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Relationship of Phylogenetic Background, Biofilm Production, and Time to Detection of Growth in Blood Culture Vials with Clinical Variables and Prognosis Associated with *Escherichia coli* Bacteremia

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In patients with *Escherichia coli* bacteremia, data on the relationship of phylogenetic background, biofilm production, and degree of bacteremia with clinical variables and prognosis are scarce. During a 1-year period, all adults with bacteremia due to *Escherichia coli* diagnosed at a university center were enrolled. Determination of phylogenetic background, biofilm production, and genotyping was performed with all strains, and the time to positivity of blood culture vials was recorded. A total of 185 episodes of diverse-source *E. coli* bacteremia was analyzed. Strains of phylogroup D were predominant (52%). Phylogroup A isolates were associated with pneumonia and prior antibiotic intake, B1 with an abdominal source of infection, B2 with the absence of urological abnormalities, and D with urological abnormalities and age below 65 years. Resistance to antibiotics and no biofilm production were concentrated in phylogroup A strains. Biofilm production was not associated with any clinical variable. An immunocompromising condition (odds ratio [OR] = 5.01, 95% confidence interval [CI] = 1.4 to 17.9), peritonitis (OR = 17, 95% CI = 3.32 to 87), pneumonia (OR = 9.97, 95% CI = 1.96 to 50.6), and ≤7 h to bacteremia detection (OR = 4.37, 95% CI = 1.38 to 13.8) were the best predictors of a fatal outcome. Results from this study suggest that the distribution of phylogenetic backgrounds among *E. coli* strains involved in diverse-source bacteremia may be subject to geographical variation and that, in afflicted individuals, some high-risk sources, the patient's underlying condition, and the degree of bacteremia are more important than microbial factors in determining the outcome. Time to positivity of blood culture vials may be a variable of potential clinical impact.

Escherichia coli continues to be the single most frequently involved gram-negative organism in adult patients with either community-acquired or nosocomial bacteremia (12, 29). Extraintestinal *E. coli* infections, including bacteremia, are caused predominantly by strains displaying a wide array of putative virulence factors, such as adhesins, iron acquisition systems, host defense-subverting mechanisms, and toxins. The genetic elements encoding these factors have been concentrated predominantly in strains derived from phylogroup B2 and, to a lesser extent, from group D, whereas isolates of groups A and B1 usually contain few virulence determinants and are essentially regarded as commensals (8, 14, 25, 26). Although certain virulence factors have been associated with invasion of specific organs and shedding into the bloodstream (14, 22, 28), there is no proof that they have any impact on prognosis of patients with bacteremia (11). As a matter of fact, mortality associated with bloodstream *E. coli* infections seems to be influenced mostly by host and treatment factors (10, 21, 27), and patients with local or general compromising conditions are less frequently infected by highly virulent strains (25).

Biofilm formation is commonly associated with infections of medical devices and is considered to be relevant in some spontaneous diseases, such as native valve endocarditis, periodontitis, chronic prostatitis, otitis media, and bronchopulmonary sepsis in patients with cystic fibrosis (7). Although the ability of *E. coli* to form biofilms is widely recognized, the frequency of this trait in strains causing clinical infection and its relationship to other clinical or bacterial characteristics is still unknown.

Classical studies suggest that the magnitude of bacteremia as determined by quantitative blood cultures may be related to mortality independently of the underlying disease's severity (19). Given the strong relationship between the inoculum size and the time to positivity, as assessed by automatic blood culture-processing devices (2), the latter may constitute a surrogate marker of the degree of bacteremia and provide useful diagnostic and prognostic information (18).

In the present study, we have analyzed the phylogenetic background of the strains involved in a series of unselected and epidemiologically unrelated episodes of *E. coli* bacteremia, the frequency of biofilm formation in these organisms, and the time to detection of growth as a surrogate marker of the degree of bacteremia in order to gain insight into the relationship of these characteristics with clinical variables and prognosis.

MATERIALS AND METHODS

Patients. The study was conducted at the Hospital Clínic, a 700-bed university hospital in Barcelona, Spain. During one year (2003), all patients with bloodstream infections due to *E. coli* were prospectively followed up from diagnosis to discharge and the strains involved were assessed for biofilm

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production, determination of their phylogenetic background, and molecular epidemiological relatedness. This study focuses on episodes of *E. coli* bacteremia, defined as bloodstream *E. coli* infections that arose de novo or, in patients with a previous bacteremic *E. coli* infection, after the apparent clinical cure and source eradication.

