Eur J Clin Microbiol Infect Dis (2016) 35:993–999
DOI 10.1007/s10096-016-2628-6

ORIGINAL ARTICLE

Innate immune evasion of *Escherichia coli* clinical strains from orthopedic implant infections

L. Crémet[1,2] · A. Broquet[1] · C. Jacqueline[1] · C. Chaillou[1] · K. Asehnoune[1,3] · S. Corvec[1,2] · N. Caroff[1]

Received: 17 December 2015 / Accepted: 16 March 2016 / Published online: 2 April 2016

Abstract *Escherichia coli* is one of the first causes of Gram-negative orthopedic implant infections (OII). Those infections, usually hematogenous, mostly originate from the urinary tract. We investigated the strategies developed by *E. coli* in this context to evade host innate immune responses, i.e. complement and polymorphonuclear neutrophils (PMN). Twenty strains from OII were compared with 20 strains from bacteremia in patients with non-infected orthopedic implant. In both groups, 6/20 (30 %) strains lysed PMNs, due to the production of the pore-forming toxin α-hemolysin (HlyA). For the others, resistance to phagocytic killing by PMN was not significantly different between both groups. In contrast, resistance to complement-mediated serum killing was significantly higher in OII strains than in the others (65 % *vs* 10 %; $P < 0.001$). In *E. coli*, different mechanisms have been involved in complement resistance. Here, serum resistance was not linked to a group 2 capsule, or a loss of outer membrane permeability, or the recruitment of the complement inhibitor C4bp, but was significantly associated with the synthesis of long-chain LPS, regardless of the O-antigen. Thus, serum resistance could promote seeding of peri-implant tissues by helping *E. coli* to either persist in blood and reach the site of infection or overcome localized complement activation.

Electronic supplementary material The online version of this article (doi:10.1007/s10096-016-2628-6) contains supplementary material, which is available to authorized users.

✉ N. Caroff
nathalie.caroff@univ-nantes.fr

[1] UFR de Médecine, EA3826, Thérapeutiques cliniques et expérimentales des infections, Université de Nantes, 1 rue G. Veil, 44000 Nantes, France

[2] Pôle de Biologie, Service de Bactériologie-Hygiène, CHU de Nantes, France

[3] Pôle Anesthésie Réanimation, Service d'Anesthésie Réanimation Chirurgicale, CHU de Nantes, France

Introduction

Orthopedic implant infections (OII) are one of the most dreaded complications in orthopedic surgery. Their incidence remains currently low (1–2 %), but has been estimated to increase with the boom of prosthetic joint implantations, as the population ages [1, 2]. Acute hematogenous infections account for approximately 35 % of all OII [1, 3]. However, this rate is often underestimated, especially in clinical observational studies not long enough to respond to the full definition of hematogenous infections (i.e. infections manifesting for more than 2 years following implantation after a previous uneventful course, or infections that occur following an episode of documented bacteremia or an infection at a distant focus) [1, 3]. *Staphylococcus aureus* bacteremia is the first cause of distant implant seeding, but according to the study of Bouvet et al., the proportion of Gram-negative bacilli after 2 years post-implantation is higher than the proportion of Gram-positive bacteria [1, 3].

Gram-negative bacilli are implicated in 10–23 % of all OII and *Escherichia coli* is the first cause of Gram-negative OII [3, 4]. This bacterium, known as a harmless commensal of the gut flora, also causes various infections, the most common ones being urinary tract infections (UTI). Uropathogenic *E. coli* (UPEC), characterized by their ability to colonize and damage the urinary tract, or evade innate immune responses, through specific sets of virulence determinants (e. g. adhesins, flagella, capsule, iron uptake systems, or toxins), cause more than 80 % of all UTI, and are also a major cause of bacteremia [5–7]. These bacteremic UTI can be complicated by distant

infections, such as secondary abscess, osteomyelitis, or septic arthritis [7]. As a matter of fact, urinary infection is a well-recognized risk factor for hematogenous prosthetic joint infections, whereas recent studies found no evidence of such relation with asymptomatic bacteriuria [8]. Accordingly, in a previous study, we showed that a large number of *E. coli* recovered from peri-implant tissues shared many similarities with UPEC, and the urinary tract was the most frequent source of infection in our patients [9]. However, this earlier study failed to find very specific combinations of virulence factors associated with OII [9].

In this study, we aimed to determine if an enhanced ability to circumvent host innate immune defenses could lend *E. coli* effective at seeding peri-implant tissues. Thus, we compared the resistance to serum complement-mediated killing and to opsonophagocytosis by human polymorphonuclear neutrophils (PMN) of 20 well-characterized OII *E. coli* strains [2] and 20 *E. coli* control strains from bacteremia not complicated by subsequent implant seeding (blood panel), and investigated mechanisms potentially involved in *E. coli* innate immune evasion.

