonal groups were not temporally clustered over the 10-year period, which strongly suggests that they represent endemic lineages rather than outbreak-related strains.

Understanding the role of bacterial characteristics in the pathogenesis of BT has obvious implications for preventive measures in patients with cirrhosis, particularly those awaiting liver transplantation. Although prophylaxis with norfloxacin is recommended in patients at high risk of infection, this may promote the emergence of fluoroquinolone-resistant organisms (3, 29). Screening patients for the presence of translocating isolates in the gut could help identify those who would benefit the most from norfloxacin prophylaxis. Moreover, the virulence profiles of clonal group-associated isolates, many of which include certain conserved VFs, suggest a range of potential targets for vaccines, particularly if a role in BT or invasion can be demonstrated for these VFs.

REFERENCES