Extra-corporeal membrane oxygenation-associated infections: implication of extra-intestinal pathogenic *Escherichia coli* clones

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**Abstract**

Extra-corporeal membrane oxygenation (ECMO) is a promising life-saving technique for critically ill patients. Bacterial infection is a frequent complication, and *Escherichia coli* the predominant causative pathogen, but little is known about the characteristics of *E. coli* strains in these infections. We therefore conducted a retrospective study of 33 *E. coli* strains responsible for 33 ECMO-related infections, in 30 subjects. Antimicrobial susceptibility, phylotyping, O-typing, clonal relatedness determination and the screening for four virulence factor genes were conducted. Polymicrobial infections were evidenced in 61.6% of episodes, irrespective of *E. coli* characteristics. Extra-intestinal pathogenic strains represented the major large (69.7%) of all *E. coli* isolates. Their advantageous genetic background may explain their predominance in this context. The potential for targeted digestive decontamination should be investigated in these patients for whom infectious complications are a heavy burden.

Extra-corporeal life support has recently gained interest in intensive care units (ICUs) [1–3], either as a circulatory support for the treatment of cardiogenic shock or as an oxygenation device in acute respiratory distress syndrome (ARDS) patients. The development of this technique has been accompanied by the description of its adverse events and complications [4]. Among those, a recent meta-analysis of published extra-corporeal membrane oxygenation (ECMO) adult studies identified associated infections as one of the main complications, occurring in 30.4% (19.5–44.0%) [4]. These infections have been found to increase the length of ECMO and ventilation, the length of ICU and hospital stay, and mortality when the infection was severe [5]. In this 6-year retrospective study of 220 patients who underwent veno-arterial ECMO for cardiogenic shock, infections were mainly cannula-associated infections [5], with *Escherichia coli* as the predominant responsible pathogen. ECMO-associated infections were defined if occurring >24 h after ECMO initiation and within 48 h after its discontinuation [5].

Seven phylogroups of *E. coli* have been described [6–8]. A, B1, E and F phylogroups are found mainly as commensals and are devoid of virulence factors and extra-intestinal virulence, whereas B2, C and D phylogroups usually possess a high content of virulence factors and extra-intestinal virulence potential [9, 10].

Owing to the frequent femoral site of catheterization, one may wonder if strains responsible for cannula-related infections have particular traits or if their presence simply reflects the anatomical proximity with the lower digestive tract. The identification of a particular trait might benefit from a selective decontamination, as in other nosocomial infections [11]. We therefore decided to investigate genetic characteristics and the susceptibility spectrum of *E. coli* strains from ECMO cannula-associated infections.
We conducted a retrospective monocentric study (November 2012–January 2015) in Pitié-Salpêtrière Medical ICU. All E. coli isolates from cannula-related infection or colonization were retrieved from the microbiology department files. The subjects’ files with an E. coli sample were reviewed and demographic, anamnestic data were collected. The reason for ECMO support, its type (veno-arterial or veno-venous), length of ECMO assistance, and status at ICU discharge were recorded. All E. coli-related episodes were qualified independently as infection or colonization by two investigators (JM/MS), irrespectively of its delay after ECMO discontinuation. Infection was defined by the presence of cutaneous inflammation or pus at cannulation site, with a positive microbiology sampling. The duration of ECMO prior to the diagnosis, its delay after ECMO explantation, local signs of infection, and therapies required for the treatment of the episode were collected. Only the first isolate from each infection episode was studied.

Susceptibility pattern of E. coli isolates was determined by the disk-diffusion method according to the French Society of Microbiology. Susceptibility score was defined as the sum of active antimicrobial agents, according to the in vitro susceptibility to 17 of those (see ESM for details) for each isolate [12]. A score of 1 was attributed for a sensitive, 0.5 for an intermediary, and 0 for a resistant isolate, a higher score thus indicating a higher antimicrobial susceptibility.

