



## **Escherichia coli bacteraemia in children: age and portal of entry are the main predictors of severity**

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### **► To cite this version:**

Charles Burdet, Olivier Clermont, Stéphane Bonacorsi, Cédric Laouénan, Edouard Bingen, et al.. Escherichia coli bacteraemia in children: age and portal of entry are the main predictors of severity. Pediatric Infectious Disease Journal, 2014, 33 (2), pp.872. 10.1097/INF.0000000000000309 . inserm-01079025

**HAL Id: inserm-01079025**

**<https://inserm.hal.science/inserm-01079025>**

Submitted on 9 Nov 2014

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# Title

*Escherichia coli* bacteraemia in children: age and portal of entry are the main predictors of severity

## Abbreviated Title

Predictors of severity in children' *E. coli* bacteraemia

## Running Title

*E. coli* bacteraemia's severity in children

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## **Authors' contribution**

CB, SB and ED wrote the article

OC performed and analysed the bacterial data

CB, CL and FM performed the statistical analyses

AL, SB, EB, YA and ED conceived the study

## **Conflicts of Interest and Source of Funding**

The COLIBAFI study was supported by grants from “Réseau de Recherche Clinique” (INSERM: RBM-03-58) and “Projet Hospitalier de Recherche Clinique” (Assistance Publique-Hôpitaux de Paris: AOR 04 053).

All authors reported no conflict of interest.

## **Meetings**

This work has partly been reported at the 32nd Réunion interdisciplinaire de chimiothérapie anti-infectieuse (RICAI), held in Paris, France on 21 – 22 November 2012 (poster 548).

## **Acknowledgements**

We are grateful to Cécile Gateau for her technical help in the genotyping of the *E. coli* strains and to Jorge Blanco from the University of Santiago de Compostela, Lugo, Spain for serotyping of some strains.

## **Key words**

Bacteraemia, *Escherichia coli*, Children, Portal of entry, Prognosis, Severity, Death, Intensive care units, Virulence

## INTRODUCTION

Severe sepsis in children remains a common cause of hospitalization (1). Bacteraemia account for 25% of hospitalization for severe bacterial infections, and mortality rate is as high as 17% (1). Few temporal changes in the distribution of bacterial species in bloodstream infections have been reported (2). In industrialized countries, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* figure among the 5 more frequently isolated organisms (3).

*E. coli* is a commensal bacteria of the gut (4). Some strains can cause however intestinal or extra-intestinal infections due to specific virulence factors (5). The structure of *E. coli* population is mainly clonal (6), with seven principal phylogenetic groups (A, B1, B2, C, D, E and F) and numerous clonal complexes (4, 7-10). Most clinical extra-intestinal pathogenic *E. coli* belong to the B2 phylogroup, and to a lesser extent to the D phylogenetic group (11). Some clones or clonal complexes have been linked to specific syndromes (12, 13).

In adults, mortality of *E. coli* bacteraemia has been reported to be as high as 13% in a recent study performed by our group (14). We showed that host factors and the portal of entry are the main drivers of mortality during *E. coli* bacteraemia. The only bacterial characteristic influencing the prognosis was the presence of virulence factor *ireA*, which was negatively associated with death. Phylogenetic belonging of the strains was not a significant predictor.

Studies of *E. coli* bloodstream infections in children, especially in urinary-source bacteraemia, linked some virulence factors to the development of bacteraemia (15-17). They include adhesins, iron uptake systems, protectins and toxins, with differences according to the portal of entry. A higher content in virulence factors was thus reported in strains causing urosepsis when compared to strains causing urinary-tract infections without bacteraemia (16). However, there is a lack of prospective studies taking into account both host and bacterial characteristics *versus* the clinical outcome of *E. coli* bacteraemia in children.

To better understand the pathophysiology and severity of *E. coli* bacteraemia in children, we analysed the cohort of children  $\leq 18$ -year-old of the COLIBAFI study (14). We compared bacterial characteristics (i) in bacteraemia from urinary vs. digestive origin, (ii) in children  $\leq 3$  vs.  $>3$ -month-old, and (iii) in community-acquired urinary-source bacteraemia in children vs. adults. Then we searched for clinical and bacterial risk factors associated with the severity of *E. coli* bacteraemia in children.

## **MATERIALS AND METHODS**

### ***Study Design and Setting***

This study is part of the COLIBAFI study, a large prospective observational study conducted in 15 French hospitals between January 2005 and November 2007 in which 1051 adults and 84 children with *E. coli* bacteraemia were enrolled. The methodology has been previously published (14). *E. coli* bacteraemia was defined as the presence of *E. coli* in  $\geq 1$  aseptically filled blood culture vial(s). The follow-up ended at hospital discharge, or at day 28 after the first positive blood culture. The study was approved by the institutional Ethics Committee (Hôpital Saint-Louis, Paris, France).

### ***Clinical and Bacterial Characteristics***

Clinical characteristics included age, gender, birth weight, preterm birth, immunodepression and origin of infection. Portal of entry, immunodepression and nosocomial bacteraemia were defined as in (14). Severity of bacteraemia was defined as the occurrence of death or transfer to ICU during follow-up.