Materials and methods

Bacterial strains

A well-characterized panel [2] of 20 clinical *E. coli* strains (Ec1 to Ec20) involved in hip (14 strains) or knee (6 strains) OII was compared with 20 clinical strains of *E. coli* (EcA to EcT) from bacteremia in patients with non-infected knee or hip prostheses (Table 1). Blood strains (EcA to EcT) were randomly selected to match on their phylogenetic group with the OII strains, thereby giving comparable panels in terms of virulence potential (13 group B2, 3 group D, 3 group A, and 1 group B1 per panel). The genetic relatedness of the 40 *E. coli* was studied by MLST analysis according to the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli). The strains were also investigated by PCR for the most common O-serotypes of UPEC, group 2 capsule with in particular K1 and K2 variants, and three virulence factors (*traT*, *hlyA*, *cnf1*) potentially contributing to serum resistance or cytotoxicity.

Isolation of human PMN

PMN were isolated from fresh heparinized venous blood of healthy consenting volunteers by 6 % dextran (Sigma) sedimentation and ficoll density gradient (Lymphocyte Separation Medium 1077, PAA) centrifugation (2500 × *g* for 3 min). The pelleted cells were treated with cold 0.83 % ammonium chloride for lysis of residual erythrocytes, and washed two times with sterile phosphate-buffered saline (PBS) containing 1 mM ethylene diamine tetraacetic acid (EDTA). PMN extracts were checked for purity (>95 %), and counted in an automated hematological analyzer Sysmex XS-800i.

E. coli-induced PMN lysis experiments

Experiments were performed as previously described [10], with some modifications. Bacteria were opsonized for 30 min at 37 °C with 10 % heat-inactivated pooled normal human serum and combined with 10^5 PMN at a multiplicity of infection (MOI) of 1:1 in 24-well culture plates containing RPMI 1640 (Lonza). The plates were centrifuged at 300 × *g* for 5 min to synchronize infection and allowed to incubate at 37 °C in 5 % CO2 for 2 hours. PMN lysis was visualized by light microscopy (Leica Microsystems; magnification × 1000), after cytocentrifugation and Gram staining. Two independent experiments were performed in duplicate for each strain.

PMN bactericidal assays

As for PMN lysis experiments, bacteria were opsonized and combined either with PMN (MOI of 1:1) or without cells (reference sample). After 1 hour of incubation, a PMN lysis solution was added in all wells (Triton X-100 at a final concentration of 0.1 %), and lysates were serially diluted and plated on Tryptic Soy Agar (TSA) plates. The bactericidal activity of PMN was expressed as the percentage of viable bacteria after exposition to PMN compared to the reference sample. Two independent experiments were performed in duplicate for each strain.

Serum bactericidal assays

A pooled normal human serum (NHS) collected from ten healthy consenting volunteers was kept frozen at –80 °C in aliquots, and used for each assay. Bacteria from logarithmic-phase cultures adjusted to 10^6 CFU/mL were mixed at a 1:3 vol/vol ratio with NHS. Viable bacteria were counted by plating dilutions on TSA plates after 0, 1, 2, and 3 h of incubation at 37 °C. Responses were graded from 1 to 6, as previously described [11]. Strains were categorized as sensitive [all counts < 100 % of the inoculum, i.e. grades 1 (1-hour count < 10 %) and 2 (1-hour count between 10 % and 100 %)], intermediate [1-hour count > 100 % and 3-hours count < 100 %, i.e. grades 3 (2-hours count < 100 %) and 4 (2-hours count > 100 %)] or resistant [3-hours count > 100 %, i.e. grades 5 (count falling at 3-hours) and 6 (progressive rise at each hourly interval)] to NHS. Each strain was tested three times.

NaCl and SDS sensitivity assays

The minimum inhibitory concentrations (MICs) of SDS and NaCl were determined by broth microdilution method as previously described [7].

