Bacteraemia caused by third-generation cephalosporin-resistant *Escherichia coli* in France: prevalence, molecular epidemiology and clinical features

A. Courpon-Claudinon[1,2], A. Lefort[3,4], X. Panhard[5], O. Clermont[2], Q. Dornic[5], B. Fantin[3,4], F. Mentré[5], M. Wolff[6], E. Denamur[2], C. Branger[1,2], on behalf of the COLIBAFI group*

1) *AP-HP, Hôpital Louis Mourier, Service de Microbiologie-Hygiène, Colombes,* 2) *INSERM U722 and Université Paris 7, Faculté de Médecine, Site Xavier Bichat,* 3) *AP-HP, Hôpital Beaujon, Service de Médecine interne, Clichy,* 4) *EA3965, Université Paris 7, Faculté de Médecine, Site Xavier Bichat,* 5) *INSERM U738 and Université Paris 7, Faculté de Médecine, Site Xavier Bichat and* 6) *AP-HP, Hôpital Bichat-Claude Bernard, Service de Réanimation Médicale et Infectieuse, Paris, France*

Abstract

Escherichia coli is one of the major pathogens responsible for bactaeremia. Empirical antibiotherapy of these infections usually relies on third-generation cephalosporins (3GCs). Thus, the occurrence and epidemiology of 3GC-resistant strains have to be monitored. The French prospective multicentre study COLIBAFI collected 1081 strains of *E. coli* responsible for bacteraemia in 2005. In the present work, the prevalence of resistance to 3GCs was evaluated, and the implicated molecular mechanisms were characterized by specific PCR and sequencing. Phylogenetic grouping, O-typing, pulsed-field gel electrophoresis and virulence factor analysis were used to investigate the genetic background of the 3GC-resistant (3GC-R) strains. Clinical features of the patients with documented data (n = 1051) were analysed. Decreased susceptibility to 3GCs was observed in 41 strains (3.8%): 19, 18 and four had extended-spectrum β-lactamase (ESBL), AmpC cephalosporinase and OXA-type penicillinase phenotypes, respectively. Pulsed-field gel electrophoresis revealed that the 3GC-R strains constitute a diverse population. All but one of the strains with an ESBL phenotype produced a CTX-M-type enzyme, and six of them belonged to the widespread intercontinental clone O25b:H4-ST131. AmpC phenotype strains harboured various chromosomal *ampC* promoter and coding region mutations and/or the bla_{CMY-2} plasmidic gene. 3GC-R strains carried fewer virulence factors and were more co-resistant to other antibiotics than 3GC-susceptible (3GC-S) strains. Infections with 3GC-R strains were mostly community-acquired and, as compared with those caused by their 3GC-S counterparts, were more severe. Underlying chronic disease and prior use of antibiotics were independent risk factors for development of a 3GC-R strain bacteraemia. The fact that the molecular support of 3GC resistance is mainly plasmid-mediated represents a potentially epidemic threat.

Keywords: β-Lactamases, bloodstream infection, epidemiology, *Escherichia coli*, multiresistance
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Corresponding author: A. Courpon-Claudinon, Hôpital Louis Mourier, Service de Microbiologie-Hygiène, 178 rue des Renouillers, 92701 Colombes, France
E-mail: aurorecourpon@gmail.com

*Members of the COLIBAFI group are listed in the Acknowledgements.

Introduction

Bacteraemias represent a major cause of death in industrialized countries such as Europe and the USA, with large increases in incidence and mortality being seen over the past 20 years [1]. For *Escherichia coli*, a leading pathogen implicated in these infections [2], an increase in the prevalence of β-lactam resistance, especially concerning the third-generation cephalosporins (3GCs), has been observed recently, according to the annual report of the European Antimicrobial Resistance Surveillance System (http://www.rivm.nl/earss). Three kinds of β-lactamase are commonly responsible for 3GC resistance: extended-spectrum β-lactamases (ESBLs), OXA-type penicillinases (or oxacillinases) and AmpC cephalosporinases (chromosomal or plasmid-mediated). Among ESBLs, the CTX-M β-lactamases have now become most prevalent [3,4].

As 3GCs form part of empirical antimicrobial chemotherapy in severe infections, the prevalence of resistance in *E. coli* is important to evaluate because of the risk of treatment failure. In 2005, a large, prospective multicentre study, COLIBAFI (http://www.colibafi.net), was conducted to identify the factors of severity associated with *E. coli* bacteraemia. The present ancillary study aimed to investigate the prevalence and molecular epidemiology of resistance to 3GCs of the COLIBAFI collection, and to analyse clinical features of patients infected by these resistant strains.