Phylogenetic group of strains was determined by a quadruplex PCR method derived from the Clermont method (18), associated to allele specific PCRs allowing the delineation of 7 main phylogroups (*i.e.*, A, B1, B2, C, D, E and F) (19). Strains exhibiting the A<sub>1</sub> genotype (*chuA*-, *yjaA*+, TspE4.C2-) (18) correspond to ST complex (STc) 10 according to the Achtman multilocus sequence typing (MLST) scheme (9). Among the D phylogroup strains, clonal group A (CGA) was identified as

previously described (20). Ten main B2 phylogenetic subgroups (I to X) were detected by allele specific PCRs (8, 21). They correspond to STc 131, 73, 127, 141, 144, 12, 14, 452, 95 and 372, respectively (9). O-typing was performed by PCR (22, 23).

The presence of 20 extraintestinal virulence factors was tested by PCR (14, 24), including adhesins/invasins (*papC*, *papG* II and III alleles, *sfa/foc*, *iha*, *hra*, and *ibeA*), toxins (*hlyC*, *cnf1*, *sat*, *clbA* and *clbQ*), iron capture systems (*fyuA*, *irp2*, *iroN*, *iucC*, and *ireA*), protectins (*neuC*, chromosomal *ompT*, and *traT*), and a gene encoding an uropathogenic-specific protein, *usp*. The presence of 5 intestinal virulence genes was also tested by PCR (23): *afaD* (diffusely adherent *E. coli*=DAEC), *eltB* and *estA* (enterotoxigenic *E. coli*=ETEC), *eae* (enteropathogenic *E. coli*=EPEC and enterohemorrhagic *E. coli*=EHEC) and *aata* (enteroaggregative *E. coli*=EAEC). The presence of 7 pathogenicity-associated islands (PAIs) was deduced from the presence of virulence factors (25, 26): PAI I<sub>CT073</sub> (*papGII*, *hlyC*, and *iucC* positive), PAI II<sub>J96</sub> (presence of at least 3 of the 4 following genes: *papGIII*, *hlyC*, *cnf1*, and *hra*), PAI III<sub>536</sub> (*sfa/foc* and *iroN* positive), PAI IV<sub>536</sub> (*irp2* and *fyuA* positive), PAI<sub>gimA</sub> (*ibeA* positive), PAI<sub>USP</sub> (*usp* positive) and PAI<sub>pks</sub> (*clbA* and *clbQ* positive). For each strain, virulence and PAIs scores were computed as the number of virulence factors and PAIs present.

Antimicrobial susceptibility was assessed using the disk diffusion method, as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie ([www.sfm-microbiologie.org](http://www.sfm-microbiologie.org)). Intermediate susceptibility was regarded as resistance. Resistance to cefotaxime and/or ceftazidime defined resistance to third-generation cephalosporin. Resistance to amoxicillin, ofloxacin and cotrimoxazole defined multidrug resistance. A resistance score was computed based on resistance to amoxicillin, cefotaxime, gentamicin, ofloxacin and cotrimoxazole.

## **Statistical Methods**

We compared bacterial characteristics i) in bacteraemia from urinary vs. digestive origin, ii) in children ≤3 vs. >3-month-old, iii) in children vs. adults presenting a community-acquired urinary-



source bacteraemia. The cut-off of 3 months was chosen as the first 3 months of life correspond to a period of age with a known increased risk of *E. coli* bacteraemia and meningitis related to the impaired innate immunity in this period (27, 28). For the third comparison, all adults from the COLIBAFI cohort presenting a community-acquired urinary-source bacteraemia were included. Characteristics tested included phylogenetic group (from the results of the triplex PCR (18), grouped as B2 vs. non-B2), virulence factors and PAIs, virulence and PAI scores, antimicrobial resistance, multidrug resistance, and resistance score. Comparisons between groups were performed using non-parametric tests (Wilcoxon or Fisher exact tests).

We searched for risk factors associated with severity (yes/no). Analyses were performed in all children and in the  $\leq 3$ -month-old subgroup. Clinical characteristics studied included gender, age ( $\leq 3$  vs.  $> 3$ -month-old), immunodepression, nosocomial infection and portal of entry. Prematurity and birth weight were also studied in  $\leq 3$ -month-old infants. The same bacterial characteristics than those described above were analyzed. Variables achieving a p-value  $< 0.20$  in univariate logistic regression analysis were entered into a multivariate logistic regression analysis to identify risk factors of severity. Using a forward selection method, we obtained a final model in which all risk factors had a p-value  $< 0.05$ . First order interaction was tested for significant variables. The model discrimination was assessed by the c-statistic and its 95% confidence interval (95%CI), and the model calibration was assessed by the Hosmer-Lemeshow goodness-of-fit test.

Analyses were performed with SAS v9.3 (SAS Institute Inc., Cary, NC). All tests were two-sided with a type-I error fixed to 0.05.

## RESULTS

### *Clinical Characteristics*

All 84 patients  $\leq 18$ -year-old from the COLIBAFI cohort were included (Table 1). The most frequent portals of entry were the urinary tract (n=51, 66.2%) and the digestive tract (n=15, 19.5%).

Eight children (10.4%) were immunocompromized: 4 had a history of hemopathy, 2 had had solid-organ transplantation, 1 had neutropenia and 1 had a congenital immunodeficiency. Fourteen children (16.7%) were transferred to ICU, 8 (9.5%) died during follow-up. In total, 17 (20.2%) presented a severe bacteraemia (95%CI, 12.2% - 30.4%).