The extended-spectrum β-lactamase (ESBL) genes were characterised among the isolates exhibiting an ESBL phenotype. A DNA amplification of β-lactamase genes was carried out, and the amplified DNA fragments were sequenced by Sanger technology [13] (see Table S1 available in the online Supplementary Material for details).

A successive refinement strain typing strategy was applied from the seven main phylogenetic groups (A, B1, B2, C, D, E, F) [8] to strain identity by a combination of PCR-based methods (clonal complex belonging and O-typing) [14–18] and PFGE [12, 19, 20]. Thus, the B1 clonal complex 87 (CC87) (Institut Pasteur MLST schema nomenclature) corresponding to the ST58 and ST155 in the Achtman schema [14], the 10 main B2 subgroups and the clonal group A (CGA) from the D phylogroup were determined among the strains. The exhaustive correspondence between this typing approach and STc membership according to the currently used Achtman MLST scheme [21] is available in [22]. Then, strains exhibiting identical phylogenetic group/subgroup and O-type were analysed by PFGE to determine their relatedness.

The presence of virulence factor genes was determined with a PCR-based method [23]. We chose a representative of main classes of E. coli extra-intestinal virulence factors: adhesins (pap), iron capture systems (fyuA), toxins (hly) and protectins (neuC) [24].

Results are expressed as median [IQR] or n (%) as appropriate. Chi-square or Fisher’s exact test were used to compare categorical variables, and t-test was used for continuous variables. GraphPad Prism 6 (GraphPad Software, San Diego, USA) was used, and P<0.05 was considered significant.

During the study period, 33 E. coli strains (Table 1), responsible for 33 infections, were collected in 30 subjects.

**Patients**

The 30 subjects (18 men), aged 50.7 (40.3–61.7) with a median Simplified Acute Physiologic Score II of 45.5 (36–81.5), had veno-arterial ECMO (n=28, 93 %) for cardiogenic shock (n=21, 75 %), cardiac arrest (n=5, 18 %) and septic shock (n=2; 7 %). The remaining two had veno-venous ECMO for ARDS.

Seven patients had more than one isolate collected (Fig. 1): two patients had two separate infectious episodes, one after a 9-month interval, the other one after a 24-day interval, and an adequate antimicrobial treatment; in one patient, two isolates were retrieved at a 5-day interval despite antibiotics; one patient had a colonization and an infection isolate; finally, three patients had more than one isolate collected during the same episode.

ICU survival was 70 %, and three deaths were partly related to E. coli infection.

**Episodes**

Median time to the collection of the strain was 13 (7.5–19) days after ECMO implantation, and 8 days (5–10) after explantation in the 11 patients in whom the episode occurred after explantation. A total of 21 episodes (61.7 %) were polymicrobial, associated to other Enterobacteriaceae (n=12), Enterococcus spp. (n=10), Pseudomonas aeruginosa (n=8) or Staphylococcus spp. (n=5) (Table 1). The infection site was mainly scarp cellulitis (n=26), of which two had concomitant bacteraemia. Cannula infection without celluli-tis occurred in five patients, including one with bacteraemia; the other infections were one bacteraemia alone, and one ruptured mycotic aneurysm.

All of them received systemic antimicrobial treatment, and surgery was mandatory in 21 % of cases (7/33), including one vascular repair. Among the 22 infection episodes occurring while ECMO was ongoing, four (17 %) required its explantation. Among the 11 episodes occurring after ECMO explantation, local signs of infection were present in all but one patient (such as scarp cellulitis in seven, foul discharge in six, scar dehiscence in five, and one ruptured mycotic aneurysm), general signs in five (fever >38.0 °C in five, septic shock in five). Although an alternative diagnosis was present for four of these 11 patients, E. coli was deemed responsible for the ECMO-associated infection, either because of local signs (n=3), or because a positive sampling in an immunocompromised patient. More details are given in Table S2.
Table 1. Main characteristics of the 33 E. coli causing ECMO-associated infections