Table 1 Characteristics of the *E. coli* strains investigated in this study

Isolate	Type of infection[a]	Positive blood cultures	Presumed source of infection[b]	Phylo-genetic group	MLST (ST)	Serotype[c]	Virulence genes				Serum[e]	LPS[f]	SDS MIC (%)	NaCl MIC (M)	PMN killing, % viable bacteria (mean ± SD)
							K antigen[d]	*traT*	*hlyA*	*cnf1*					
OII *E. coli*															
Ec1	Late	Yes	Urinary tract	B2	95	O2	K1	+	−	−	6	LC	0.125	1	79.94 ± 5.93
Ec2	Late	−	Urinary tract	B2	80	O75	K1	−	+	+	6	LC	0.125	1	62.70 ± 7.90
Ec3	Late	Yes	Urinary tract	B2	537	O75	Group 2	−	+	+	6	LC	0.125	1	PMN Lysis
Ec4	Delayed	−	Urinary tract	B2	126	−	Group 2	−	+	+	6	LC	0.125	1	PMN Lysis
Ec5	Late	Yes	Urinary tract	B2	141	O2	K1	−	+	+	6	LC	0.125	1	PMN Lysis
Ec6	Late	−	Urinary tract	B2	404	O75	Group 2	−	−	−	6	LC	0.063	0.6	48.70 ± 8.14
Ec7	Early	−	Not searched	B2	73	−	Group 2	−	+	+	6	LC	0.125	1	PMN Lysis
Ec8	Early	−	Urinary tract	B2	73	O6	Group 2	−	+	−	6	LC	0.125	1	PMN Lysis
Ec9	Delayed	−	Not searched	B2	550	O75	Group 2	−	−	−	6	LC	0.125	1	92.92 ± 1.73
Ec10	Early	−	Unknown	B2	95	O1	K1	+	−	−	5	LC	0.125	1	79.84 ± 9.01
Ec11	Delayed	−	Not searched	B2	141	O2	K1	−	+	+	4	LC	0.125	1	PMN Lysis
Ec12	Early	−	Not searched	B2	1618	O4	Group 2	−	−	−	2	LC	0.125	1	46.80 ± 9.40
Ec13	Delayed	−	Urinary tract	B2	95	O1	K1	+	−	−	1	LA	0.250	1	82.74 ± 2.10
Ec14	Early	Yes	Urinary tract	D	354	O1	−	+	−	−	6	LC	0.125	1	52.71 ± 9.66
Ec15	Late	−	Urinary tract	D	405	O2	Group 2	+	+	−	5	LC	0.063	1	81.05 ± 8.41
Ec16	Delayed	−	Not searched	D	420	−	K1	−	−	−	4	LC	0.125	1	82.47 ± 9.90
Ec17	Early	Yes	Unknown	A	361	−	K2	+	−	−	5	LC	0.063	0.8	68.12 ± 7.58
Ec18	Late	−	Not searched	A	88	O8	−	−	−	−	2	LC	0.063	1.2	74.64 ± 6.19
Ec19	Delayed	−	Unknown	A	10	−	−	+	−	−	1	LA	0.063	1.2	79.43 ± 6.43
Ec20	Late	−	Not searched	B1	1167	O21	−	−	−	−	1	LA	0.125	1	75.98 ± 5.33
Blood *E. coli*															
EcA	n/a	Yes	Urinary tract	B2	95	O1	K1	+	−	−	5	LC	0.063	1	78.50 ± 3.21
EcB	n/a	Yes	Urinary tract	B2	95	O1	K1	+	−	−	4	LC	0.125	1	43.17 ± 2.25
EcC	n/a	Yes	Urinary tract	B2	12	O4	Group 2	−	+	+	4	LC	0.063	1.2	PMN Lysis
EcD	n/a	Yes	Unknown	B2	73	−	Group 2	−	+	+	3	LC	0.125	1	PMN Lysis
EcE	n/a	Yes	Urinary tract	B2	12	O4	−	−	+	+	3	LC	0.125	1	PMN Lysis
EcF	n/a	Yes	Abscess	B2	95	O1	K1	+	−	−	3	LC	0.125	1	56.74 ± 10.05
EcG	n/a	Yes	Urinary tract	B2	144	O16	K1	+	−	−	2	LC	0.063	1	98.94 ± 3.07
EcH	n/a	Yes	Biliary tract	B2	131	O25	Group 2	+	−	−	2	LC	0.125	1	91.25 ± 2.42
EcI	n/a	Yes	Urinary tract	B2	144	O16	K1	+	+	−	1	LC	0.063	1	PMN Lysis
EcJ	n/a	Yes	Urinary tract	B2	95	O1	K1	+	−	−	1	LA	0.125	1	90.18 ± 3.42
EcK	n/a	Yes	Urinary tract	B2	1193	O75	Group 2	−	−	−	1	LA	0.031	0.4	38.00 ± 7.05
EcL	n/a	Yes	Urinary tract	B2	83	O83	Group 2	−	+	+	1	LA	0.125	1	PMN Lysis
EcM	n/a	Yes	Urinary tract	B2	95	O1	K1	−	−	−	1	LC	0.125	1	35.88 ± 8.55
EcN	n/a	Yes	Urinary tract	D	69	−	Group 2	+	−	−	6	LC	0.125	1	66.58 ± 8.87
EcO	n/a	Yes	Respiratory tract	D	624	O25	−	+	−	−	2	LA	0.063	0.8	64.23 ± 4.88
EcP	n/a	Yes	Urinary tract	D	59	O1	K1	+	−	−	2	LC	0.125	1	95.89 ± 7.87
EcQ	n/a	Yes	Urinary tract	A	540	−	−	+	+	−	2	LC	0.250	1.2	PMN Lysis
EcR	n/a	Yes	Digestive tract	A	23	−	−	+	−	−	2	LC	0.125	0.6	79.84 ± 7.76
EcS	n/a	Yes	Urinary tract	A	10	−	−	+	−	−	1	LA	0.125	1.2	32.59 ± 3.34
EcT	n/a	Yes	Urinary tract	B1	58	−	−	+	−	−	1	LC	0.125	1.2	61.99 ± 4.47