### **Bacterial Characteristics**

Three children had a polymicrobial bacteraemia. The most frequent *E. coli* phylogenetic group was B2 group (63.1%), followed by D, F and A groups (11.9%, 9.5% and 8.3%, respectively). No strain belonged to the E phylogenetic group. Among the B2 isolates, most represented clonal groups were the II (STc73) and IX (STc95) subgroups (37.7% for each group). Other clonal groups were I, IV, V, VI, VII and X groups (5.6%, 9.4%, 1.9%, 1.9%, 3.8% and 1.9%, respectively). All except 3 strains from the D phylogroup corresponded to the CGA. Three among the 7 strains of phylogroup A belonged to the STc10. Only 3 strains were not O-typed. Four O-types were predominant: O6a (20.2%), O1 (19.0%), O2a and O2b (15.4%) and O7 (8.3%). Individual data for phylogenetic groups, clonal groups and O-types are presented in Supplemental Digital Content 1 (Table S1).

The proportion of extraintestinal virulence factors ranged from 6.0% for *ibeA* to 94.1% for *fyuA* and *irp2* (see Table S1 in Supplemental Digital Content 1 and Figure S1 in Supplemental Digital Content 2). Likely, the frequency of PAIs ranged from 6.0% for the PAI<sub>gimA</sub> to 94.1% for the PAI IV<sub>536</sub>, with frequencies of 17.9%, 25.0%, 27.4%, 32.1% and 65.5% for PAI II<sub>J96</sub>, PAI I<sub>CFT073</sub>, PAI III<sub>536</sub>, PAI<sub>pks</sub> and PAI<sub>USP</sub>, respectively. Median (min-max) virulence and PAI scores were 12 (1-9) and 2 (0-7), respectively. Only one strain belonging to the A<sub>1</sub> clonal group (STc10) exhibited intraintestinal virulence genes, *i.e.* *afaD* and *aatA*. Most isolates were resistant to amoxicillin (63.1%) and susceptible to ofloxacin and gentamicin (96.4% and 96.4%, Figure S1). Three isolates were resistant to third-generation cephalosporins (3.6%) but none to imipenem, and 29 (34.5%) were resistant to cotrimoxazole. Three isolates (3.6%) were multidrug resistant.

### ***Comparison of urinary vs. digestive origin***

The aerobactin gene *iucC*, the *papGII* allele and the PAI I<sub>CF1073</sub> were more frequent in strains responsible of bacteraemia from urinary origin than from digestive origin ( $p=0.003$ ,  $p=0.03$  and  $p=0.05$ , respectively). There was no difference in phylogroup repartition or antimicrobial susceptibility according to portal of entry (Table 2).

### ***Comparison of $\leq 3$ vs. $>3$ -Month-Old Children***

Children  $\leq 3$ -month-old ( $n=43$ , 51%) were more frequently males than patients  $>3$ -month-old (72.1% vs. 41.5%,  $p<0.01$ ) and less frequently immunocompromized (0.0% vs. 21.1%,  $p=0.002$ ). There was no significant difference found in portals of entry. Severe bacteraemia were more frequently observed in children  $\leq 3$ -month-old (32.6% vs. 7.3%,  $p=0.006$ ). Thirteen children  $\leq 3$ -month-old and 1 children  $>3$ -month-old were transferred to ICU, while 6 children  $\leq 3$ -month-old and 2 children  $>3$ -month-old died during follow-up. Bacterial characteristics are reported in Table 3. Virulence score was higher in  $\leq 3$ -month-old children (median [min-max] = 15 [5-18] vs. 10 [1-19],  $p=0.02$ ), with 3 virulence factors being more frequently observed: *neuC* (K1 antigen, 55.8% vs. 26.8%,  $p=0.009$ ), *irp2* (100% vs. 87.8%,  $p=0.02$ ) and *fyuA* (100% vs. 87.8%,  $p=0.02$ ). Antimicrobial resistance score was lower in  $\leq 3$ -month-old infants (median [min-max] = 1 [0-4] vs. 1 [0-5],  $p=0.01$ ), with lower rates of resistance to amoxicillin (51.2% vs. 75.6%,  $p=0.02$ ) and cotrimoxazole (23.3% vs. 46.3%,  $p=0.04$ ).

### ***Children vs. Adults Community-Acquired Urinary-Source Bacteraemia***

Community-acquired urinary-source bacteraemia concerned 45 children and 513 adults from the COLIBAFI cohort (Table 4). We observed more females (73.1% vs. 44.4%,  $p<0.001$ ) and a higher rate of immunodepression in adults than in children (23.9% vs. 4.9 %,  $p=0.003$ ). Severe bacteraemia was more frequent in adults (11.9% vs. 0%,  $p=0.01$ ). There was no significant difference in phylogenetic groups (B2 vs. non-B2) nor in virulence scores, but 5 virulence factors were significantly more frequent in *E. coli* strains isolated from children (*i.e.*, *iucC*, *iha*, *papC*, *papGII* and *sat*), whereas

*hrra* was significantly less frequent. Adult isolates had a higher resistance score (median [min-max] = 1 [0-5] vs. 1 [0-4],  $p=0.04$ ).

### ***Risk Factors of Severity***

Results of the univariate and multivariate analyses to identify risk factors of severity in all the included children are reported Table 5. Because of some missing characteristics, the multivariate analysis was performed in 77 children among which 16 (20.8%) had a severe bacteraemia. Non-urinary-source bacteraemia (OR = 0.01, 95%CI = 0.001-0.1) and being  $\leq 3$ -month-old (OR = 7.7, 95%CI = 1.4-42.8) were the only risk factors of severity. The c-statistic of the final model was 0.93 (95%CI = 0.87-0.99). The p-value of the Hosmer-Lemeshow test was 0.93, showing no model misspecification.