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<th>Subgroup</th>
<th>O-type</th>
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<th>papC</th>
<th>hlyC</th>
<th>fyuA</th>
<th>PFGE pattern</th>
<th>Antibiotype</th>
<th>Susceptibility score</th>
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<th>Bacteraemia</th>
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STRAINS

Antimicrobial susceptibility

Median susceptibility score of all 33 strains was 13.5 (10.5–17). Among those, 11 (32.3%) were susceptible to all tested antibiotics. Of the 33 *E. coli* strains collected, 13 (39.4%) had a wild-type phenotype towards beta-lactams, 15 (36.5%) produced a penicillinase, four (11.7%) an ESBL, and one a cephalosporinase (Table 1). The ESBL genotype variants were CTX-M-1 (*n* = 2) or CTX-M-14 (*n* = 2), and all of those were associated with TEM-1.

Phylogenetic groups/subgroups, strain relatedness and virulence factors

Phylogenetic groups associated to extra-intestinal pathogenic strains [9, 10] were predominant, accounting for 23 strains (69.7%; phylogroup B2 *n* = 12; D *n* = 8; C *n* = 3). Breakdown figures for B2 subgroups were the following: II (*n* = 5; 41.7%), I (*n* = 4, 33.3%) and IV (*n* = 1; 8.3%), two remaining strains were unassigned. All subgroup I strains belonged to the O16 ST131 clade. Subgroup II strains exhibited an O2a type (*n* = 2) and an O6 type (*n* = 3). All but two B2 II-O6 strains had highly different PFGE profiles (Table 1). Seven D strains (78%) belonged to CGA (O15 *n* = 3; O17 *n* = 2; O2b *n* = 1; and non-typed *n* = 1). Ten strains (30.3%) belonged to phylogroups associated to commensalism [9, 10] (phylogroup A *n* = 4; B1 *n* = 4; E *n* = 1; F=1). One of the B1 strains belonged to the CC87 [14] (Table 1). No difference in mono or polymicrobial pattern was found according to the extra-intestinal pathogenic or commensal-associated genotype (nine monomicrobial out of 25 extra-intestinal pathogenic strains (36%) vs three out of 10 commensal (30%; *P* = 0.74)).

The virulence potential of the B2, D and C strains [24] was attested by the presence of one virulence factor in 13 strains (39.4% – namely *fyuA* for 12 and *papC* for one), and of two in six (18.2% – all with *fyuA*, associated to *hlyC* in three; to *papC* in two; and to *neuC* in one). Three D and one C strain did not carry any of the virulence factor gene screened. On the other hand, a single isolate (10%) belonging to commensalism-associated phylogroups harboured *fyuA* as the only virulence factor gene (Table 1), reinforcing this extra-intestinal/commensal dichotomy.

Susceptibility score did not differ significantly between extra-intestinal pathogenic and commensalism-associated isolates (13 (10.5–17) for B2, D or F vs 17 (10.4–17) for A, B1, C, or E, *P* = 0.08). Interestingly, all O16 ST131 strains produced an ESBL, one was trimethoprim-sulfamethoxazole resistant, two resistant to gentamicin and all evidenced a reduced susceptibility to fluoroquinolones. ESBL enzyme harboured by ST131 isolates were *bla* _CTX-M-1_ (*n* = 2) and *bla* _CTX-M-14_ (*n* = 1) (Table 1).

DISCUSSION

*E. coli* has been described as the most worrisome pathogen [25], and most frequently implicated in ECMO-related infections. In this study, we characterized their clinical
pattern, susceptibility profile and the genetic characteristics of E. coli isolates of ECMO-related infections.

Interestingly, 69.7% of all causative isolates harboured phylogenetic characteristics of extra-intestinal pathogenic strains. These strains are known for their virulence potential as has been described in other infections such as in bacteremia [26, 27] and ventilator-associated pneumonia [12]. These particular traits are linked to the presence of virulence factor genes, as was the case in many of the strains isolated here.

