JCM
Journals.ASM.org

Antimicrobial Resistance and Virulence Characterization among *Escherichia coli* Clinical Isolates Causing Severe Obstetric Infections in Pregnant Women

Elisabet Guiral,[a] Emma Sáez-López,[a] Jordi Bosch,[a,b] Anna Goncé,[c] Marta López,[c] Sergi Sanz,[a,d] Jordi Vila,[a,b] Sara M. Soto[a]

Barcelona Centre for International Health Research (CRESIB), Hospital Clínic-Universitat de Barcelona, Barcelona, Spain[a]; Department of Clinical Microbiology, Hospital Clinic, School of Medicine, University of Barcelona, Barcelona, Spain[b]; Department of Maternal-Fetal Medicine, Institut Clinic de Ginecologia, Obstetricia i Neonatologia, Hospital Clinic-IDIBAPS, University of Barcelona, Barcelona, Spain[c]; Unit of Biostatistics of Department of Public Health, Faculty of Medicine, University of Barcelona, Barcelona, Spain[d]

The virulence markers and the antimicrobial resistance profiles of 78 *Escherichia coli* isolates causing obstetric infections accompanied by sepsis or not were studied. Adhesion-related virulence factors were the most prevalent markers. Low rates of resistance to the antimicrobial agents used as first-line therapy suggest their correct implementation in stewardship guidelines.

Escherichia coli is the enteric Gram-negative bacillus most frequently found in the genital tract of women. Despite its commensal role, this microorganism can become pathogenic, colonizing new environments. Extraintestinal *E. coli* is the second most prevalent etiologic agent causing obstetric infections (1). *E. coli* possesses several virulence factor genes (VFG) that enhance vaginal and/or endocervical colonization in pregnant women. This colonization can lead to different infections in obstetric patients, such as intra-amniotic infection (IAI) or endometrial and urinary tract infections (UTIs), sometimes accompanied by sepsis. In addition, these microorganisms can cause neonatal infections, leading to maternal and fetal morbidity and mortality (2, 3). It has been estimated that 15% of pregnant and 12% of nonpregnant women in our hospital present *E. coli* in the genital tract (4).

The treatment of choice for maternal sepsis includes the administration of different antimicrobial agents, depending on the infection focus, being limited by the low number of antimicrobial agents considered to be safe to the fetus (5). In our hospital, the treatment of choice in patients with IAI consists of ceftriaxone, ampicillin-gentamicin, or ampicillin-cefoxitin, while the treatment of endometritis involves the use of ampicillin-gentamicin-metronidazole.

Briefly, among the virulence factors involved in UTIs, it is well known that adhesins, fimbriae, and toxins are the most important, as they allow the bacteria to adhere to the uroepithelium and cause tissue damage. However, further knowledge is necessary regarding their prevalences and the roles of other families of virulence factors in the specific field of obstetric infections derived from UTIs.

For this purpose, 78 *E. coli* isolates obtained from pregnant women attending the Hospital Clinic of Barcelona from 1987 to 2010 were included in the study; 56 were isolated from the blood samples of patients with sepsis from a genital or urinary origin, and 22 were isolated from amniotic fluid or placenta samples of patients with nonbacteremic IAI.

The resistance profiles were determined using the disk diffusion method. The antimicrobial agents tested are listed in Table 1 and include the first therapeutic options used to treat UTIs and genital infections. The results were interpreted according to CLSI guidelines (6), and the *E. coli* ATCC 25922 strain was used as the control.

The VFG profiles of the isolates were analyzed by PCR using gene-specific primers for the virulence genes coding for the adhesins, toxins, and invasins most prevalent in the uropathogenic *E. coli* (UPEC) isolates described, from which the isolates causing the obstetric infections studied potentially come. The isolates were also screened for 5 specific virulence markers for extraintestinal pathogenic *E. coli* (ExPEC) or non-ExPEC classification (7). The PCR conditions used were 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, with the corresponding annealing temperature (55 to 63°C) for 30 s, 72°C for 2 min, and a final elongation cycle of 72°C for 5 min. The samples were run in 1.5% agarose gels and stained with SYBR Safe DNA gel stain (Invitrogen, Spain). The *E. coli* phylogenetic group was determined using the 3-locus PCR-based method described previously (8). In order to determine if any isolate belonged to sequence type 131 (ST131), serotype O25b was identified in the collection, according to the methodology proposed by Clermont et al. (9), and the multilocus sequence typing (MLST) methodology was carried out with these isolates using the University of Warwick database for assigning sequence types (ST).

Statistical analysis was performed using Stata version 13.1 (Stata Corp., TX, USA). *P* values of <0.05 were accepted as significant, and statistical correction for multiple comparisons was applied.

