ARTICLE TYPE: Short-Form Paper

TITLE

Antimicrobial resistance and virulence characterisation among *Escherichia coli* clinical isolates causing severe obstetric infections in pregnant women.

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RUNNING TITLE: *E. coli* resistance and virulence in obstetric infections.

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ABSTRACT

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

Escherichia coli are the enteric Gram-negative bacilli most frequently found in the genital tract of women. Despite their commensal role, these microorganisms 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 infection (UTI), sometimes accompanied by sepsis. In addition, these microorganisms can cause neonatal infections leading to mother and fetal morbidity and mortality (2, 3). It has been estimated that 15% of pregnant and 12% of non-pregnant women in our hospital present *E. coli* in the genital tract (4).

The treatment of choice in maternal sepsis includes the administration of different antimicrobial agents depending on the focus, being limited by the low number of the 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/metronidazol.

In general lines, among the virulence factors involved in UTI, it is well-known that adhesins, fimbriaes 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 its prevalence and the role of other families of virulence factors in the specific field of obstetric infections derived from UTIs.

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

Resistance profiles were determined using the disk diffusion method. The antimicrobial agents tested are listed in Table 1 and include the first therapeutic options to treat UTI and genital infections. The results were interpreted following CLSI guidelines (6) and the *E. coli* ATCC25922 strain was used as the control.

The VFG profile of the isolates was analyzed by PCR using gene-specific primers for the virulence genes encoding 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 from. 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 94ºC for 4 minutes, followed by 30 cycles of 94ºC for 30 seconds, with the corresponding annealing temperature (55-63ºC) for 30 seconds, 72ºC for 2 minutes and a final elongation cycle of 72ºC for 5 minutes. 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 ST131, serotype O25b was identified in the collection according to the methodology proposed by Clermont et al. (9), and the multi-locus 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 less than 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 multi-drug resistant (MDR) phenotype. Sixty-three percent of all the isolates were resistant to ampicillin whereas only 13% were resistant to amoxicillin-clavulanic acid. Most of the isolates were susceptible to 2^{nd} and 3^{rd} generation cephalosporins, imipenem, aminoglycosides, ciprofloxacin and chloramphenicol, with higher rates of resistance for tetracycline, trimethoprim/sulphametoxazole, cefazolin, and nalidixic acid. Isolates causing sepsis had a lower prevalence of resistance to nalidixic acid, with a higher percentage of resistance being observed with cephazolin (Table 1).

The most prevalent VFG found among the isolates were adhesion-related, with percentages between 56 and 86%. The isolates harboring the greatest number of VFG were those causing sepsis, with a significantly higher percentages of *hly*A, *cnf*1, *pap*A, *iha, fyu*A or *pap*GII, all contained in pathogenicity islands. Regarding virulence factors related to iron recruitment, the *iut*A gene was found significantly more frequently in IAI causing isolates (p=0.0001) whereas the *iro*N gene was the most common in sepsis producing isolates (p=0.0284). Multivariate analysis of VFG showed the presence of the *fim*A, *iuc*C, *iro*N, *iut*A, *iha and hra* genes as independent predictors for 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.

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 among 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 ESBLs-carrying strains in the last years 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 to gentamicin and cephalosporins in *E. coli* causing obstetric infections in each hospital, 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 *hly*A and *cnf*1 among the isolates causing sepsis could be related to the tissue damage involved in these infections. Concerning the iron recruitment systems, the yersiniabactin receptor encoded by *fyu*A and the siderophores receptors Iha and IroN encoding genes 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 in UPEC (12). On

the other hand, *iut*A 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 TE-resistant isolates and the lower presence of several VFG included in PAIs, similar to the previously described relationship between 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 not carrying ESBL. 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 (REIPI RD06/0008), from the *Ministerio de Economía y Competitividad, Instituto de Salud Carlos III* (ISCIII), by the Instituto de Salud Carlos III (FIS 10/01579), co-financed by *European Regional Development Fund* 'A way to achieve Europe' (ERDF). 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.