Among the 43 children  $\leq 3$ -month-old, the bacteraemia was severe in 14 (32.6%). Birth weight was lower (median [min-max] = 1560g [595-3580] vs. 3285g [910-5090],  $p<0.01$ ) and a preterm birth was more frequent (71.4% vs. 13.8%,  $p<0.001$ ) in  $\leq 3$ -month-old children with severe bacteraemia. Children  $\leq 3$ -month-old with a severe bacteraemia had a more frequently non-urinary (92.9% vs. 17.2%,  $p<0.001$ ) but digestive (50.0% vs. 10.3%,  $p<0.01$ ) portal of entry than those without. Because of some missing characteristics, the multivariate analysis was performed in 41 children among which 13 (31.7%) had a severe bacteraemia. A non-urinary source of bacteraemia was the only risk factor associated with severity (OR = 72.0, CI95% = 7.2-796.9). Prematurity and birth weight were not found significantly associated with severity, nor any of the bacterial characteristics tested. The c-statistic for this final model was 0.89 (CI95% = 0.80-0.99). The p-value of the Hosmer-Lemeshow test was  $>0.99$ , showing no model misspecification.

## **DISCUSSION**

Few data are available about *E. coli* bacteraemia in children despite its frequency and severity. To our knowledge, this is the first report combining bacterial and host characteristics in *E. coli* bacteraemia in children.

We found a high proportion of strains belonging to the B2 and D phylogroups. Main clonal groups delineated from the B2 strains were the II and IX clonal groups, representing 23.8% each of all the strains. They correspond respectively to the STc73 and STc95 (8, 9). Strains belonging to STc131 (clonal group I) accounted for 3.6% in our study. We found a high proportion of CGA (STc69) among the D phylogroup strains, which accounted for 8.3% of all strains. All these CGA strains were resistant to cotrimoxazole. These results are similar to those obtained in adults. In a study of 300 uropathogenic *E. coli*, most common STc retrieved were STc73 (16.6%), STc131 (13.3%), STc69 (9%) and STc95 (6.3%) (29). We also observed a predominance of 4 specific O-types, *i.e.* O1, O2a/b, O6 and O7. These data indicate that some specific clones among the huge clonal diversity of the *E. coli* species (4) are involved in extraintestinal infections. At the opposite, a high diversity in the pattern of presence or absence of virulence genes was evidenced, with 61 observed combinations (Table S1). Altogether, these data support the fact that extraintestinal virulence in *E. coli* is due to multiple combinations of virulence genes (6) arriving on specific genetic backgrounds (30). However, some specific virulence factors have been associated to specific syndromes. In their study of 123 children, Cheng et al. showed that *E. coli* strains causing urinary-source bacteraemia harboured more frequently *papGI*, *iutA*, *traT*, *focG*, *afa*, *bmaE*, *kpsMT III*, *rfa* and *cvaL* than strains causing acute pyelonephritis or acute lobar nephronia (16). Bonacorsi and colleagues found that, in infants younger than 90 days, the presence of *hly* and/or *iroN*, as was that of *hly* and/or antigen K1, was associated in strains causing urinary tract infections with bacteraemia (15). This group also found that, in young infants, *papGII*, *sfa/foc* and *hly* were more frequent in strains isolated from urosepsis than from meningitis (12, 13). Similarly, in a study of 100 infants  $\leq 3$ -month-old with bacteraemia, these authors concluded that *papGII* and *tcpC* were more frequent, but *ibeA* less frequent, in *E. coli* originating from urinary tract infection than from gut translocation (17). In the same line, we found that *iucC*, which belongs to the aerobactin operon as *iutA*, and *papGII* were more frequent in urinary-source bacteraemia (Table 2).

We took the opportunity of the large COLIBAFI cohort to compare community-acquired urinary-source bacteraemia between children and adults. We found that bacteraemia in children were less severe, but involved strains exhibiting a specific repertoire of virulence genes and being more resistant than those isolated in adults. Children are usually considered to be more susceptible to infections due to an immature immune system. This apparently contra intuitive result might be explained by comorbidities in the adult group (23.9% of adults were immunocompromized (14), and only 4.9% of children). To our knowledge, no study compared the prevalence of resistant bacteria in children and in adults. Data of the European Antimicrobial Resistance Surveillance Network for the period 2005 – 2007 show that about 55% of *E. coli* strains were resistant to aminopenicillins (31). The higher prevalence of resistance observed in our cohort of children (63.1%) might be explained by the difference in antibiotic consumption among children. In France in 2004, the number of antimicrobial prescriptions for respiratory infections was almost 3 times higher in infants <2.5-year-old than in adults (32). This suggests that children have a higher selective pressure than adults, which could result in higher rates of infection with resistant bacteria.

In our study, we found a case-fatality rate of 9.5%, similar to that observed in a cohort of 2300 *E. coli* bacteraemia in Canada (2). No bacterial characteristic was associated with severity defined as death or transfer to ICU during follow-up. The severity outcome was driven by portal of entry and age. This observation was also made in adults, in whom we showed that host factors and the portal of entry outweighed bacterial determinants for predicting death from *E. coli* bacteraemia (14). Houdouin et al. examined patients' characteristics and bacterial virulence mechanisms in lethal and non-lethal *E. coli* meningitis (27). In their retrospective study of 99 children  $\leq 3$ -month-old, no pre-onset clinical factor was significantly associated with a fatal outcome. The only bacterial characteristic significantly associated with lethality was the absence of the aerobactin gene *iucC*. However, no multivariate analysis was performed.