[a] Time interval between index surgery and diagnosis of infection: Early (<3 months after surgery); Delayed (3-24 months after surgery); Late (>2 years after surgery)

[b] The source of infection was determined on the basis of the isolation of the same *E. coli* (same susceptibility to antibiotics) from the presumed portal of entry and/or clinical evaluation

[c] 14 *E. coli* serotypes associated with UPEC (O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75, and O83) were searched by PCR

[d] Group 2 capsular antigens were searched by PCR, and among the various group 2 capsular types, the K1 and K2 variants were searched by PCR

[e] Grades of response in serum bactericidal assays

[f] LPS patterns were coded as follows: *LC* long chain O antigen (35-60 kb), *LA* only the band for lipid A-core

LPS analysis

LPS was isolated by a hot water-phenol extraction method as described previously [12]. Samples were separated by SDS-PAGE and then silver-stained.

Statistical analysis

Differences in responses to serum or PMN between OII and blood strains, and associations between cytolytic effect, or serum resistance, and the examined parameters, were calculated using the Fisher's exact test. *P* values less than 0.05 were considered statistically significant.

Results

Characterization of the *E. coli* strains from both panels

The 40 strains belonged to 27 different sequence types. The retrospective review of the 40 patient's records and the molecular analysis of the different *E. coli* strains revealed that UTI was a common finding among both panels. A high frequency of O-serotypes preferentially associated with UPEC, was documented in the strains of both panels [15/20 (75 %) in the OII panel and 14/20 (70 %) in the blood panel], and group 2 capsular polysaccharides, also mostly associated with UPEC, were highly prevalent in each panel [16/20 (80 %) in the OII panel and 14/20 (70 %) in the blood panel] (Table 1). UTI was the most common presumed source of infection in both panels [15/20 (75 %) in the blood panel and 10/20 (50 %) in the OII panel, taking into consideration that the source of OII was not investigated in 7 cases] (Table 1). Finally, 10/20 (50 %) OII were considered as hematogenously acquired, according to the definition of Zimmerli et al. [1].

OII *E. coli* and *E. coli* from bacteremia have the same ability to circumvent killing by PMN

PMN are endowed with an array of toxic weapons highly effective at killing most bacteria. However, some pathogens, including many UPEC, employ various strategies to inhibit PMN migration and function [5, 10, 13]. Accordingly, we found in each panel of the study that 30 % (6/20) of the strains induced PMN lysis *in vitro*. The cytolytic effect was significantly associated with the detection of two virulence genes by PCR, i.e. alpha-hemolysin (*hlyA*) and cytotoxic necrotizing factor 1 (*cnf1*) ($P < 0.001$; Fisher's exact test) (Table 1). Both virulence factors, often associated in approximately one third of UPEC, are commonly linked with hemorrhagic UTI, and known to promote bacterial invasion of the bladder and kidneys tissues and to modulate host inflammatory responses [5, 13]. CNF1 mediates uroepithelial cell apoptosis, and reduces PMN phagocytosis through targeting Rho-family GTPases [5, 13]. At high concentrations, the pore-forming toxin HlyA can trigger rapid epithelial cells lysis, while sublytic concentrations affect host cell signaling pathways [5, 13]. We recently demonstrated that 6 HlyA-producing strains of the OII panel (Ec3, Ec4, Ec5, Ec7, Ec8 and Ec11) were highly cytotoxic toward human osteoblastic cells *in vitro*, while when the *hlyA* gene was very slightly expressed (strains Ec2 and Ec15) or absent, no osteoblast lysis was observed [2]. Results presented here (Table 1, Fig. 1a-f) show that HlyA-producing *E. coli* also induce PMN lysis *in vitro*. These data are consistent with those of Russo et al. who found by studying one extraintestinal pathogenic strain of *E. coli* and its isogenic derivatives, that HlyA, but not CNF1, mediated PMN lysis/necrosis [14].

Finally, we sought to determine whether the non-cytolytic strains from OII were more resistant to killing by PMN than those from bacteremia. After 1 hour of co-culture with PMN, less than one third of the strains of both panels were efficiently killed (viable bacteria < 50 %). No significant difference in survival was found between both panels (Table 1, Fig. 2a). These findings were not surprising, since most of the infections originated from the urinary tract, and since it is now well-established that virulence of UPEC lies in part, in their ability to subvert the antimicrobial properties of PMN in the urinary tract [5, 10].