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199 **TABLE 1.** Percentage of resistance to antimicrobial agents in *E. coli* isolates according

200 to the clinical features.

Antimicrobial agent	**Non sepsis-isolates (n=22)**	**Sepsis- isolates (n=56)**	**Total isolates (n=78)**	**p-value**
Ampicillin	13 (59%)	36 (64%)	49 (63%)	0.6692 [1]
Amoxicillin /clavulanic acid	3 (14%)	7 (12%)	10 (13%)	1.0000 [2]
Cefazolin	3 (14%)	14 (25%)	17 (22%)	0.3680 [2]
Cefuroxime	1 (5%)	2 (4%)	3 (4%)	1.0000 [2]
Cefoxitin	0 (0%)	1 (2%)	1 (1%)	1.0000 [2]
Cefotaxime	1 (5%)	1 (2%)	2 (3%)	0.4872 [2]
Ceftazidime	0 (0%)	0 (0%)	0 (0%)	-
Imipenem	0 (0%)	0 (0%)	0 (0%)	-
Piperacillin /tazobactam	0 (0%)	1 (2%)	1 (1%)	1.0000 [2]
Nalidixic acid	5 (23%)	9 (16%)	14 (18%)	0.5216 [2]
Ciprofloxacin	0 (0%)	2 (4%)	2 (3%)	1.0000 [2]
Chloramphenicol	2 (9%)	5 (9%)	7 (9%)	1.0000 [2]
Gentamicin	0 (0%)	3 (5%)	3 (4%)	0.5547 [2]
Amikacin	0 (0%)	1 (2%)	1 (1%)	1.0000 [2]
Kanamycin	1 (5%)	5 (9%)	6 (8%)	0.6697 [2]
Tetracycline	7 (32%)	20 (36%)	27 (35%)	0.7448 [1]
Trimethoprim/sulfa-methoxazole	6 (27%)	16 (29%)	22 (28%)	0.9087 [1]

1: Chi-squared test
2: Fisher's exact test
bold: Statistically significant results

201 **TABLE 2.** Prevalence of virulence factor genes according to the clinical features.

					UNIVARIATE ANALYSIS			MULTIVARIATE ANALYSIS		
Virulence factor	**Non sepsis-isolates (n=22)**	**Sepsis-isolates (n=56)**	**Total isolates (n=78)**	**p-value**	**Odds Ratio[3]**	**95% Conf. Interval**	**p-value**	**Odds Ratio[3]**	**95% Conf. Interval**	**p-value**
*hly*A	**5 (23%)**	**28 (50%)**	**33 (42%)**	**0.0282[1]**	**3.40**	**(1.10; 10.49)**	**0.0332**	-	-	-
*cnf*1	**2 (9%)**	**19 (34%)**	**21 (27%)**	**0.0261[1]**	**5.14**	**(1.08; 24.32)**	**0.0392**	-	-	-
*sat*1	9 (41%)	23 (41%)	32 (41%)	0.9895[1]	1.01	(0.37; 2.74)	0.9895	-	-	-
*fim*A	17 (77%)	50 (89%)	67 (86%)	0.2757[2]	2.45	(0.66; 9.07)	0.1792	**32.20**	**(1.28; 809.78)**	**0.0349**
*pap*A	**8 (36%)**	**40 (71%)**	**48 (62%)**	**0.0042[1]**	**4.37**	**(1.54; 12.43)**	**0.0056**	-	-	-
*pap*C	11 (50%)	33 (59%)	44 (56%)	0.4742[1]	1.43	(0.53; 3.86)	0.4752	-	-	-
*pap*EF	12 (55%)	38 (68%)	50 (64%)	0.2701[1]	1.76	(0.64; 4.83)	0.2727	-	-	-
*pap*GI	0 (0%)	0 (0%)	0 (0%)	-	1.00	-	-	-	-	-
*pap*GII	**8 (36%)**	**42 (75%)**	**50 (64%)**	**0.0014[1]**	**5.25**	**(1.82; 15.13)**	**0.0021**	-	-	-
*pap*GIII	9 (41%)	14 (25%)	23 (29%)	0.1656[1]	0.48	(0.17; 1.37)	0.1697	-	-	-

