VIRULENCE FACTORS AND PHYLOGROUPS ARE NOT ASSOCIATED WITH PATIENTS' FEATURES OR SOURCE OF INFECTIONS IN BACTERAEMIC ESBL-PRODUCING *ESCHERICHIA COLI:* A PROSPECTIVE MULTICENTER COHORT

Jesús Rodríguez-Baño (1), Jesús Mingorance (2), Natalia Fernández-Romero (2), Lara Serrano (1), Lorena López-Cerero (1), Alvaro Pascual (1), and the ESBL-REIPI Group

(1)Unidad de Enfermedades Infecciosas y Microbiología, Hospital Universitario Virgen Macarena, Seville, Spain

(2) Servicio de Microbiología, Hospital Universitario La Paz, Madrid, Spain.

ECCMID 2012
P1477

INTRODUCTION

-Extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates exhibit more virulence factors (VF) than commensal isolates. Some of these VF have been associated with the ability of ExPEC to cause such infections, particularly in patients without predisposing conditions. Most *E. coli* isolates causing extraintestinal infections belong to phylogroups (PG) B2 and D, which harbour more VF than A and B1 PG.

-Bloodstream infections (BSI) caused by extended-spectrum beta-lactamase-producing *E. coli* (ESBLEC) frequently affect patients with predisposing conditions. There is some controversy about the virulence of ESBLEC; also, the importance of PG and VF in BSI due to ESBLEC is not well characterised.

-The objective of this study was to investigate the association of phylogenetic groups (PG) and VF with the epidemiology and clinical features of bloodstream infections (BSI) due by ESBL-producing *Escherichia coli* (ESBLEC).

METHODS

Design: Prospective, multicenter cohort including all 191 patients with BSI due to ESBLEC in 13 Spanish hospitals during 2004-2006. The clinical and prognostic features of these patients, and the characterisation of ESBL and antibiotic susceptibility has been previously reported (Rodríguez-Baño et al, Clin Infect Dis 2011 and J Clin Microbiol 2011).

The study was approved by the local Ethic Committees.

Microbiological studies: ESBLs were detected using CLSI recommendations, and characterised by isoelectric focusing, PCR, and sequencing. Susceptibility testing was performed by microdilution as recommended by CLSI. PG and genes codifying for 25 VF (adhesins: *papC, papGI, papGII, papGIII, sfaD/E, afaB/C, iha, fimH;* toxins: *hlyA, cnf1, cnf2, cdtB, sat;* iron-related: *fyuA, iutA, iucD, iroN;* capsule and other protectines: *kps, traT; and* miscellaneous: *cvaC, ibeA, usp, malX, svg, ompT)* were studied by PCR.

Variables: Demographics, type of acquisition (community, healthcare-associated, nosocomial), underlying conditions and severity (Charlson index), source of BSI (according to clinical and microbiological criteria). A VF score (number of VF genes detected) was calculated for all isolates.

Statistical analysis: The association of PG and VF with epidemiological and clinical features was analysed. Fisher or chi squared tests were used for dichotomous variables, as appropriate, and Mann-Whitney U test was used for continuous variables.

RESULTS

The distribution of PG were: B2, 30 (15.7%); D, 51 (26.7%); A, 55 (28.8%), and B1, 55 (28.8%); groups A and B1 will be analysed together. The microbiological features of the isolates and VF according to PG is shown in Table 1. The epidemiological and clinical features according to PG is shown in Table 2. The association between PG, VF and ESBL with the source of BSI is shown in Table 3.