Our study has several limitations. First, our prospective cohort included a rather small number of children. Bacteraemia was severe only in 17 children, thus diminishing the power to

determine risk factors of severity. However, we obtained a model with good predictive capacity (c-statistic of 0.93). Second, our study was performed in 2005 – 2007. Only 3 strains resistant to third generation cephalosporins were isolated. In the recent years, the prevalence of resistant bacteria has increased. A study performed in Sweden in 2010 showed that 2.9% of healthy children were colonized with extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*. This figure rose to 8.4% in ill children (33). This could weaken our results, as bacteraemia due to ESBL-producing *E. coli* have been associated with an increased risk of mortality in adults (34). Despite these limitations, our multicenter study with a prospective design is the first one focusing on *E. coli* bacteraemia in children. Further studies should be performed on larger cohorts to confirm our findings.

## REFERENCES

1. Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. *Am J Respir Crit Care Med*. 2003;167:695-701.
2. Laupland KB, Gregson DB, Church DL, Ross T, Pitout JD. Incidence, risk factors and outcomes of *Escherichia coli* bloodstream infections in a large Canadian region. *Clin Microbiol Infect*. 2008;14:1041-1047.
3. Doit C, Mariani-Kurkdjian P, Mahjoub-Messai F, et al. Epidemiology of pediatric community-acquired bloodstream infections in a children hospital in Paris, France, 2001 to 2008. *Diagn Microbiol Infect Dis*. 2010;66:332-335.
4. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *Escherichia coli*. *Nat Rev Microbiol*. 2010;8:207-217.
5. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol*. 2004;2:123-140.
6. Touchon M, Hoede C, Tenaillon O, et al. Organised genome dynamics in the *Escherichia coli* species results in highly diverse adaptive paths. *PLoS Genet*. 2009;5:e1000344.

7. Jaureguy F, Landraud L, Passet V, et al. Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains. *BMC Genomics*. 2008;9:560.
8. Le Gall T, Clermont O, Gouriou S, et al. Extraintestinal virulence is a coincidental by-product of commensalism in B2 phylogenetic group *Escherichia coli* strains. *Mol Biol Evol*. 2007;24:2373-2384.
9. Wirth T, Falush D, Lan R, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol*. 2006;60:1136-1151.
10. Moissenet D, Salauze B, Clermont O, et al. Meningitis caused by *Escherichia coli* producing TEM-52 extended-spectrum beta-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. *J Clin Microbiol*. 2010;48:2459-2463.
11. Johnson JR, Russo TA. Extraintestinal pathogenic *Escherichia coli*: "the other bad *E. coli*". *J Lab Clin Med*. 2002;139:155-162.
12. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev*. 1998;11:142-201.
13. Bidet P, Mahjoub-Messai F, Blanco J, et al. Combined multilocus sequence typing and O serogrouping distinguishes *Escherichia coli* subtypes associated with infant urosepsis and/or meningitis. *J Infect Dis*. 2007;196:297-303.
14. Lefort A, Panhard X, Clermont O, et al. Host factors and portal of entry outweigh bacterial determinants to predict the severity of *Escherichia coli* bacteremia. *J Clin Microbiol*. 2011;49:777-783.
15. Bonacorsi S, Houdouin V, Mariani-Kurkdjian P, Mahjoub-Messai F, Bingen E. Comparative prevalence of virulence factors in *Escherichia coli* causing urinary tract infection in male infants with and without bacteremia. *J Clin Microbiol*. 2006;44:1156-1158.
16. Cheng CH, Tsau YK, Kuo CY, Su LH, Lin TY. Comparison of extended virulence genotypes for bacteria isolated from pediatric patients with urosepsis, acute pyelonephritis, and acute lobar nephronia. *Pediatr Infect Dis J*. 2010;29:736-740.
17. Mahjoub-Messai F, Bidet P, Caro V, et al. *Escherichia coli* isolates causing bacteremia via gut translocation and urinary tract infection in young infants exhibit different virulence genotypes. *J Infect Dis*. 2011;203:1844-1849.



18. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol.* 2000;66:4555-4558.
19. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep.* 2013;5:58-65.
20. Johnson JR, Owens K, Manges AR, Riley LW. Rapid and specific detection of *Escherichia coli* clonal group A by gene-specific PCR. *J Clin Microbiol.* 2004;42:2618-2622.
21. Clermont O, Christenson J, Daubié A, Gordon D, Denamur E. Development of an allele-specific PCR for *Escherichia coli* B2 sub-typing, a rapid and easy to perform substitute of multilocus sequence typing. *submitted.* 2014.
22. Clermont O, Johnson JR, Menard M, Denamur E. Determination of *Escherichia coli* O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia. *Diagn Microbiol Infect Dis.* 2007;57:129-136.
23. Clermont O, Olier M, Hoede C, et al. Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. *Infect Genet Evol.* 2011;11:654-662.
24. Johnson JR, Johnston B, Kuskowski MA, Nougayrede JP, Oswald E. Molecular epidemiology and phylogenetic distribution of the *Escherichia coli* pks genomic island. *J Clin Microbiol.* 2008;46:3906-3911.
25. Hacker J, Blum-Oehler G, Muhldorfer I, Tschape H. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. *Mol Microbiol.* 1997;23:1089-1097.
26. Bingen-Bidois M, Clermont O, Bonacorsi S, et al. Phylogenetic analysis and prevalence of urosepsis strains of *Escherichia coli* bearing pathogenicity island-like domains. *Infect Immun.* 2002;70:3216-3226.
27. Houdouin V, Bonacorsi S, Bidet P, et al. Association between mortality of *Escherichia coli* meningitis in young infants and non-virulent clonal groups of strains. *Clin Microbiol Infect.* 2008;14:685-690.