prs	15 (68%)	36 (64%)	51 (65%)	0.7448[1]	0.84	(0.29; 2.40)	0.7450	-	-	-
*fyu*A	**4 (18%)**	**29 (52%)**	**33 (42%)**	**0.0069[1]**	**4.83**	**(1.45; 16.10)**	**0.0103**	-	-	-
hra	8 (36%)	11 (20%)	19 (24%)	0.1216[1]	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[1]	1.61	(0.51; 5.06)	0.4142	-	-	-
*ibe*A	5 (23%)	9 (16%)	14 (18%)	0.5216[2]	0.65	(0.19; 2.22)	0.4926	-	-	-
*iuc*C	14 (64%)	44 (79%)	58 (74%)	0.1740[1]	2.10	(0.71; 6.16)	0.1787	**53.38**	**(2.31; 1233.37)**	**0.0130**
*iut*A	**15 (68%)**	**10 (18%)**	**25 (32%)**	**< 0.0001[1]**	**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[1]**	**3.90**	**(1.17; 13.00)**	**0.0267**	**20.61**	**(1.77; 240.12)**	**0.0157**
*iro*N	**8 (36%)**	**36 (64%)**	**44 (56%)**	**0.0252[1]**	**3.15**	**(1.13; 8.79)**	**0.0284**	**6.47**	**(1.30; 32.15)**	**0.0225**
*ag*43	10 (45%)	27 (48%)	37 (47%)	0.8261[1]	1.12	(0.42; 3.01)	0.8262	-	-	-
*mal*X	**9 (41%)**	**38 (68%)**	**47 (60%)**	**0.0286[1]**	**3.05**	**(1.10; 8.44)**	**0.0319**	-	-	-

1: Chi-squared test
2: Fisher's exact test
3: Odds Ratio for present vs. absent
bold: Statistically significant results

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TABLE 1 *E. coli* isolates resistance profile according to the clinical features

Antimicrobial agent		Non sepsis-causing isolates (n=22)	Sepsis-causing isolates (n=56)	Total isolates (n=78)	p-value
Ampicillin	susceptible	9 (41%)	20 (36%)	29 (37%)	0.6692 [1]
	resistant	13 (59%)	36 (64%)	48 (62%)	
Amoxicillin /clavulanic acid	susceptible	19 (86%)	49 (88%)	68 (87%)	1.0000 [2]
	resistant	3 (14%)	7 (12%)	10 (13%)	
Cefazolin	susceptible	19 (86%)	42 (75%)	61 (78%)	0.3680 [2]
	resistant	3 (14%)	14 (25%)	17 (22%)	
Cefuroxime	susceptible	21 (95%)	54 (96%)	75 (96%)	1.0000 [2]
	resistant	1 (5%)	2 (4%)	3 (4%)	
Cefoxitin	susceptible	22 (100%)	55 (98%)	77 (99%)	1.0000 [2]
	resistant	0 (0%)	1 (2%)	1 (1%)	
Cefotaxime	susceptible	21 (95%)	55 (98%)	76 (97%)	0.4872 [2]
	resistant	1 (5%)	1 (2%)	2 (3%)	
Ceftazidime	susceptible	22 (100%)	56 (100%)	78 (100%)	-
	resistant	0 (0%)	0 (0%)	0 (0%)	
Imipenem	susceptible	22 (100%)	56 (100%)	78 (100%)	-
	resistant	0 (0%)	0 (0%)	0 (0%)	
Piperacillin /tazobactam	susceptible	22 (100%)	55 (98%)	77 (99%)	1.0000 [2]
	resistant	0 (0%)	1 (2%)	1 (1%)	
Nalidixic acid	susceptible	17 (77%)	47 (84%)	64 (82%)	0.5216 [2]
	resistant	5 (23%)	9 (16%)	14 (18%)	
Ciprofloxacin	susceptible	22 (100%)	54 (96%)	76 (97%)	1.0000 [2]
	resistant	0 (0%)	2 (4%)	2 (3%)	
Chloramphenicol	susceptible	20 (91%)	51 (91%)	71 (91%)	1.0000 [2]
	resistant	2 (9%)	5 (9%)	7 (9%)	
Gentamicin	susceptible	22 (100%)	53 (95%)	75 (96%)	0.5547 [2]
	resistant	0 (0%)	3 (5%)	3 (4%)	
Amikacin	susceptible	22 (100%)	55 (98%)	77 (99%)	1.0000 [2]
	resistant	0 (0%)	1 (2%)	1 (1%)	
Kanamycin	susceptible	21 (95%)	51 (91%)	72 (92%)	0.6697 [2]
	resistant	1 (5%)	5 (9%)	6 (8%)	
Tetracycline	susceptible	15 (68%)	36 (64%)	51 (65%)	0.7448 [1]
	resistant	7 (32%)	20 (36%)	27 (35%)	
Trimethoprim/sulfa-methoxazole	susceptible	16 (73%)	40 (71%)	56 (72%)	0.9087 [1]
	resistant	6 (27%)	16 (29%)	22 (28%)	