Clinical variables. Clinical variables collected from patients with bacteremia included the following: demographics, place of acquisition (hospital or community), primary source of infection, underlying diseases, selected extrinsic risk factors (orotracheal intubation, bladder catheterization, corticosteroids, or other immunosuppressive therapy), selected clinical aspects (fever and shock), antibiotic therapy-related issues (previous administration of antibiotics, ongoing therapy at the time of drawing blood cultures, and empirical and definitive therapies), susceptibility of the infecting strain, and in-hospital mortality. Patients with solid or hematological cancer, human immunodeficiency virus infection, solid organ or bone marrow transplantation, neutropenia, or splenectomy or patients receiving corticosteroids or other immunosuppressive therapy were considered to be immunocompromised. The source of infection was established according to common clinical, analytical, and radiographic criteria and was considered either probable, when cultures from the suspected site were not obtained or negative, or definitive, when an *E. coli* strain with the same susceptibility pattern as the one isolated from blood was recovered from the apparent source. To consider a venous catheter the source of infection, a positive culture of the catheter tip by the roll plate method and no evidence of other putative origin were required. Antimicrobial therapy given for at least 48 h in the 30 days before bacteremia diagnosis was considered a previous antibiotic treatment. Treatment was considered appropriate when the isolated organism was susceptible in vitro to at least one of the antibiotics given and when the dosage and route of administration were adequate. Detection of growth in a lapse equal to or lower than the 25th percentile of the growth time distribution of the isolates was considered indicative of high-degree bacteremia.

Microbiological procedures. Blood cultures were processed by the BACTEC 9240 system (Becton-Dickinson, Sparks, Md.). When using media without resins, the standard aerobic bottle (BACTEC Standard/10 Aerobic/F) was always paired with the BACTEC Lytic/10 Anaerobic/F bottle. Aerobic and anaerobic resin bottles were BACTEC PLUS Aerobic/F and BACTEC PLUS Anaerobic/F, respectively. The volume of blood cultured was not verified. The exact time from the start of incubation to a positive reading was recorded, considering time to positivity to be that for the bottle in the processed set or sets that became positive first. Antibiotic susceptibility to ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin, and gentamicin was determined by a microdilution method following CLSI (formerly NCCLS) standards (20). The *E. coli* phylogenetic background was determined by using a three-locus PCR-based method (4). In vitro biofilm formation was analyzed by growing the bacteria into microtiter plates with a minimal glucose medium (M63), stained with a 1% violet crystal solution, and read using a spectrophotometer (6). Molecular epidemiology analysis was performed by pulsed-field gel electrophoresis by using XbaI endonuclease as described elsewhere (9). The subset of phylogroup D strains was serotyped by an agglutination test using specific antiserum from O15, K52, and H1 antigens (Statens Serum Institute, Denmark).

Statistical analysis. Proportions were compared by the χ^2 test or the Fisher test when appropriate. Quantitative variables were compared by the *t* test or the Mann-Whitney test. Logistic regression was used to select the best predictors of the phylogenetic background, high-degree bacteremia, and in-hospital mortality. In models predicting mortality, shock was not included as an explanatory variable, due to the fact that it may be a common intermediate condition in the path through which other important prognostic factors lead to death.

TABLE 1. Clinical and microbiological features of 185 episodes of *E. coli* bacteremia