28. Bachur R, Caputo GL. Bacteremia and meningitis among infants with urinary tract infections. *Pediatr Emerg Care*. 1995;11:280-284.
29. Gibreel TM, Dodgson AR, Cheesbrough J, Fox AJ, Bolton FJ, Upton M. Population structure, virulence potential and antibiotic susceptibility of uropathogenic *Escherichia coli* from Northwest England. *J Antimicrob Chemother*. 2012;67:346-356.
30. Escobar-Paramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguenec C, Denamur E. A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. *Mol Biol Evol*. 2004;21:1085-1094.
31. EARS-Net. European Antimicrobial Resistance Surveillance Network - Annual Reports. Accessed on 04/12/2013 at : <http://ecdc.europa.eu>.
32. Chahwakilian P, Huttner B, Schlemmer B, Harbarth S. Impact of the French campaign to reduce inappropriate ambulatory antibiotic use on the prescription and consultation rates for respiratory tract infections. *J Antimicrob Chemother*. 2011;66:2872-2879.
33. Kaarme J, Molin Y, Olsen B, Melhus A. Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in healthy Swedish preschool children. *Acta Paediatr*. 2013;102:655-660.
34. Rodriguez-Bano J, Picon E, Gijon P, et al. Community-onset bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli*: risk factors and prognosis. *Clin Infect Dis*. 2010;50:40-48.

**TABLE 1.** Clinical and bacteriological characteristics of the 84 children with *E. coli* bacteraemia.

<b>Characteristic</b>	<b>n [%]<sup>1</sup></b>
Age, median (min-max), months	2.4 (0-204)
Males	48 [57.1]
Nosocomial infection <sup>2</sup>	25 [30.1]
Immunocompromized <sup>2</sup>	8 [10.4]
Portal of entry <sup>2</sup>	
Urinary tract	51 [66.2]
Digestive tract	15 [19.5]
Respiratory tract	2 [2.6]
Cutaneous	3 [3.9]
Venous catheter	5 [6.5]
Surgical site	1 [1.3]
Not determined	7 [9.1]
Two portals of entry	7 [9.1]
Polymicrobial infection	3 [3.6]
Phylogenetic group	
A	7 [8.3]
B1	3 [3.6]
B2	53 [63.1]
C	3 [3.6]
D	10 [11.9]
F	8 [9.5]
Antibiotic resistance	
Amoxicillin	53 [63.1]
Amoxicillin + clavulanate	38 [45.2]
Gentamicin	3 [3.6]
Ofloxacin	3 [3.6]
Cotrimoxazole	29 [34.5]
3rd generation cephalosporins	3 [3.6]
Multidrug resistance	3 [3.6]
Resistance score, median (min-max)	1 (0-5)
Virulence score, median (min-max)	12 (1-19)

Notes:

<sup>1</sup>Except when otherwise notified.

<sup>2</sup>Because of missing data, percentages are calculated based on available data.

**TABLE 2.** Characteristics of the *E. coli* isolates according to the portal of entry (urinary- vs. digestive-source bacteraemia).

Characteristic	n [%] <sup>1</sup>		p
	Urinary tract (n=47)	Digestive tract (n=11)	
Phylogenetic group			
B2	31 [66]	5 [45.5]	0.3
non-B2	16 [34]	6 [54.5]	
Extraintestinal virulence gene			
<i>neuC</i> (K1)	16 [34]	7 [63.6]	0.09
<i>sfa/soc</i>	12 [25.5]	2 [18.2]	>.99
<i>iroN</i>	25 [53.2]	5 [45.5]	0.7
<i>iucC</i>	42 [89.4]	5 [45.5]	0.003
<i>iha</i>	25 [53.2]	4 [36.4]	0.5
<i>papC</i>	39 [83]	7 [63.6]	0.2
<i>papGII</i>	38 [80.9]	5 [45.5]	0.03
<i>papGIII</i>	2 [4.3]	2 [18.2]	0.2
<i>hlyC</i>	17 [36.2]	2 [18.2]	0.3
<i>cnf1</i>	7 [14.9]	2 [18.2]	>.99
<i>hra</i>	26 [55.3]	6 [54.6]	>.99
<i>sat</i>	31 [66]	4 [36.4]	0.09
<i>ire</i>	22 [46.8]	4 [36.4]	0.7
<i>usp</i>	31 [66]	7 [63.6]	>.99
<i>ompT</i>	40 [85.1]	10 [90.9]	>.99
<i>ibeA</i>	2 [4.3]	2 [18.2]	0.2
<i>fyuA</i>	45 [95.7]	10 [90.9]	0.5
<i>irp2</i>	45 [95.7]	10 [90.9]	0.5
<i>traT</i>	38 [80.9]	9 [81.8]	>.99
<i>clbA</i>	12 [25.5]	4 [36.4]	0.5
<i>clbQ</i>	12 [25.5]	4 [36.4]	0.5
Virulence score, median (min-max)	12 (1-18)	11 (3-15)	0.3
PAI			
PAI I <sub>CFT073</sub>	14 [29.8]	0 [0]	0.05
PAI II <sub>J96</sub>	7 [14.9]	2 [18.2]	>.99
PAI III <sub>536</sub>	12 [25.5]	2 [18.2]	>.99
PAI IV <sub>536</sub>	45 [95.7]	10 [90.9]	0.5
PAI <sub>gimA</sub>	2 [4.3]	2 [18.2]	0.2
PAI <sub>USP</sub>	31 [66]	7 [63.6]	>.99
PAI <sub>pks</sub>	12 [25.5]	4 [36.4]	0.5
PAI score, median (min-max)	2 (0-6)	2 (0-5)	0.8
Antibiotic resistance			
Amoxicillin	34 [72.3]	6 [54.6]	0.3
Gentamicin	1 [2.1]	0 [0]	>.99
Ofloxacin	2 [4.3]	0 [0]	>.99
Cotrimoxazole	19 [40.4]	4 [36.4]	>.99
3rd generation cephalosporins	1 [2.1]	0 [0]	>.99
Multidrug resistance	2 [4.3]	0 [0]	>.99
Resistance score, median (min-max)	1 (0-4)	1 (0-2)	0.3