Materials and Methods

Study protocol and bacterial strains

The prospective multicentre study COLIBAFI was conducted in 15 hospitals in different areas in France: Paris (eight hospitals), Angers, Brest, Caen, Dijon, Nantes, Rennes and Tours. All except one were university hospitals. During the year 2005, all cases of *E. coli* bacteraemia, defined on the basis of the isolation of *E. coli* from one or more sets of blood culture bottles, were collected by the local bacteriology laboratory. Only patients receiving vasopressors before the bacteraemia or patients already included in the study for a previous episode were not considered for inclusion. Overall, 1099 adults were included. Forty-eight patients were excluded either because the *E. coli* isolate was not available (n = 18) or because clinical data were lacking (n = 30). Thus, the microbiological study was conducted on 1081 strains, and the clinical study concerned 1051 patients. Bacterial identification was performed with the API20E system (bioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibility was determined by the disk diffusion method on Mueller–Hinton agar, and interpreted according to the 2005 guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (http://www.sfm.asso.fr). The strains were sent to a central laboratory (INSERM U722) with clinical features of the patients and antimicrobial susceptibility data. Clinical characteristics, collected by a tandem of senior investigators (an infectious disease clinician and a bacteriologist) included age, sex, underlying chronic disease, immunosuppression, antibiotherapy received within 2 weeks before the bacteraemia, community-acquired or nosocomial infection, portal of entry and clinical outcome. Bacteraemia episodes were defined as community-acquired if the first positive blood culture was obtained <48 h following hospital admission. The full description of the cohort will be published elsewhere (A. Lefort, X. Panhard, O. Clermont, P. L. Woerther, C. Branger, F. Mentré, B. Fantin, M. Wolff, E. Denamur and the COLIBAFI group; personal communication). Strains with decreased susceptibility to cefotaxime and/or ceftazidime according to the 2005 guidelines of the CA-SFM were selected. Strains with decreased susceptibility to cefoxitin were also considered, as this can be a helpful marker with which to detect AmpC production [5].

Antimicrobial susceptibility testing of the selected strains

All selected strains were tested for ESBL production by the double-disk synergy test [6]. For the strains with a negative test result, MICs of cefoxitin, cefotaxime, ceftazidime and cefepime were determined by the Etest diffusion method (AB Biodisk, Solna, Sweden).

Molecular characterization of β-lactamases

For strains with a positive double-disk synergy test result, characterization of ESBLs was performed by specific PCR amplification and sequencing [7].

For strains with a negative double-disk synergy test result, the chromosomal *ampC* gene, its promoter and its attenuator were amplified and sequenced with primers Int-B2 and Int-HN [8]. Mutations were studied by comparison with the published *ampC* gene sequence of *E. coli* K-12 (GenBank accession number NC_000913). Plasmid-mediated AmpC cephalosporinase was detected with a multiplex PCR method [9] and identified by sequencing.

The strains were also screened for the presence of an OXA-type β-lactamase by PCR [10], and this was followed by sequencing to identify the bla_{OXA} gene.

Strain genetic background analysis

Phylogenetic grouping of the *E. coli* strains was determined by a PCR-based method [11]. The strains were screened for 17 genes encoding putative virulence factors (*sfa/foc*, *iroN*, *iutA*, *iha*, *papC*, *papG* (II and III alleles), *hlyC*, *cnf1*, *hra*, *sat*, *ire*, *usp*, chromosomal *ompT*, *ibeA*, *fyuA*, *irp2* and *traT*) by PCR [12]. For each strain, a virulence score was defined as the sum of virulence factors present over the 17 tested. Twenty-five O-types were determined with a molecular approach based on allele-specific PCR [13] in the 3GC-resistant (3GC-R) strains (see Table S1 for a list of the primers used). They include the O-types most frequently found in extra-intestinal pathogenic [13] and ESBL-producing [14,15] strains. An allele-specific PCR of the *pabB* gene was used to detect strains belonging to the O25b:H4-ST131 clone [16]. Pulsed-field gel electrophoresis was performed with a CHEF DRII System (BioRad, Marnes-la-Coquette, France) using genomic DNA digested with *XbaI* [17]. A dendrogram was constructed using the Dice similarity coefficient, and the UPGMA algorithm was used to cluster the strains.