Table 1	All cases (n=191)	B2 (n=30)	D (n=51)	A/B1 (n=110)
Virulence score, median (IQR)	6 (4-8)	9 (8-13)[a,b]	7 (6-9)[d]	6 (2-7)
Virulence factors				
papC	45 (24)	8 (27)	23 (45)[d]	14 (13)
papGI	0	0	0	0
papGII	31 (16)	3 (10)	20 (39)[c,d]	8 (7)
papGIII	7 (4)	4 (13)[a]	1 (2)	2 (2)
sfaD/E	4 (2)	1 (3)	1 (2)	2 (2)
afaB/C	13 (7)	9 (30)[a,b]	4 (8)[d]	0
iha	30 (16)	16 (53)[a,b]	10 (20)[d]	4 (4)
fimH	160 (84)	28 (93)[a]	48 (94)[d]	84 (76)
hlyA	4 (2)	3 (10)[a]	1 (2)	0
cnf1	4 (2)	3 (10)[a]	1 (2)	0
cdtB	4 (2)	3 (10)[a]	1 (2)	0
sat	39 (20)	19 (63)[a,b]	17 (33)[d]	2 (3)
fyuA	98 (51)	28 (93)[a,b]	37 (73)[d]	33 (30)
iutA	157 (82)	28 (93)[a]	46 (90)[d]	83 (76)
iucD	140 (73)	23 (77)	41 (80)	76 (69)
iroN	118 (62)	17 (57)	29 (57)	72 (66)
kps	44 (23)	20 (67)[a,b]	19 (37)[d]	5 (5)
traT	141 (74)	28 (83)	37 (72)	79 (72)
cvaC	66 (35)	7 (23)	16 (31)	43 (39)
ibeA	15 (8)	7 (23)[a]	7 (14)[d]	1 (1)
usp	33 (17)	26 (87)[a,b]	7 (14)[d]	3 (3)
malX	75 (39)	28 (93)[a,b]	34 (67)[d]	13 (12)
svg	3 (2)	2 (7)	0	1 (1)
ireA	29 (15)	3 (10)	19 (37)[c,d]	7 (6)
ompT	102 (53)	27 (90)[a,b]	32 (63)[d]	43 (39)
ESBL group*				
CTX-M-9 group**	122 (64)	10 (33)	41 (80)[c, d]	71 (65)
CTX-M-1 group***	42 (22)	17 (57)[a,b]	8 (16)	17 (16)
SHV group	33 (17)	4 (13)	5 (10)	24 (22)
Resistance to				
Amoxicillin/clavulanic acid	73 (38)	19 (63)[a,b]	20 (39)	34 (31)
Piperacillin/tazobactam	16 (8)	5 (17)	3 (6)	8 (7)
Ciprofloxacin	129 (68)	18 (60)	36 (71)	75 (68)
Gentamycin	39 (20)	4 (13)	14 (28)	21 (19)
Tobramicin	34 (18)	13 (43)[a,b]	10 (20)	11 (10)
Amikacin	3 (2)	2 (7)[a]	1 (2)	0
Co-trimoxazole	115 (60)	19 (63)	38 (75)[d]	58 (53)
No. of resistances****	5 (4-6)	6 (4-7)[a]	5 (4-6)	5 (4-5)

[a]Higher in B2 vs A/B1 (p<0.05). [b] Higher in B vs D (p<0.05). [c]Higher in D vs B2 (p<0.05).
[d]Higher in D vs A/B1 (p<0.05). [e]Higher in A/B1 vs D (p<0.05).
*7 isolates produced >1 ESBL; ony 1 isolate produced a TEM ESBL. **Mainy CTX-M-14.
***Mainly CTX-M-15.

Table 2	B2 (n=30)	D (n=51)	A/B1 (n=110)
Male gender	20 (67.7)	28 (54.9)	59 (53.6)
Median age in years (IQR)	72 (58-82)	71 (58-78)	69 (54-77)
Nosocomial acquisition	14 (46.7)	24 (47.1)	58 (52.7)
Strict community acquired	4 (13.3)	3 (5.9)	16 (14.5)
Nursing home resident	2 (6.7)	7 (13.7)	3 (2.7)
Diabetes mellitus	9 (30)	12 (23.5)	31 (28.2)
Chronic pulmonary disease	4 (13.3)	9 (17.6)	21 (19.1)
Cancer	4 (13.3)[a,b]	15 (29.4)	36 (32.7)
Liver cirrhosis	5 (16.7)	4 (7.8)	9 (8.2)
Chronic renal insufficiency	3 (10)	5 (9.8)	20 (18.2)
Inmunosuppresive therapy	4 (13.3)	9 (17.6)	14 (12.7)
Obstructive urinary disease	7 (23.3)	7 (13.7)[c]	29 (26.4)
Systemic predisposing factors*	16 (53.3)	24 (47.1)	60 (54.5)
Local predisposing factors**	19 (63.3)	30 (58.8)	73 (66.4)
Any predisposing factor	24 (80)	41 (80.4)	93 (84.5)
Biliary tract disease	2 (6.7)	3 (5.9)	13 (11.8)
Neutropenia	1 (3.3)	3 (5.9)	6 (5.5)
Urinary catheter	13 (43.3)	17 (33.3)	36 (32.7)
Central venous catheter	5 (16.7)	12 (23.5)	36 (32.7)
Mechanical ventilation	1 (3.3)	3 (5.9)	4 (3.6)
Previous surgery	6 (20)	14 (27.5)	24 (21.8)
McCabe			
Non fatal	16 (53.3)	28 (54.9)	62 (56.4)
Ultimately fatal	12 (40)	19 (37.3)	38 (34.5)
Rapidly fatal	2 (6.7)	4 (7.8)	10 (9.1)
Previous antibiotics			
Previous fluoroquinolones	7 (23.3)	12 (23.5)	30 (27.3)
Previous cephalospororins	9 (30)	15 (29.4)	29 (26.4)