OII *E. coli* are more serum resistant than *E. coli* from bacteremia

An ability to escape serum complement-mediated killing is another important determinant of *E. coli* pathogenicity during both localized and systemic infections, and has been proposed to contribute to the pathogenic success of the dominant *E. coli* ST131 clonal lineage [7]. Our data suggest that this property could promote seeding of peri-implant tissues. In fact, we show that 65 % of our OII strains were resistant to serum killing (three grade 5 and ten grade 6), whereas only 10 % of the *E. coli* strains from blood were categorized as serum-resistant (one grade 5 and one grade 6) (Table 1, Fig. 2b). The sensitive strains were fully resistant to heat-inactivated serum, demonstrating that killing was indeed complement-mediated.

Such a difference between both panels was somewhat unexpected, firstly because strains from blood were chosen to resemble the OII strains in term of virulence, and secondly because several reports stated that *E. coli* should avoid complement attack to survive in the blood [7, 15–18]. However, few recent studies of large clinical cohorts corroborate this last point [18]. Hughes et al. and Devine et al. reported about 20 % of serum-resistant strains among hundreds of UPEC, under the same experimental conditions as ours [11, 19]. In fact, several technical factors could influence results of serum bactericidal assays, and a lower amount of serum (20–60 %) and/

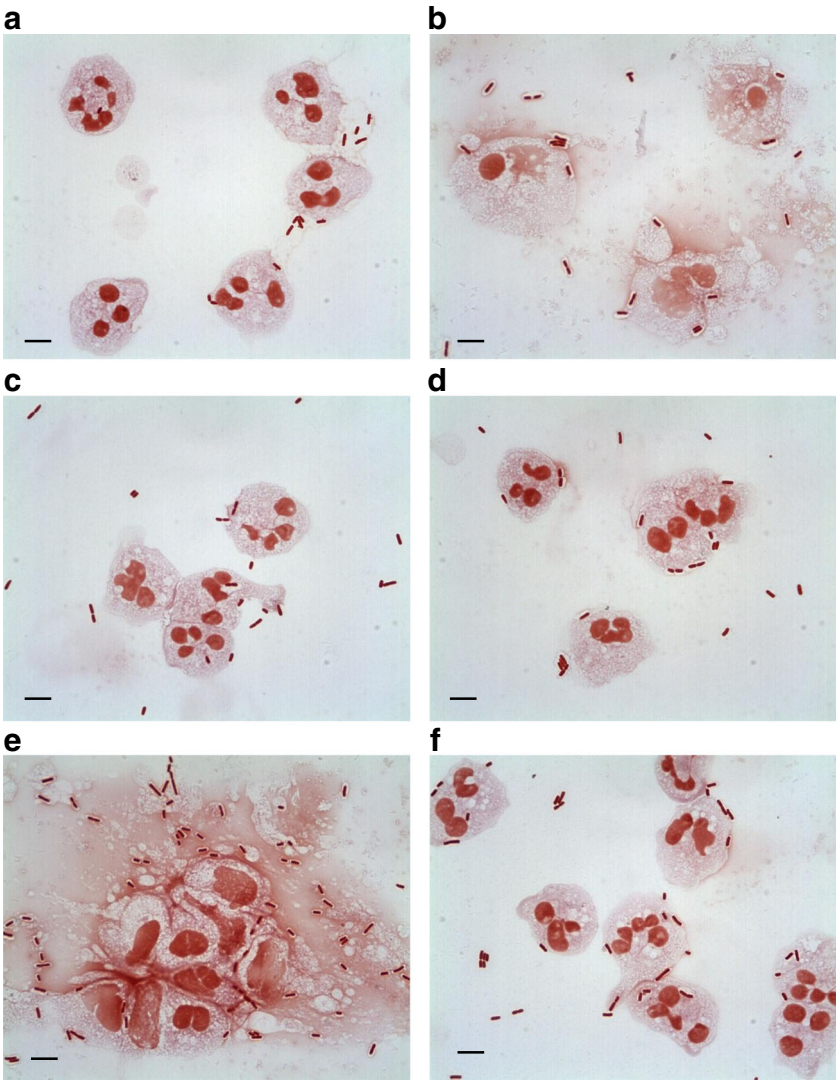

Fig. 1 HlyA-producing *E. coli* strains induce PMN lysis/necrosis *in vitro*. PMN were exposed to *E. coli* strains (MOI of 1:1) for 2 hours, including (**a**) *E. coli* Ec1, (**b**) *E. coli* Ec3, (**c**) *E. coli* Ec2, (**d**) *E. coli* Ec15, (**e**) *E. coli* ZKLR^{+} (HlyA^{+}), and (**f**) *E. coli* Zhly- (Δ*hlyA* mutant of *E. coli* ZKLR^{+}). The examination under light microscopy of PMN–*E. coli* interaction on Gram-stained cytospins, showed phagocytosing PMN and engulfed bacteria (**a**, **c**, **d**, and **f**) when the *hlyA* gene was absent (**a** and **f**) or very slightly expressed by the *E. coli* strains (**c** and **d**), and lysed PMN (**b** and **e**) for the HlyA-producing strains (**b** and **e**). Scale bars: 10 μm

or shorter incubation times might lead to overestimate serum resistance in some studies [6, 15–17, 20]. Thus, overall, our data underscore the peculiar tolerance of the OII *E. coli* against the first line of defense of the host innate immune system induced by non-self surfaces (e.g. bacteria or biomaterials).