Characteristic	Value[f]
Demographics	
Age (yr) (mean [SD])	65.27 (17)
Female gender	106 (57)
Underlying conditions	
Hospital-acquired infection	31 (17)
Any prior antibiotic	42 (23)
Prior quinolones	22 (12)
Urological abnormalities[a]	55 (30)
Chronic renal insufficiency	8 (4)
Diabetes	41 (22)
Liver cirrhosis	17 (9)
Obstructive pulmonary disease	12 (7)
Immunocompromising condition[b]	74 (40)
Other[c]	26 (14)
Any underlying condition	152 (82)
Clinical and laboratory aspects	
Fever	181 (98)
Shock	32 (17)
White blood cell count (median)	10,290
Sources of infection[d]	
Urinary tract infection	106 (57)
Unknown	27 (15)
Intra-abdominal infection[e]	34 (18)
Pneumonia	9 (5)
Venous catheter	5 (3)
Skin and soft tissue infections	4 (2)
Microbiological variables	
Nalidixic acid susceptibility	101 (55)
Phylogroup A	32 (17)
Phylogroup B1	22 (12)
Phylogroup B2	34 (18)
Phylogroup D	97 (52)
Time to positivity (h) (median)	9
Biofilm production	79 (43)
Polymicrobial bacteremia	5 (3)
Outcome	
In-hospital mortality	20 (11)

[a] Includes indwelling bladder catheterization in 29 episodes (16%).
[b] Includes corticosteroid therapy (n = 26, 14%), immunosuppressive therapy (n = 25, 13%), solid (n = 33, 18%) or hematological (n = 16, 9%) cancer, solid organ transplantation (n = 10, 5%), neutropenia (n = 15, 8%), human immunodeficiency virus infection (n = 3, 2%), and splenectomy (n = 3, 2%).
[c] Includes heart disease (n = 23, 12%) and stroke or severe dementia (n = 3, 2%).
[d] The source was definitive in 96 episodes (52%) and probable in 62 episodes (34%).
[e] Includes cholangitis/cholecystitis (n = 25), secondary peritonitis (n = 7), and spontaneous bacterial peritonitis (n = 2).
[f] All results are given in number (%) of instances, unless otherwise indicated.

RESULTS

One hundred seventy-seven patients had a total of 185 episodes of *E. coli* bacteremia, of which 96 (52%) had a definitive source. Clinical and microbiological characteristics are shown in Table 1. Almost two-thirds of the episodes occurred in patients older than 65 years, and there was a slight overrepresentation of females. Most individuals suffered from some underlying disease, and more than half of the episodes originated in the urinary tract. Median time to detection of growth by the continuous monitoring blood culture system was 9 h (interquartile interval of 7 to 11 h). Pulsed-field gel electrophoresis confirmed that each isolate corresponded to a unique strain.

Regarding the phylogenetic background of isolates, there was a predominance of strains derived from group D (52%), followed in decreasing order of frequency by those from groups B2 (18%), A (17%), and B1 (12%). The relationship between the phylogroup and clinical or other microbiological variables is shown in Table 2. Strains belonging to group D were the most frequently found among most sources of bacteremia and other clinical characteristics. Only in patients with

TABLE 2. Univariate analysis of the association of demographic, clinical, and microbiological variables with phylogenetic background