[a]B2 vs A/B1, P=0.03. [b]B2 vs D, P=0.09. [c]D vs A/B1, P=0.07.

Table 3	Urinary tract (n=90)	Biliary tract (n=24)	Other sources (n=77)
Virulence score, Median (IQR)	6 (4-9)	6 (4-8)	6 (4-9)
Phylogroup			
B2	12 (13.3)	3 (12.5)	15 (19.4)
D	25 (27.7)	5 (20.8)	21 (27.2)
A/B1	53 (58.8)	16 (66.6)	41 (53.2)
Virulence factors*			
hlyA	0	2 (8.3)[a]	2 (2.8)
cnf1	0	2 (8.3)[a]	2 (2.8)
cnf2	0	2 (8.3)[a]	2 (2.8)
usp	12 (13.3)	7 (29.1)	20 (26)[b]
ESBL group**			
CTX-M-9	61 (67.8)	14 (58.3)	47 (61)
CTX-M-1	20 (22.2)	4 (16.6)	18 (23.3)
SHV	10 (11.1)	7 (29.1)	16 (20.7)[c]

*Only VF with p value <0.1 for any comparison are included. **Some isolates produced >1 ESBL. All p values >0.05 except: [a]biliary vs urinary, p=0.04; [b]others vs urinary p=0.04; [c]others vs urinary, p=0.05.

Other data

Overall, no association between specific VF or VF score and local or systemic predisposing features, acquisition, or previous antibiotic use was found with 2 exceptions: *papGII* was more frequent among those without than among those with any predisposing feature (25% vs 12%, p=0.02), while *sat* was less frequent (25% vs 40%, p=0.03).

Among patients with urinary tract BSI, isolates from unpredisposed patients had a higher prevalence than those predisposed of: *papC* (46% vs 10%, p=0.01), and *papGII* (36% vs 7%, p=0.001). No significant differences were found among patients with biliary tract BSI or other sources.

CONCLUSIONS

- The virulence score of ESBLEC causing BSI was lower than expected because isolates belonging to "low virulent" phylogroups A/B1 caused most episodes, in contrast with previous studies of BSI due to *E. coli*, in which B2 ad D were predominant (Johnson et al, J Infect Dis 2002; Sannes et al. J Infect Dis 2004; Cooke et al, J Clin Microbiol 2010. Lefort et al, J Clin Microbiol 2011).
- We hypothesize that this may be related to the fact that BSI due to ESBLEC frequently occurred in patients with local or systemic predisposing factors, and/or in patients who previously received antibiotics. Previous antibiotics would select for ESBLEC because of their multidrug-resistant nature, and such resistant strains would be able to cause invasive disease despite their lower virulence (in the case of isolates from the A/B1 phylogroups) because of the predisposing features of the hosts.
- An exception to this would be ESBLEC from the B2 and D phylogroups. However, we did not find that B2 and D isolates caused infections in clearly less predisposed patients than A/B1 isolates, with the exception of cancer (less frequent among B2) and obstructive urinary tract disease (somehow less frequent among D). Also, we did not find that specific virulence fators were clearly associated with the epidemiological or clinical features of the infections with the exception of *papGII* that was more common among unpredisposed patients.
- This would mean that host factors and previous antimicrobial use would be more important than virulence determinants in the pathogenicity of ESBLEC. Clusters of isolates according to their virulence profile are now being analysed to investigate if groups of FV makes any difference.

ESCMID COLLABORATIVE CENTRE
Hospital Universitario Virgen Macarena y Área

Contact information: Jesús Rodríguez-Baño (jesusrb@us.es)