Serum resistance of OII *E. coli* lies on varied complement evasion strategies

In *E. coli*, multiple strategies have been described to block complement activation or control complement cascade on self surfaces [6, 7, 11, 15–20]. Most of these mechanisms are closely tied to the bacterial envelope [6, 7, 18]. Thus, certain types of group 2 capsular antigens (K1 and K2) or an additional colanic acid capsule, several O-antigen serotypes (for example, O1, O6, and O18) or lipopolysaccharide (LPS) containing long O-polysaccharide chains, or specific proteins affecting outer membrane permeability (TraT, Prc, outer membrane proteins [OMPs]) or interacting with the complement inhibitor C4bp (C4b-binding protein) (OmpA, NlpI) might confer protection against complement killing [6, 7, 15–18]. It is however noteworthy that a number of important insights gained in recent years about complement evasion strategies are issued from studies of deletion mutants derived from prototypic *E. coli* (CFT073, RS218, EC958) [6, 7, 15–18]. The strength of the inhibitory effect of several factors against complement-mediated killing remains to be confirmed in a wider range of clinical strains of different lineages, since serum-resistance appears to be resolutely multifactorial.

In our study (see Table 1), as previously shown by Phan et al. [7], neither the K1 capsular antigen, nor even a group 2 capsular antigen was found to provide a survival advantage in serum (56 % of the strains with a group 2 K-antigen were

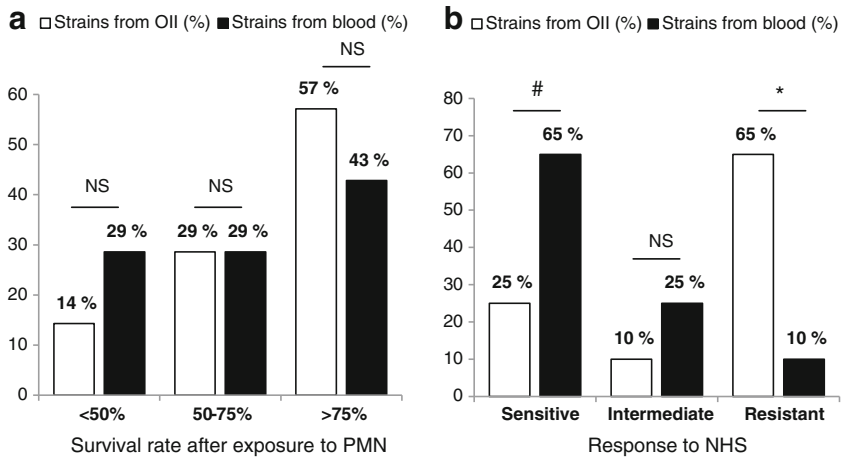

Fig. 2 Effects of PMN and NHS on the survival of the 20 OII *E. coli* (*white columns*) and 20 *E. coli* from blood (*black columns*). **a** The strains were ranked in three categories, according to the percentage of surviving bacteria (<50 %, 50–75 %, or >75 %) after 1 hour of co-culture with PMN (MOI of 1:1). **b** Serum bactericidal assays performed with 75 % NHS for 3 hours allowed the categorization of the strains as sensitive (grades 1 and 2), intermediate (grades 3 and 4), or resistant (grades 5 and 6) to serum. # $P < 0.05$ or * $P < 0.001$; Fisher's exact test

serum-sensitive). Similarly, while the O1-antigen was found to be protective by Sarkar et al. [6], the largest proportion of our O1 strains (44 %) was categorized as serum-sensitive. Reduced serum survival was also mostly not due to damaged outer membranes, since under environmental stress conditions, like high salt concentrations (NaCl) or osmotic stress (SDS), serum-sensitive strains did not show significantly lower MICs than serum-resistant strains. Additionally, the outer membrane lipoprotein TraT, known to inhibit the membrane attack complex [18], was more prevalent in serum-sensitive *E. coli* (67 % of *traT*-positive strains) than in the serum-resistant ones (47 %). Furthermore, while the periplasmic protease Prc had been demonstrated to change OMPs profiles when inactivated [16]; further analysis of six serum-sensitive (EcI, EcJ, EcK, EcL, EcM, Ec13) and five serum-resistant (Ec1, Ec2, Ec3, Ec4, Ec8) strains of our study, did not reveal a reduced expression of the *prc* gene in the six serum-sensitive strains (Fig. S1), and no inactivating mutation was found in the Prc active site of these strains (data not shown). This further analysis also failed to demonstrate the protective role of C4bp deposition on the bacteria. Thus, although there were differences in C4bp binding between the 11 strains, serum resistance did not positively correlate with C4bp deposition levels (Fig. S2).