Twenty isolates (26%) were resistant to three or more antimicrobial classes, presenting a multidrug-resistant (MDR) phenotype. Sixty-three percent of all the isolates were resistant to ampicillin, whereas only 13% were resistant to amoxicillin-clavulanic

Received 20 February 2015 **Returned for modification** 20 February 2015
Accepted 23 February 2015

Accepted manuscript posted online 4 March 2015

Citation Guiral E, Sáez-López E, Bosch J, Goncé A, López M, Sanz S, Vila J, Soto SM. 2015. Antimicrobial resistance and virulence characterization among *Escherichia coli* clinical isolates causing severe obstetric infections in pregnant women. J Clin Microbiol 53:1745–1747. doi:10.1128/JCM.00487-15.

Editor: R. Patel

Address correspondence to Sara M. Soto, sara.soto@cresib.cat.

doi:10.1128/JCM.00487-15

TABLE 1 Resistance to antimicrobial agents in *E. coli* isolates, according to the clinical features

Antimicrobial agent	No. (%) resistant in isolate group			
	Non-sepsis causing ($n = 22$)	Sepsis causing ($n = 56$)	Total ($n = 78$)	*P* value
Ampicillin	13 (59)	36 (64)	49 (63)	0.6692[a]
Amoxicillin-clavulanic acid	3 (14)	7 (12)	10 (13)	1.0000[b]
Cefazolin	3 (14)	14 (25)	17 (22)	0.3680[b]
Cefuroxime	1 (5)	2 (4)	3 (4)	1.0000[b]
Cefoxitin	0 (0)	1 (2)	1 (1)	1.0000[b]
Cefotaxime	1 (5)	1 (2)	2 (3)	0.4872[b]
Ceftazidime	0 (0)	0 (0)	0 (0)	
Imipenem	0 (0)	0 (0)	0 (0)	
Piperacillin-tazobactam	0 (0)	1 (2)	1 (1)	1.0000[b]
Nalidixic acid	5 (23)	9 (16)	14 (18)	0.5216[b]
Ciprofloxacin	0 (0)	2 (4)	2 (3)	1.0000[b]
Chloramphenicol	2 (9)	5 (9)	7 (9)	1.0000[b]
Gentamicin	0 (0)	3 (5)	3 (4)	0.5547[b]
Amikacin	0 (0)	1 (2)	1 (1)	1.0000[b]
Kanamycin	1 (5)	5 (9)	6 (8)	0.6697[b]
Tetracycline	7 (32)	20 (36)	27 (35)	0.7448[a]
Trimethoprim-sulfamethoxazole	6 (27)	16 (29)	22 (28)	0.9087[a]

[a] Chi-square test.
[b] Fisher's exact test.

acid. Most of the isolates were susceptible to second- and third-generation cephalosporins, imipenem, aminoglycosides, ciprofloxacin, and chloramphenicol, with higher rates of resistance for tetracycline, trimethoprim-sulfamethoxazole, cefazolin, and nalidixic acid. The isolates causing sepsis had a lower prevalence of resistance to nalidixic acid, with a higher percentage of resistance to cefazolin being observed (Table 1).

The most prevalent VFG found among the isolates were adhesion related, with prevalences between 56 and 86%. The isolates harboring the greatest number of VFG were those causing sepsis, with a significantly higher percentages of *hlyA*, *cnf1*, *papA*, *iha*, *fyuA*, or *papG*II, all of them contained in pathogenicity islands. Regarding virulence factors related to iron recruitment, the *iutA* gene was found significantly more frequently in IAI-causing isolates ($P = 0.0001$), whereas the *iroN* gene was the most common in sepsis-causing isolates ($P = 0.0284$). A multivariate analysis of VFG showed the presence of the *fimA*, *iucC*, *iroN*, *iutA*, *iha*, and *hra* genes as being independent predictors of sepsis-causing isolates (Table 2). Seventy-eight percent of the isolates (with no significant differences between the sepsis- and non-sepsis-causing isolates) were classified as ExPEC according to the virulence markers harbored, and only two of these isolates belonged to ST131.

An analysis of the presence of each VFG among the resistance profiles of the isolates to each of the antimicrobial agents tested was carried out, showing that susceptible isolates had a higher carriage of VFG.