1: Chi-squared test

2: Fisher's exact test

bold: Statistically significant results

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

						UNIVARIATE ANALYSIS			MULTIVARIATE ANALYSIS		
Virulence factor		Non sepsis-causing isolates (n=22)	Sepsis-causing isolates (n=56)	Total isolates	p-value	Odds Ratio[3]	(95% Conf. Interval)	p-value	Odds Ratio[3]	(95% Conf. Interval)	p-value
Biofilm	non-forming	10 (45%)	34 (61%)	44 (56%)	0.2213[1]	0.54	(0.20; 1.46)	0.2242	-	-	-
	forming	12 (55%)	22 (39%)	34 (44%)							
S. cerevisiae agglutination	negative	6 (27%)	8 (14%)	14 (18%)	0.2007[2]	2.25	(0.68; 7.47)	0.1854	-	-	-
	positive	16 (73%)	48 (86%)	64 (82%)							
*hly*A	absent	17 (77%)	28 (50%)	45 (58%)	**0.0282[1]**	**3.40**	**(1.10; 10.49)**	**0.0332**	-	-	-
	present	5 (23%)	28 (50%)	33 (42%)							
*cnf*1	absent	20 (91%)	37 (66%)	57 (73%)	**0.0261[1]**	**5.14**	**(1.08; 24.32)**	**0.0392**	-	-	-
	present	2 (9%)	19 (34%)	21 (27%)							
*sat*1	absent	13 (59%)	33 (59%)	46 (59%)	0.9895[1]	1.01	(0.37; 2.74)	0.9895	-	-	-
	present	9 (41%)	23 (41%)	32 (41%)							
*fim*A	absent	5 (23%)	6 (11%)	11 (14%)	0.2757[2]	2.45	(0.66; 9.07)	0.1792	**32.20**	**(1.28; 809.78)**	**0.0349**
	present	17 (77%)	50 (89%)	67 (86%)							
*pap*A	absent	14 (64%)	16 (29%)	30 (38%)	**0.0042[1]**	**4.37**	**(1.54; 12.43)**	**0.0056**	-	-	-
	present	8 (36%)	40 (71%)	48 (62%)							
*pap*C	absent	11 (50%)	23 (41%)	34 (44%)	0.4742[1]	1.43	(0.53; 3.86)	0.4752	-	-	-
	present	11 (50%)	33 (59%)	44 (56%)							
*pap*EF	absent	10 (45%)	18 (32%)	28 (36%)	0.2701[1]	1.76	(0.64; 4.83)	0.2727	-	-	-
	present	12 (55%)	38 (68%)	50 (64%)							
*pap*GI	absent	22 (100%)	56 (100%)	78 (100%)	-	1.00	-	-	-	-	-
*pap*GII	absent	14 (64%)	14 (25%)	28 (36%)	**0.0014[1]**	**5.25**	**(1.82; 15.13)**	**0.0021**	-	-	-
	present	8 (36%)	42 (75%)	50 (64%)							
*pap*GIII	absent	13 (59%)	42 (75%)	55 (71%)	0.1656[1]	0.48	(0.17; 1.37)	0.1697	-	-	-
	present	9 (41%)	14 (25%)	23 (29%)							
prs	absent	7 (32%)	20 (36%)	27 (35%)	0.7448[1]	0.84	(0.29; 2.40)	0.7450	-	-	-
	present	15 (68%)	36 (64%)	51 (65%)							
*fyu*A	absent	18 (82%)	27 (48%)	45 (58%)	**0.0069[1]**	**4.83**	**(1.45; 16.10)**	**0.0103**	-	-	-