The proximity of the infection site (Scarpa’s triangle is the cannulation site for all patients) with the faecal flora very probably explains why a majority of infections were polymicrobial. It certainly also explains why less virulent strains (A, B1 and F), because of the bacterial load and the patients’ frailty, were also usually responsible for infections as also observed in ventilator-associated pneumonia.

We performed PFGE to confirm the absence of relatedness of isolates, all collected in a single ICU. We show that only two B2 II-O6 E. coli strains had some genetic similarities with only two different bands [19, 28]. Nevertheless, they had been collected at very different periods in two different subjects, pleading for the absence of nosocomial cross-transmission.

In our series, most subtype I B2 strains (n=3, 75% of B2 subtype I strains) belong to the specific O16 ST131 subgroup. The O16 ST131 subset of E. coli B2 strains has been identified [18, 29] to constitute a distinct phylogenetic clade than typical O25b ST131. ST131 is known to have emerged as the most prevalent human extra-intestinal pathogen E. coli [30]. O16 ST131 specific antimicrobial susceptibility has recently been described [18], and whereas O25b ST131 are deemed to exhibit a high antimicrobial resistance pattern, O16 ST131 has been associated with a susceptibility to fluoroquinolones and third generation cephalosporins [18], and resistance to gentamicin and trimethoprim-sulfamethoxazole. Unlike the described antimicrobial susceptibility pattern [18], our O16 ST131 strains produced ESBL and have a reduced susceptibility to fluoroquinolones. Gentamicin resistance was seen in two out of three strains, and one strain was trimethoprim-sulfamethoxazole susceptible. The ESBL variants of the O16 ST131 strains were CTX-M-1 (n=2) and CTX-M-14 for one, when all of those carried the blTEM-1 gene. Our findings of an important proportion of ST131 strains are consistent with the highly virulent feature of this subgroup [18, 31]. To note, subgroup II strains were the most frequently collected B2 subgroup (n=5; 41.7%). This finding is consistent with the predominance of subgroup II in commensal strains collected in healthy Paris-area subjects [32] and in bacteraemic strains from the same area [10].

Two of the isolates responsible for a bacteraemia were D isolates, belonging to CGA (O-non typed and O15) and the two others were B2 strains (one subgroup I-O25b, and one subgroup II-O2a isolate). These clones are able to cause severe sepsis in mice [9] and are retrieved in bacteraemia in humans [10]. The clinical pattern was of major severity for
three out of four, as the bacteraemia were associated with a septic shock, and multi-organ failure. One of the patients died 5 days after the occurrence of the bacteraemia, in spite of an effective antimicrobial therapy, after the withdrawal of life-sustaining therapies.

It is worth pointing out that 11 ECMO-related infections occurred after 8 days (5–10) of ECMO explantation. ECMO-related infections have been defined as those occurring within 48 h after ECMO discontinuation [5]. Nevertheless, in our series, the analysis of the patients’ charts confirmed the direct link between the ECMO cannula and the infection as described in the ESM, Table S2. We therefore did not exclude isolates even if the infection occurred more than 48 h after ECMO explantation. We suggest, in the same way as surgical site infections [33], that a period of 30 days is used for the diagnosis of an ECMO-related infection.

Our findings support, without confirming it, the hypothesis that the patients host their own E. coli pathogen strain in their faecal microbiota, and secondarily develop the infection. This phenomenon has been previously described in ventilator-associated pneumonia [12]. In a similar manner as shown in these ICU-acquired infections [11] and other E. coli infections [34, 35], a selective preventive decontamination using bacteriophages could be considered, targeting the predominant faecal isolate.

As for other extra-intestinal infectious sites as urine, blood, cerebrospinal fluid and lung, our results show the predominance of extra-intestinal E. coli pathogenic strains in ECMO-associated infections. Their advantageous genetic background may explain their predominance in this context. The potential for targeted digestive decontamination should be investigated in these patients for whom infectious complications are a heavy burden.

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Conflicts of interest
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Ethical statement
The ethics committee of the French Intensive Care Society approved the design of the study (CE SRLF 15–15).

References