The phenotypic results of antimicrobial resistance observed in the present study indicated high levels of ampicillin-resistant isolates in the collection, in accordance with those found in *E. coli* isolates causing neonatal sepsis and in extraintestinal *E. coli* in general (10). On the other hand, the low rates of resistance to amoxicillin-clavulanic acid and second- and third-generation cephalosporins observed in the present study are in contrast with the increasing appearance of strains carrying extended-spectrum

TABLE 2 Prevalence of virulence factor genes according to the clinical features[a]

Virulence factor	No. (%) with VFG in isolate group				Univariate analysis			Multivariate analysis		
	Non-sepsis causing ($n = 22$)	Sepsis causing ($n = 56$)	Total ($n = 78$)	*P* value	Odds ratio[d]	95% confidence interval	*P* value	Odds ratio[d]	95% confidence interval	*P* value
hlyA	**5 (23)**	**28 (50)**	**33 (42)**	**0.0282**[b]	**3.40**	**(1.10, 10.49)**	**0.0332**			
cnf1	**2 (9)**	**19 (34)**	**21 (27)**	**0.0261**[b]	**5.14**	**(1.08, 24.32)**	**0.0392**			
sat1	9 (41)	23 (41)	32 (41)	0.9895[b]	1.01	(0.37, 2.74)	0.9895			
fimA	17 (77)	50 (89)	67 (86)	0.2757[c]	2.45	(0.66, 9.07)	0.1792	**32.20**	**(1.28, 809.78)**	**0.0349**
papA	**8 (36)**	**40 (71)**	**48 (62)**	**0.0042**[b]	**4.37**	**(1.54, 12.43)**	**0.0056**			
papC	11 (50)	33 (59)	44 (56)	0.4742[b]	1.43	(0.53, 3.86)	0.4752			
papEF	12 (55)	38 (68)	50 (64)	0.2701[b]	1.76	(0.64, 4.83)	0.2727			
*papG*I	0 (0)	0 (0)	0 (0)		1.00					
*papG*II	**8 (36)**	**42 (75)**	**50 (64)**	**0.0014**[b]	**5.25**	**(1.82, 15.13)**	**0.0021**			
*papG*III	9 (41)	14 (25)	23 (29)	0.1656[b]	0.48	(0.17, 1.37)	0.1697			
prs	15 (68)	36 (64)	51 (65)	0.7448[b]	0.84	(0.29, 2.40)	0.7450			
fyuA	**4 (18)**	**29 (52)**	**33 (42)**	**0.0069**[b]	**4.83**	**(1.45, 16.10)**	**0.0103**			
hra	8 (36)	11 (20)	19 (24)	0.1216[b]	0.43	(0.14, 1.27)	0.1270	**0.13**	**(0.02, 0.96)**	**0.0452**
sfa	5 (23)	18 (32)	23 (29)	0.4118[b]	1.61	(0.51, 5.06)	0.4142			
ibeA	5 (23)	9 (16)	14 (18)	0.5216[c]	0.65	(0.19, 2.22)	0.4926			
iucC	14 (64)	44 (79)	58 (74)	0.1740[b]	2.10	(0.71, 6.16)	0.1787	**53.38**	**(2.31, 1,233.37)**	**0.0130**
iutA	**15 (68)**	**10 (18)**	**25 (32)**	**<0.0001**[b]	**0.10**	**(0.03, 0.31)**	**0.0001**	**0.01**	**(0.00, 0.13)**	**0.0016**
iha	**4 (18)**	**26 (46)**	**30 (38)**	**0.0210**[b]	**3.90**	**(1.17, 13.00)**	**0.0267**	**20.61**	**(1.77, 240.12)**	**0.0157**
iroN	**8 (36)**	**36 (64)**	**44 (56)**	**0.0252**[b]	**3.15**	**(1.13, 8.79)**	**0.0284**	**6.47**	**(1.30, 32.15)**	**0.0225**
ag43	10 (45)	27 (48)	37 (47)	0.8261[b]	1.12	(0.42, 3.01)	0.8262			
malX	**9 (41)**	**38 (68)**	**47 (60)**	**0.0286**[b]	**3.05**	**(1.10, 8.44)**	**0.0319**			

[a] Statistically significant results are in bold type.
[b] Chi-square test.
[c] Fisher's exact test.
[d] Odds ratio for present versus absent.

β-lactamases (ESBLs) in the last years and causing infections from other sources, suggesting that the implementation of these antimicrobial agents as first-line therapy in these types of infections is correct (11). Nonetheless, the treatment administered should still be chosen depending on the rates of resistance in each hospital to gentamicin and cephalosporins in *E. coli* causing obstetric infections, as well as the prophylaxis or previous treatment with these antimicrobial agents, which have led to the development of resistant bacteria.

Regarding the VFG present in *E. coli* involved in the obstetric infections studied, it was found that adhesins and fimbriae may play an important role in the development of these infections, allowing the bacteria to colonize different environments. The higher prevalence of *hlyA* and *cnf1* among the isolates causing sepsis might be related to the tissue damage involved with these infections. Concerning the iron recruitment systems, the yersiniabactin receptor encoded by *fyuA* and the genes encoding the siderophore receptors Iha and IroN were also more prevalent among the isolates causing sepsis, due to the need for UPEC to capture iron from the host within the hostile environment of urine. These virulence factors have been largely described as characteristic of UPEC (12). On the other hand, *iutA* was more frequently found in isolates causing IAI, elucidating a high adaptation capacity according to the particular microenvironmnent colonized.