Variable[a]	Total no. of strains	No. (%) of strains of phylogroup A	*P*	No. (%) of strains of phylogroup B1	*P*	No. (%) of strains of phylogroup B2	*P*	No. (%) of strains of phylogroup D	*P*
Age (yr)									
≥65	112	21 (19)		17 (15)		22 (20)		52 (46)	
<65	73	11 (15)	0.5	5 (7)	0.09	12 (16)	0.6	45 (62)	0.04
Any prior antibiotic									
Yes	42	15 (36)		5 (12)		4 (10)		18 (43)	
No	143	17 (12)	0.0003	17 (12)	1	30 (21)	0.09	79 (55)	0.2
Prior quinolone									
Yes	22	9 (41)		2 (9)		2 (9)		9 (41)	
No	163	23 (14)	0.002	20 (12)	1	32 (20)	0.4	98 (54)	0.2
Urological abnormality									
Yes	55	8 (15)		5 (9)		5 (9)		37 (67)	
No	130	24 (19)	0.5	17 (13)	0.4	29 (22)	0.03	60 (46)	0.008
Immunocompromising condition									
Yes	74	18 (24)		8 (11)		12 (16)		36 (49)	
No	111	14 (13)	0.04	14 (13)	0.7	22 (20)	0.5	61 (55)	0.4
Urinary tract infection									
Yes	106	17 (16)		9 (9)		23 (22)		57 (54)	
No	79	15 (19)	0.6	13 (17)	0.1	11 (14)	0.2	40 (51)	0.7
Unknown source									
Yes	27	6 (22)		5 (19)		4 (15)		12 (44)	
No	158	26 (17)	0.4	17 (11)	0.3	30 (19)	0.8	85 (54)	0.4
Intra-abdominal infection									
Yes	34	5 (15)		8 (24)		5 (15)		16 (46)	
No	151	27 (18)	0.7	14 (9)	0.002	29 (19)	0.5	81 (54)	0.5
Pneumonia									
Yes	9	4 (44)		0		2 (22)		3 (33)	
No	176	28 (16)	0.049	22 (13)	0.6	32 (19)	0.7	94 (53)	0.2
Venous catheter									
Yes	5	0		0		0		5 (100)	
No	180	32 (18)	0.3	22 (12)	1	34 (19)	0.6	92 (51)	0.06
Soft tissue infection									
Yes	4	0		0		0		4 (100)	
No	181	32 (18)	1	22 (12)	1	34 (19)	1	93 (51)	0.1
Nalidixic acid susceptibility									
Yes	101	7 (7)		11 (11)		25 (25)		58 (57)	
No	84	25 (30)	0.0001	11 (13)	0.6	9 (11)	0.01	39 (46)	0.1
Biofilm production									
Yes	79	5 (6)		11 (14)		18 (23)		45 (57)	
No	106	27 (26)	0.0007	11 (10)	0.5	16 (15)	0.2	52 (49)	0.3
Time to positivity (h)									
≤7	40	7 (18)		7 (18)		4 (10)		22 (55)	
>7	145	25 (17)	1	15 (10)	0.2	30 (21)	0.1	75 (52)	0.7
In-hospital mortality									
Yes	20	6 (30)		1 (5)		3 (15)		10 (50)	
No	165	26 (16)	0.1	21 (13)	0.5	31 (19)	1	87 (53)	0.8

[a] Other variables not associated with any phylogenetic group included gender, hospital acquisition of the infection, chronic renal failure, diabetes, liver cirrhosis, chronic obstructive pulmonary disease, and shock.

pneumonia did strains from phylogroup A prevail over group D isolates. Sixteen out of 97 (16%) group D strains belonged to the O15:K52:H1 serotype.

Patients infected with phylogroup A strains more frequently received antibiotics during the last month, had an immunocompromising disease, and had pneumonia as the source of infection. However, only pneumonia (odds ratio [OR] = 4.89, 95% confidence interval [CI] = 1.11 to 21) and prior antibiotic

intake (OR = 4.33, 95% CI = 1.88 to 9.95) were independent predictors. Group A strains were also more resistant to any antibiotic except trimethoprim-sulfamethoxazole (data not shown), an association not confounded by recent previous antibiotic exposure. Even for patients who had not received any antibiotic within the last month, rates of resistance to at least one of the drugs tested were significantly higher among phylogroup A strains than among those of other phylogenetic backgrounds (100% versus 74%, respectively; $P = 0.01$).

Group B1 strains were more frequent in episodes with an abdominal source of infection (peritonitis and biliary tract infections combined) than in those with other origins (OR = 3.01, 95% CI = 1.14 to 9.95). The only independent predictor of group B2 strains was the absence of urological abnormalities (OR = 2.94, 95% CI = 1.04 to 8.33). This association was stronger among patients with a urinary source of infection (OR = 4, 95% CI = 1.02 to 10) than in those without it (OR = 1.75, 95% CI = 0.31 to 17). Group B2 strains were also less resistant to quinolones, an association not confounded by prior quinolone administration. In patients who had not received these drugs within the last month, resistance to nalidixic acid was still lower with B2 strains than with those of other phylogenetic backgrounds (22% versus 42%, respectively; $P = 0.04$). Group D strains were independently associated with urological abnormalities (OR = 1.96, 95% CI = 1.04 to 3.7) and age below 65 years (OR = 2.48, 95% CI = 1.26 to 4.87). It is of note that the association of group D isolates with urological abnormalities was even stronger for the episodes considered not to have originated in the urinary tract (OR = 3.3, 95% CI = 1.01 to 12; $P = 0.03$) than for those having a urinary tract source (OR = 1.9, 95% CI = 0.8 to 4.9; $P = 0.12$). O15:K52:H1 strains did not differ from other group D isolates, except in their higher susceptibility to nalidixic acid (88% versus 54%, respectively; $P = 0.01$).