Notes:

<sup>1</sup>Except when otherwise notified.

PAI, pathogenicity-associated island

**TABLE 3.** Bacterial characteristics in the ≤3 vs. >3-month-old groups.

Characteristic	n [%] <sup>1</sup>		p
	≤3-month (n=43)	>3-month (n=41)	
Polymicrobial infection	2 [4.7]	1 [2.4]	>.99
Phylogenetic group			
B2	29 [67.4]	24 [58.5]	0.5
non-B2	14 [32.6]	17 [41.5]	
Extraintestinal virulence gene			
<i>neuC</i> (K1)	24 [55.8]	11 [26.8]	0.009
<i>sfa/foc</i>	12 [27.9]	11 [26.8]	>.99
<i>IroN</i>	25 [58.1]	25 [61]	0.8
<i>iucC</i>	34 [79.1]	36 [87.8]	0.4
<i>iha</i>	24 [55.8]	17 [41.5]	0.2
<i>papC</i>	37 [86]	30 [73.2]	0.2
<i>papGII</i>	34 [79.1]	28 [68.3]	0.3
<i>papGIII</i>	6 [14]	3 [7.3]	0.5
<i>hlyC</i>	17 [39.5]	12 [29.3]	0.4
<i>cnf1</i>	10 [23.3]	5 [12.2]	0.3
<i>hra</i>	29 [67.4]	19 [46.3]	0.1
<i>sat</i>	26 [60.5]	23 [56.1]	0.8
<i>ire</i>	25 [58.1]	18 [43.9]	0.3
<i>usp</i>	31 [72.1]	24 [58.5]	0.3
<i>ompT</i>	39 [90.7]	32 [78]	0.1
<i>ibeA</i>	3 [7]	2 [4.9]	>.99
<i>fyuA</i>	43 [100]	36 [87.8]	0.02
<i>irp2</i>	43 [100]	36 [87.8]	0.02
<i>traT</i>	36 [83.7]	29 [70.7]	0.2
<i>clbA</i>	15 [34.9]	12 [27.9]	0.6
<i>clbQ</i>	15 [34.9]	12 [27.9]	0.6
Virulence score, median (min-max)	15 (5-18)	10 (1-19)	0.02
PAI			
PAI I <sub>CFT073</sub>	12 [27.9]	9 [22]	0.6
PAI II <sub>J96</sub>	10 [23.3]	5 [12.2]	0.3
PAI III <sub>536</sub>	12 [27.9]	11 [26.8]	>.99
PAI IV <sub>536</sub>	43 [100]	36 [87.8]	0.02
PAI <sub><i>gimA</i></sub>	3 [7]	2 [4.9]	>.99
PAI <sub>USP</sub>	31 [72.1]	24 [58.5]	0.3
PAI <sub><i>pks</i></sub>	15 [34.9]	12 [27.9]	0.6
PAI score, median (min-max)	3 (1-16)	2 (0-7)	0.1
Antibiotic Resistance			
Amoxicillin	22 [51.2]	31 [75.6]	0.02
Gentamicin	1 [2.3]	2 [4.9]	0.6
Ofloxacin	1 [2.3]	2 [4.9]	0.6
Cotrimoxazole	10 [23.3]	19 [46.3]	0.04

3rd generation cephalosporins	1 [2.3]	2 [4.9]	0.6
Multidrug resistance	1 [2.3]	2 [4.9]	0.6
Resistance score, median (min-max)	1 (0-4)	1 (0-5)	0.01

Notes:

<sup>1</sup>Except when otherwise notified.

PAI, pathogenicity-associated island.

**TABLE 4.** Clinical and bacterial characteristics in children and adults with community-acquired urinary-source bacteraemia.