Long-chain LPS (35-60 kD) was the only bacterial factor significantly associated with serum resistance, regardless of the O-antigen (100 % of serum-resistant strains with long-chain LPS *vs* 56 % of serum-sensitive strains with long-chain LPS; $P = 0.0036$, Fisher's exact test) (Table 1, Fig. S3). The critical role of this factor in serum resistance had already been underlined in several studies [7, 18]. Here, long-chain LPS did not alone explain the difference in serum resistance observed between both panels.

Concluding remarks

Jointly, our results highlight the formidable subversive capacities of OII *E. coli* against two major components of the innate immune response. Evasion to PMN-mediated killing contributes to UPEC pathogenesis in the urinary tract. This survival advantage could promote bacteremia and complications, like OII. Moreover, in our study, the pore-forming toxin HlyA appeared as an essential attribute of *E. coli* to lyse PMN. Our findings further suggested that resistance to serum complement-mediated killing could provide an important advantage for establishing OII. We encountered difficulties in finding bacterial determinants mediating complement resistance among the numerous previously described, even though the critical role of long-chain LPS was confirmed here. Thus, OII *E. coli* probably use a redundancy of factors to survive to complement attack and reach a high bacterial load for subsequent implant seeding. Nevertheless, we cannot exclude the involvement in serum resistance of a still undetermined component of the bacterial envelope, that could contribute to the physiopathological process of OII. In fact, specific "Microbial Surface Components Recognizing Adhesive Matrix Molecules" (MSCRAMMs) on *S. aureus* surface, namely, the collagen-binding protein Cna, and the bone sialoprotein binding protein Bbp, which mediate staphylococcal adherence to the cartilage and bone tissues, have recently been shown to interfere with different components of the complement cascade [21–24]. Future studies should address whether similar adhesins with dual functions are crucial in *E. coli* OII.

Acknowledgments We thank Dr J. Holubova and Pr P. Sebo (Laboratory of Molecular Biology of Bacterial Pathogens, Institute of Microbiology of the Academy of Sciences of the Czech Republic,

Prague, Czech Republic) for kindly providing the ZKLR$^+$ and Zhly- *E. coli* control strains used in this study, and Pr S. Bonacorsi (Laboratory of Microbiology, Robert-Debré hospital, AP-HP, Paris, France) for serotyping of a part of the *E. coli* isolates.

Compliance with ethical standards

Funding This work was supported by the French "Ministère de l'Enseignement Supérieur et de la Recherche".