Biofilm production was not associated with any demographic or clinical variables and was significantly less common in strains of phylogroup A (5 out of 32, or 16%, for phylogroup A strains versus 74 out of 153, or 48%, for non-phylogroup A strains; $P = 0.0007$). Strains from patients with bladder catheter-associated urinary tract infection or intravenous catheter-related bacteremia or from men with urinary tract infection without bladder catheters, considered either individually or all together, did not produce biofilm more frequently than strains recovered from other patients (data not shown).

Forty bacteremic episodes were detected by the continuous monitoring blood culture system in 7 h or less of incubation. A univariate analysis of the relationship between time to positivity and clinical or microbiological characteristics is shown in Table 3. Multiple logistic regression identified neutropenia (OR = 4.14, 95% CI = 1.85 to 20.4), a catheter source (OR = 22, 95% CI = 2.22 to 219), liver cirrhosis (OR = 5.06, 95% CI = 1.69 to 15.1), and pneumonia (OR = 6.16, 95% CI = 1.42 to 26.7) as independent predictors of high-degree bacteremia.

Twenty patients (11%) died during hospitalization, and the factors associated with in-hospital mortality are shown in Table 4. Multivariate analysis selected an immunocompromising condition (OR = 5.01, 95% CI = 1.4 to 17.9), peritonitis (OR = 17, 95% CI = 3.32 to 87), pneumonia (OR = 9.97, 95% CI = 1.96 to 50.6), and a time to positivity of ≤7 h (OR = 4.37, 95% CI = 1.38 to 13.8) as the factors independently associated with a fatal outcome. Appropriate empirical antimicrobial therapy was not associated with a better outcome, and there was no evidence of a significant modifying effect of any clinical or microbiological variable on the association between the appropriateness of initial therapy and mortality.

TABLE 3. Univariate analysis of the relationship of clinical and microbiological characteristics with high-degree bacteremia (time to detection of growth of ≤7 h)

Variable	No. (%) of episodes of bacteremia at time to detection of growth of:		*P*
	≤7 h (*n* = 40)	>7 h (*n* = 145)	
Age of >65 yr	23 (58)	89 (61)	0.7
Male gender	19 (48)	87 (60)	0.2
Hospital-acquired infection	11 (28)	20 (14)	0.04
Any prior antibiotic	12 (30)	30 (21)	0.2
Appropriate antibiotics at the time of blood culture	0	7 (5)	0.3
Vials with resins	6 (15)	32 (22)	0.3
Urological abnormalities	13 (33)	42 (29)	0.6
Chronic renal insufficiency	1 (3)	7 (5)	1
Diabetes	6 (15)	35 (24)	0.2
Liver cirrhosis	8 (20)	9 (6)	0.01
Obstructive pulmonary disease	4 (10)	8 (6)	0.3
Immunocompromising condition	22 (55)	52 (36)	0.03
Neutropenia	9 (23)	6 (4)	0.0008
Other underlying diseases	8 (20)	18 (12)	0.2
Shock	13 (33)	19 (13)	0.008
Urinary tract infection	14 (35)	92 (63)	0.001
Intra-abdominal infection	10 (25)	24 (17)	0.2
Unknown source	6 (15)	20 (14)	0.8
Pneumonia	5 (13)	4 (3)	0.02
Catheter-related infection	4 (10)	1 (1)	0.008
Skin and soft tissue infection	1 (3)	3 (2)	1
Nalidixic acid susceptibility	18 (45)	66 (46)	0.9
Phylogroup A	7 (18)	25 (17)	1
Phylogroup B1	7 (18)	15 (10)	0.3
Phylogroup B2	4 (10)	30 (21)	0.1
Phylogroup D	22 (55)	75 (52)	0.7
Biofilm production	15 (38)	64 (44)	0.5
In-hospital mortality	11 (28)	9 (6)	0.0005

DISCUSSION

In the present study, we have evaluated the phylogenetic background of the strains involved in a series of unselected and epidemiologically unrelated episodes of diverse-source *E. coli* bacteremia, the prevalence of biofilm production in those organisms, the time to detection of growth as a surrogate marker of the degree of bacteremia, and the association of these characteristics with several clinical variables and prognosis.