	present	4 (18%)	29 (52%)	33 (42%)							
hra	absent	14 (64%)	45 (80%)	59 (76%)	0.1216[1]	0.43	(0.14; 1.27)	0.1270	**0.13**	**(0.02; 0.96)**	**0.0452**
	present	8 (36%)	11 (20%)	19 (24%)							
sfa	absent	17 (77%)	38 (68%)	55 (71%)	0.4118[1]	1.61	(0.51; 5.06)	0.4142	-	-	-
	present	5 (23%)	18 (32%)	23 (29%)							
*ibe*A	absent	17 (77%)	47 (84%)	64 (82%)	0.5216[2]	0.65	(0.19; 2.22)	0.4926	-	-	-
	present	5 (23%)	9 (16%)	14 (18%)							
*iuc*C	absent	8 (36%)	12 (21%)	20 (26%)	0.1740[1]	2.10	(0.71; 6.16)	0.1787	**53.38**	**(2.31; 1233.37)**	**0.0130**
	present	14 (64%)	44 (79%)	58 (74%)							
*iut*A	absent	7 (32%)	46 (82%)	53 (68%)	**< 0.0001[1]**	**0.10**	**(0.03; 0.31)**	**0.0001**	**0.01**	**(0.00; 0.13)**	**0.0016**
	present	15 (68%)	10 (18%)	25 (32%)							
iha	absent	18 (82%)	30 (54%)	48 (62%)	**0.0210[1]**	**3.90**	**(1.17; 13.00)**	**0.0267**	**20.61**	**(1.77; 240.12)**	**0.0157**
	present	4 (18%)	26 (46%)	30 (38%)							
*iro*N	absent	14 (64%)	20 (36%)	34 (44%)	**0.0252[1]**	**3.15**	**(1.13; 8.79)**	**0.0284**	**6.47**	**(1.30; 32.15)**	**0.0225**
	present	8 (36%)	36 (64%)	44 (56%)							
*ag*43	absent	12 (55%)	29 (52%)	41 (53%)	0.8261[1]	1.12	(0.42; 3.01)	0.8262	-	-	-
	present	10 (45%)	27 (48%)	37 (47%)							
*mal*X	absent	13 (59%)	18 (32%)	31 (40%)	**0.0286[1]**	**3.05**	**(1.10; 8.44)**	**0.0319**	-	-	-
	present	9 (41%)	38 (68%)	47 (60%)							

1: Chi-squared test
2: Fisher's exact test
3: Odds Ratio for present vs. absent

bold: Statistically significant results

TABLE 3 Phylogenetic group distribution by clinical features

Phylogenetic group		Clinical feature: URS (n=32)	GFS (n=24)	NII (n=22)	Total isolates (N=78)	p-value
Non B2 vs. B2	Non-B2	12 (38%)	5 (21%)	12 (55%)	29 (37%)	0.0612 [1]
	B2	20 (62%)	19 (79%)	10 (45%)	49 (63%)	
A/B2/D	A	1 (3%)	2 (8%)	3 (14%)	6 (8%)	
	B2	20 (62%)	19 (79%)	10 (45%)	49 (63%)	0.0793 [2]
	D	11 (34%)	3 (12%)	9 (41%)	23 (29%)	

1: Chi-squared test

2: Fisher's exact test

URS: UTI-related sepsis, GFS: Genital-focus sepsis, NII: Non-bacteraemic intra-amniotic infection

bold: Statistically significant results