Ethical approval and informed consent Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Zimmerli W (2014) Clinical presentation and treatment of orthopaedic implant-associated infection. J Inter Med 276:111–119
2. Crémet L, Broquet A, Brulin B, Jacqueline C, Dauvergne S, Brion R, Asehnoune K, Corvec S, Heymann D, Caroff N (2015) Pathogenic potential of *Escherichia coli* clinical strains from orthopedic implant infections towards human osteoblastic cells. Pathog Dis 73(8):ftv065
3. Bouvet C, Tchernin D, Seirafi M, Stern R, Lew D, Hoffmeyer P, Uçkay I (2011) No need to search for the source of haematogenous arthroplasty infections. Swiss Med Wkly 141:w13306
4. Rodríguez-Pardo D, Pigrau C, Lora-Tamayo J, Soriano A, del Toro MD, Cobo J, Palomino J, Euba G, Riera M, Sánchez-Somolinos M, Benito N, Fernández-Sampedro M, Sorli L, Guio L, Iribarren JA, Baraia-Etxaburu JM, Ramos A, Bahamonde A, Flores-Sánchez X, Corona PS, Ariza J, REIPI Group for the Study of Prosthetic Infection (2014) Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics, and implant retention approach. A large multicenter study. Clin Microbiol Infect 20:O911–O909
5. Ulett GC, Totsika M, Schaale K, Carey AJ, Sweet MJ, Schembri MA (2013) Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection. Curr Opin Microbiol 16:100–107
6. Sarkar S, Ulett GC, Totsika M, Phan MD, Schembri MA (2014) Role of capsule and O antigen in the virulence of uropathogenic *Escherichia coli*. PLoS One 9:e94786
7. Phan MD, Peters KM, Sarkar S, Lukowski SW, Allsopp LP, Gomes Moriel D, Achard ME, Totsika M, Marshall VM, Upton M, Beatson SA, Schembri MA (2013) The serum resistome of a globally disseminated multidrug resistant uropathogenic *Escherichia coli* clone. PLoS Genet 9:e1003834
8. Sousa R, Munoz-Mahamud E, Quayle J, Dias da Costa L, Casals C, Scott P, Leite P, Vilanova P, Garcia S, Ramos MH, Dias J, Soriano A, Guyot A (2014) Is asymptomatic bacteriuria a risk factor for prosthetic joint infection ? Clin Infect Dis 59:41–47
9. Crémet L, Corvec S, Bémer P, Bret L, Lebrun C, Lesimple B, Miegeville AF, Reynaud A, Lepelletier D, Caroff N (2012) Orthopædic-implant infections by *Escherichia coli*: molecular and phenotypic analysis of the causative strains. J Infect 64:169–175
10. Loughman JA, Hunstad DA (2011) Attenuation of human neutrophil migration and function by uropathogenic bacteria. Microbes Infect 13:555–565
11. Hughes C, Phillips R, Roberts AP (1982) Serum resistance among *Escherichia coli* strains causing urinary tract infection in relation to O type and the carriage of hemolysin, colicin, and antibiotic resistance determinants. Infect Immun 35:270–275
12. Grozdanov L, Zähringer U, Blum-Oehler G, Brade L, Henne A, Knirel YA, Schombel U, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, Rietschel ET, Dobrindt U (2002) A single nucleotide exchange in the *wzy* gene is responsible for the semirough O6 lipopolysaccharide phenotype and serum sensitivity of *Escherichia coli* strain Nissle 1917. J Bacteriol 184:5912–5925
13. Garcia TA, Ventura CL, Smith MA, Merrell DS, O'Brien AD (2013) Cytotoxic necrotizing factor 1 and hemolysin from uropathogenic *Escherichia coli* elicit different host responses in the murine bladder. Infect Immun 81:99–109
14. Russo TA, Davidson BA, Genagon SA, Warholic NM, Macdonald U, Pawlicki PD, Beanan JM, Olson R, Holm BA, Knight PR 3rd (2005) *E. coli* virulence factor hemolysin induces neutrophil apoptosis and necrosis/lysis *in vitro* and necrosis/lysis and lung injury in a rat pneumonia model. Am J Physiol Lung Cell Mol Physiol 289:L207–L216
15. Tseng YT, Wang SW, Kim KS, Wang YH, Yao Y, Chen CC, Chiang CW, Hsieh PC, Teng CH (2012) NlpI facilitates deposition of C4bp on *Escherichia coli* by blocking classical complement-mediated killing, which results in high-level bacteremia. Infect Immun 80: 3669–3678
16. Wang CY, Wang SW, Huang WC, Kim KS, Chang NS, Wang YH, Wu MH, Teng CH (2012) Prc contributes to *Escherichia coli* evasion of classical complement-mediated serum killing. Infect Immun 80:3399–3409
17. Miajlovic H, Cooke NM, Moran GP, Rogers TRF, Smith SG (2014) Response of extraintestinal pathogenic *Escherichia coli* to human serum reveals a protective role for Rcs-regulated exopolysaccharide colonic acid. Infect Immun 82:298–305
18. Miajlovic H, Smith SG (2014) Bacterial self-defense: how *Escherichia coli* evades serum killing. FEMS Microbiol Lett 354: 1–9
19. Devine DA, Roberts AP (1994) K1, K5 and O antigens of *Escherichia coli* in relation to serum killing *via* the classical and alternative complement pathways. J Med Microbiol 41:139–144
20. Debnath I, Northon JP, Barber AE, Ott EM, Dhakal BK, Kulesus RR, Mulvey MA (2013) The Cpx stress response system potentiates the fitness and virulence of uropathogenic *Escherichia coli*. Infect Immun 81:1450–1459
21. Tung HS, Guss B, Hellman U, Persson L, Rubin K, Ryden C (2000) A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of the staphylococcal Sdr family. Biochem J 345:611–619
22. Hair PS, Foley CK, Krishna NK, Nyalwidhe JO, Geoghegan JA, Foster TJ, Cunnion KM (2013) Complement regulator C4BP binds to *Staphylococcus aureus* surface proteins SdrE and Bbp inhibiting bacterial opsonization and killing. Res Immunol 3:114–121
23. Kalinka J, Hachmeister M, Geraci J, Sordelli D, Hansen U, Niemann S, Oetermann S, Peters G, Löffler B, Tuchscherr L (2014) *Staphylococcus aureus* isolates from chronic osteomyelitis are characterized by high host cell invasion and intracellular adaptation, but still induce inflammation. Int J Med Microbiol 304: 1038–1049
24. Kang M, Ko YP, Liang X, Ross CL, Liu Q, Murray BE, Höök M (2013) Collagen-binding microbial surface components recognizing adhesive matrix molecule (MSCRAMM) of Gram-positive bacteria inhibit complement activation via the classical pathway. J Biol Chem 288:20520–20531