Previous studies have shown that bacteremic *E. coli* infections in adults from different geographical areas are caused by organisms belonging, in decreasing order of frequency, to phylogroups B2, D, A, and B1. This distribution corresponds with that of the number of putative virulence factors present in those strains (8, 14, 25). In general terms, our data agree with

TABLE 4. Univariate analysis of putative prognostic factors

Variable	No. (%) of episodes resulting or not resulting in in-hospital mortality		*P*
	Yes (n = 20)	No (n = 165)	
Age of >65 yr	8 (40)	104 (63)	0.046
Male gender	9 (55)	68 (41)	0.2
Hospital-acquired infection	4 (20)	27 (16)	0.7
Any prior antibiotic	5 (25)	37 (22)	0.8
Urological abnormalities	7 (35)	48 (29)	0.6
Chronic renal insufficiency	2 (10)	6 (4)	0.2
Diabetes	4 (20)	37 (22)	1
Liver cirrhosis	6 (30)	11 (7)	0.004
Obstructive pulmonary disease	2 (10)	10 (6)	0.6
Immunocompromising condition	16 (80)	58 (35)	0.0001
Shock	16 (80)	16 (10)	0.0000
Urinary tract infection	4 (20)	102 (62)	0.0004
Unknown source	4 (20)	22 (13)	0.5
Peritonitis	5 (25)	4 (2)	0.0009
Cholecystitis/cholangitis	1 (5)	24 (15)	0.3
Pneumonia	5 (25)	4 (2)	0.0009
Catheter-related infection	0	5 (3)	1
Skin and soft tissue infection	1 (5)	3 (2)	0.4
Nalidixic acid susceptibility	10 (50)	74 (45)	0.7
Phylogroup A	6 (30)	26 (16)	0.1
Phylogroup B1	1 (5)	21 (13)	0.5
Phylogroup B2	3 (15)	31 (19)	1
Phylogroup D	10 (50)	87 (53)	0.8
High-degree bacteremia	11 (50)	29 (18)	0.0005
Biofilm production	8 (40)	71 (43)	0.8
Appropriate empirical therapy	19 (95)	155 (94)	1

these observations, but some differences are of note. First, strains of group D, instead of group B2, were predominant. Second, a high proportion of strains causing pneumonia derived from phylogroup A, in contrast with previous observations (25). The reasons for these discrepancies are unclear. The relative predominance of different phylogroups may be subject to geographical variations. Fully pathogenic group D strains, such as those belonging to serotype O15:K52:H1, have been described to occur in our geographical area, where they have been endemic for the last decade (5, 23). The prevalence of serotype O15:K52:H1 among bacteremic strains found in this study (8.6%) is at least double the figures previously reported and supports its well-established presence in our region and its increasing involvement in diverse-source bloodstream infections. Regarding the phylogenetic background of strains causing pneumonia, it is conceivable that the relative prevalences of the different groups could vary according to the number of primary versus metastatic lung infections included in different series. Since primary *E. coli* pneumonia has usually been regarded as caused by commensal organisms belonging to the patient's own flora, there is a possibility that cases due to group A strains may represent true primary pneumonia.

The present data also support the general hypothesis that less virulent strains required some predisposing host conditions to become fully pathogenic and provide some insight on the nature of such precipitating factors. For commensal strains that have become more intrinsically resistant to antibiotics, such as those of group A, recent antibiotic exposure seems to be the most important conditioning event. The relationship between non-B2 phylogenetic background (A or D in particular) and resistance to ciprofloxacin or trimethoprim-sulfamethoxazole or multidrug resistance has previously been recognized (15, 16). The association of B1 strains with an abdominal focus of infection may reflect the overwhelming challenge imposed by either a high inoculum associated with peritonitis or obstruction of the biliary tract. Conversely, presumably virulent B2 strains were associated particularly with bacteremia originating in the urinary tracts of patients without urological abnormalities. This is consistent with previous observations showing the universal predominance of phylogroup B2 among bloodstream or urinary isolates from noncompromised patients with urinary tract infections (1, 13, 17, 32). A plausible explanation for the observed independent association of phylogroup D with younger age and urological abnormalities is, however, not readily apparent. Particularly disturbing is the fact that the association with urological abnormalities was stronger in patients with non-urinary tract infections. Although some misclassification of the actual infection source may have influenced the data, a more plausible reason is that urological abnormalities constitute a surrogate marker for a patient's other conditions, predisposing the patient to bloodstream infections due to relatively virulent strains.