A specific relationship was found between tetracycline-resistant isolates and the lower presence of several VFG included in pathogenicity islands (PAIs), similar to the previously described relationship between the acquisition of quinolone resistance and the loss of VFG (13).

In conclusion, to date, *E. coli* isolates causing obstetric infections present similar rates of antimicrobial resistance to those described for extraintestinal *E. coli* infections, except for a lower prevalence of resistance to third-generation cephalosporins, thereby those not carrying ESBLs. These results demonstrate that the administration of antimicrobials in our hospital is correct. However, it is important to establish surveillance networks specific for these kinds of infections in order to adapt stewardship programs when appropriate.

ACKNOWLEDGMENTS

This work was supported by the Spanish Network for Research in Infectious Diseases (grant REIPI RD06/0008), from Instituto de Salud Carlos III (ISCIII), by the Instituto de Salud Carlos III (grant FIS 10/01579), cofinanced by the European Regional Development Fund (ERDF) 'A way to achieve Europe.' It was also funded by the grant for research groups support (SGR09-1256) of the Agència de Gestió d'Ajuts Universitaris i de Recerca from Generalitat de Catalunya and by European Commission funding (TROCAR contract HEALTH-F3-2008-223031). Sara M. Soto has a fellowship from program I3 of the ISCIII.

REFERENCES

1. **Wait RB.** 1984. Urinary tract infection during pregnancy. Asymptomatic bacteriuria, acute cystitis, and acute pyelonephritis. Postgrad Med **75:**153–157, 161.
2. **Andreu A, Sanfeliu I, Vinas L, Barranco M, Bosch J, Dopico E, Guardia C, Juncosa T, Lite J, Matas L, Sanchez F, Sierr M, Grupo de Microbiólogos pare el Esduio de las Infecciones de Transmissión Vertical, Societat Catalana de Malaties Infeccioses I Microbiologia Clínica.** 2003. Decreasing incidence of perinatal group B streptococcal disease (Barcelona 1994–2002). Relation with hospital prevention policies. Enferm Infecc Microbiol Clin **21:**174–179. (In Spanish.) http://dx.doi.org/10.1157/13045447.
3. **Connolly A, Thorp JM, Jr.** 1999. Urinary tract infections in pregnancy. Urol Clin North Am **26:**779–787. http://dx.doi.org/10.1016/S0094-0143(05)70218-4.
4. **Guiral E, Bosch J, Vila J, Soto SM.** 2011. Prevalence of *Escherichia coli* among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbiol Lett **314:**170–173. http://dx.doi.org/10.1111/j.1574-6968.2010.02160.x.
5. **Burke C.** 2009. Perinatal sepsis. J Perinat Neonatal Nurs **23:**42–51. http://dx.doi.org/10.1097/JPN.0b013e31819614ed.
6. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing: 17th informational supplement. CLSI document M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Johnson JR, Stell AL.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis **181:**261–272. http://dx.doi.org/10.1086/315217.
8. **Clermont O, Bonacorsi S, Bingen E.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol **66:**4555–4558. http://dx.doi.org/10.1128/AEM.66.10.4555-4558.2000.
9. **Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, Mulvey MR, Nordmann P, Ruppe E, Sarthou JL, Frank T, Vimont S, Arlet G, Branger C, Woodford N, Denamur E.** 2009. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. J Antimicrob Chemother **64:**274–277. http://dx.doi.org/10.1093/jac/dkp194.
10. **Guiral E, Bosch J, Vila J, Soto SM.** 2012. Antimicrobial resistance of *Escherichia coli* strains causing neonatal sepsis between 1998 and 2008. Chemotherapy **58:**123–128. http://dx.doi.org/10.1159/000337062.
11. **García-Hernández AM, García-Vázquez E, Hernández-Torres A, Ruiz J, Yague G, Herrero JA, Gomez J.** 2011. Bacteraemia due to *Escherichia coli* producing extended-spectrum beta-lactamases (ESBL): clinical relevance and today's insights. Rev Esp Quimioter **24:**57–66. (In Spanish.)
12. **Porcheron G, Garenaux A, Proulx J, Sabri M, Dozois CM.** 2013. Iron, copper, zinc, and manganese transport and regulation in pathogenic *Enterobacteria*: correlations between strains, site of infection and the relative importance of the different metal transport systems for virulence. Front Cell Infect Microbiol **3:**90. http://dx.doi.org/10.3389/fcimb.2013.00090.
13. **Soto SM, Jimenez de Anta MT, Vila J.** 2006. Quinolones induce partial or total loss of pathogenicity islands in uropathogenic *Escherichia coli* by SOS-dependent or -independent pathways, respectively. Antimicrob Agents Chemother **50:**649–653. http://dx.doi.org/10.1128/AAC.50.2.649-653.2006.