Characteristic	n [%] <sup>1</sup>		p
	Children (n=45)	Adults (n=513)	
Clinical			
Female	20 [44.4]	375 [73.1]	<0.001
Immunocompromized <sup>2</sup>	2 [4.9]	120 [23.9]	0.003
Transfer to ICU	0 [0]	35 [6.9]	0.1
Death	0 [0]	33 [6.4]	0.1
Severe bacteraemia <sup>3</sup>	0 [0]	61 [11.9]	0.01
Bacterial			
Polymicrobial infection	1 [2.2]	11 [2.1]	>.99
Phylogenetic group			
B2	29 [64.4]	322 [62.8]	0.9
non-B2	16 [35.6]	191 [37.2]	
Virulence factor			
<i>neuC</i> (K1)	15 [33.3]	138 [26.9]	0.4
<i>sfa/foc</i>	12 [26.7]	154 [30.0]	0.7
<i>IroN</i>	25 [55.6]	315 [61.4]	0.4
<i>iucC</i>	41 [91.1]	367 [71.6]	0.004
<i>iha</i>	24 [53.3]	185 [36.1]	0.03
<i>papC</i>	37 [82.2]	345 [67.3]	0.04
<i>papGII</i>	36 [80.0]	288 [56.3]	0.02
<i>papGIII</i>	2 [4.4]	63 [12.3]	0.1
<i>hlyC</i>	17 [37.8]	173 [33.7]	0.6
<i>cnf1</i>	7 [15.6]	119 [23.2]	0.3
<i>hra</i>	24 [53.3]	354 [69.0]	0.04
<i>sat</i>	30 [66.7]	191 [37.2]	<0.001
<i>ire</i>	20 [44.4]	202 [39.4]	0.5
<i>usp</i>	29 [64.4]	324 [63.1]	>.99
<i>ompT</i>	38 [84.4]	398 [77.6]	0.3
<i>ibeA</i>	2 [4.4]	29 [5.7]	>.99
<i>fyuA</i>	43 [95.6]	438 [85.4]	0.07
<i>irp2</i>	43 [95.6]	440 [85.8]	0.07
<i>traT</i>	36 [80.0]	338 [65.9]	0.07
Virulence score, median (min-max)	11 (1-16)	11 (0-17)	0.1
PAI			
PAI I <sub>CFT073</sub>	14 [31.1]	102 [19.9]	0.09
PAI II <sub>J96</sub>	7 [15.6]	119 [23.2]	0.3
PAI III <sub>536</sub>	12 [26.7]	152 [29.6]	0.7
PAI IV <sub>536</sub>	43 [95.6]	438 [85.4]	0.07
PAI <sub><i>gimA</i></sub>	2 [4.4]	29 [5.7]	>.99
PAI <sub>USP</sub>	29 [64.4]	324 [63.2]	>.99
PAI score, median (min-max)	2 (0-5)	1 (0-4)	<0.001
Resistance to 3rd generation cephalosporins	1 [2.2]	15 [2.9]	>.99
Multidug resistance	2 [4.4]	38 [7.4]	0.8
Resistance score, median (min-max)	1 (0-4)	1 (0-5)	0.04

Notes:

<sup>1</sup>Except when otherwise notified.

<sup>2</sup>Because of missing data, percentages are calculated based on available data  
PAI, pathogenicity-associated island.

<sup>3</sup>Death or transfer to ICU.



**TABLE 5.** Risk factors of severity (transfer in ICU or death) from *E. coli* bacteraemia identified by univariate and multivariate analysis in all included children.

	n [%]		Univariate analysis		Multivariate analysis <sup>2</sup>	
Risk factor	Severity		OR (IC 95%)	p	OR (IC 95%)	p
	no (n =67)	yes (n =17)				
Clinical						
Younger than 3 months	29 [43.3]	14 [82.4]	6.1 (1.6 - 23.3)	0.008	7.7 (1.4 - 42.8)	0.02
Nosocomial infection <sup>1</sup>	17 [25.8]	8 [47.1]	2.6 (0.9 - 7.7)	0.09		
Portal of entry <sup>1</sup>						
Urinary tract	50 [82.0]	1 [6.2]	0.01 (0.002 - 0.1)	<0.001	0.01 (0.001 - 0.1)	<0.001
Digestive tract	8 [13.1]	7 [43.7]	5.2 (1.5 - 17.7)	0.009		
Venous catheter	2 [3.3]	3 [18.7]	6.8 (1.0 - 44.9)	0.05		
Not determined	3 [4.9]	4 [25.0]	6.4 (1.3 - 32.6)	0.02		
Bacterial						
Resistance to amoxicillin	45 [67.2]	8 [47.1]	0.4 (0.2 - 1.3)	0.1		
Resistance to 3rd generation cephalosporins	1 [1.5]	2 [11.8]	8.8 (0.8 - 103.5)	0.08		
Resistance to gentamicin	1 [1.5]	2 [11.8]	8.8 (0.8 - 103.5)	0.08		
B2 Phylogenetic group	42 [62.7]	11 [64.7]	1.1 (0.4 - 3.3)	0.9		
Virulence factor						
<i>neuC</i> (K1)	24 [35.8]	11 [64.7]	3.3 (1.1 - 10.0)	0.04		
<i>iucC</i>	59 [88.1]	11 [64.7]	0.3 (0.1 - 0.9)	0.03		
<i>ire</i>	31 [46.3]	12 [70.6]	2.8 (0.9 - 8.8)	0.08		
<i>clbA</i>	18 [26.9]	9 [52.9]	3.1 (1.0 – 9.1)	0.08		
<i>clbQ</i>	18 [26.9]	9 [52.9]	3.1 (1.0 – 9.1)	0.08		

Note:

<sup>1</sup>Because of missing data, percentages are calculated based on available data.

<sup>2</sup>Because of missing characteristics, the multivariate analysis was performed in 77 children.