The capacity to produce biofilm was not associated with any clinical characteristic. Even the strains involved in infections that presumably are or may be associated with a biofilm type of growth at the source level, such as venous catheter-related bacteremia, bladder catheter-related urinary tract infection, and urinary tract infection in men without bladder catheters (a population at increased risk for prostatitis), were not more likely biofilm producers than the strains involved in other processes. Although the exact meaning of this observation is uncertain, it suggests that biofilm formation does not foster the ability of *E. coli* to spread into the bloodstream.

In the present study, we have introduced the time to detection of growth by the continuous monitoring blood culture system as a surrogate marker of the degree of bacteremia and a possible prognostic factor. This measurement has proved to be useful for the diagnosis of venous catheter-related bacteremia (3), and in a recent study, a time to positivity of ≤14 h was associated with an endovascular source of infection, metastatic infection, and attributable mortality in patients with *Staphylococcus aureus* bacteremia (18). In fact, three of the factors independently associated with a time to detection equal to or less than 7 h are conditions associated with either severe defects in the mechanisms involved in clearing the blood of microorganisms (neutropenia or liver cirrhosis) or continuous shedding into the bloodstream (catheter-related bacteremia). The association of high-degree bacteremia with pneumonia could reflect the metastatic nature of some of the cases. High-degree bacteremia has been reported to be an important predisposing factor for metastatic shedding of *E. coli*, particularly in the central nervous system (31). The lack of association of the phylogenetic background with high-degree bacteremia suggests that virulence determinants that promote inflammatory responses, invasion of epithelial cells, and resistance to phago-

cytosis (such as P fimbriae) may be less relevant in determining the degree of bacteremia (24, 30).

Prognosis of *E. coli* bacteremia was related to the patient's underlying condition, to some well-known high-risk sources (pneumonia and peritonitis), and to the degree of bacteremia. Neither the phylogenetic background nor the ability to produce biofilm was important in this respect. Present data suggest that the source of infection and host factors, rather than the inferred amount of virulence traits, are the major determinants of prognosis of *E. coli* bacteremia. This observation agrees with the data from a previous study, in which patients with hemolysin-producing strains in fact had a better prognosis (11). Evidence that high-degree bacteremia as defined in this study was associated with some sources and had prognostic significance may have practical consequences. If these data are confirmed, the microbiology laboratory should consider alerting the physician about any blood culture that becomes positive for a gram-negative bacilli in ≤7 h. Giving this input on a real-time basis could provide valuable diagnostic information and contribute to the prompt optimization of antimicrobial therapy and other care measures (such as removal of a venous catheter) which may improve prognosis. We do not believe that the lack of association between appropriate initial therapy and prognosis observed in this particular series rules out the potential benefit of optimizing the antimicrobial therapy as soon as possible for patients with bacteremia.

This study has several drawbacks. The most obvious one is that we did not determine the presence of putative virulence factors in the involved strains, and hence we cannot rule out that they actually have prognostic significance, either individually or collectively. Any suggestion in this regard is inferential, based on the well-known association of virulence score with phylogenetic background. On the other hand, only half of the episodes were microbiologically documented at the infection focus and the isolates retrieved from the source were not available for analysis; hence, misclassification of the sources of bacteremia may have biased the results. In addition, we did not verify the volume of blood cultured, assuming that variations occurred randomly. The value of our proposed marker of high-degree bacteremia needs to be confirmed in larger series and with different continuous monitoring blood culture devices.

In summary, the present data suggest that the distribution of phylogenetic backgrounds among strains causing diverse-source *E. coli* bacteremia may be subject to geographical variation, extend the evidence about the concentration of resistance in group A strains, explore for the first time the clinical significance of biofilm production, and support the concept that prognosis is related mainly to host factors and a putative marker of the degree of bacteremia. Regarding the latter, a time to detection of growth in blood culture vials lower than or equal to the 25th percentile of the time to positivity distribution of all isolates arises as a measurement of possible clinical impact.

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