Antimicrobial Original Research Paper

Maternal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in Argentina

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The aim of this study was to determine the prevalence of vaginal *Escherichia coli* colonization and perianal carriage of Enterobacteriaceae resistant to third generation cephalosporins in pregnant women. Vaginal and perianal samples from 259 pregnant women were studied. Vaginal swabs were inoculated onto MacConkey agar plates and perianal swabs were inoculated onto CHROMagar extended-spectrum beta-lactamase (ESBL) plates. The minimal inhibitory concentration of the isolates was determined using the Epsilometer test method. The phenotypic detection of ESBLs was performed by the combined disc method using cefotaxime versus cefotaxime plus clavulanate. The prevalence of vaginal *E. coli* colonization during pregnancy was 14.3%. The resistance rate to ampicillin, gentamicin, and cefotaxime was 48.6, 10.8, and 0.8%, respectively. Enterobacteriaceae resistant to third generation cephalosporins were recovered in 7.3% of all perianal specimens. Among them, 5.4% of pregnant women were colonized with *E. coli* ESBL-producer strains. The present study revealed that colonization with Enterobacteriaceae resistant to third generation cephalosporins is significant in pregnancy. ESBL-producing *E. coli* were the most prevalent organisms. Screening strategies designed to monitor for ESBL-producing *E. coli* could be useful in endemic areas to prevent perinatal transmission and the introduction of multiresistant strains to the maternity ward.

Keywords: Extended-spectrum beta-lactamase, *E. coli*, Maternal carriage

Introduction

Escherichia coli is the most common cause of Gram-negative neonatal bacterial meningitis and septicemia. Mortality from neonatal meningitis in developing countries is estimated to be 40–58%, compared to 10% in developed countries.[1] The recommended initial empiric therapy for a neonate with suspected bacterial sepsis and/or meningitis includes ampicillin and an aminoglycoside such as gentamicin. In treating meningitis, many centres administer cefotaxime in addition to or instead of gentamicin, particularly when Gram-negative infections are suspected.

Recently, the number of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae isolated from clinical material derived from either hospitalized patients or those living in the community has increased.[2] *E. coli* strains involved in neonatal infections originate from the vagina, which is colonized by a rectal source.[3] A recent study revealed a high prevalence (26.8%) of faecal carriage of Enterobacteriaceae resistant to third generation cephalosporins, including ESBL-producing *E. coli*, within community settings in Argentina.[4] To date, no studies have investigated the prevalence of these multidrug-resistant bacteria in pregnant patients. Therefore, the aim of this study was to determine the prevalence of vaginal and perianal carriage of Enterobacteriaceae resistant to third generation cephalosporins in pregnant women.

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Methods

Setting, patient selection, and collection of surveillance specimens.

The study was conducted at the Laboratorio Hidalgo, Buenos Aires, Argentina, from August 2012 to November 2012. Consecutives perianal and vaginal samples from 259 pregnant women at term (35–37 weeks gestational age) who had not been hospitalized within the previous 30 days, were processed for detection of *Streptococcus agalactiae* according to routine laboratory protocols. Additionally, vaginal samples were screened for the presence of Gram-negative bacilli, and perianal samples were screened for the presence of Enterobacteriaceae resistant to

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third generation cephalosporins. Briefly, vaginal swabs were inoculated onto MacConkey agar plates and perianal swabs were inoculated onto CHROMagar ESBL (CHROMagar Company, Paris, France) plates, which were incubated for 48 hours at 35°C in aerobiosis. All colonies obtained were selected for subsequent characterization.

Identification and antimicrobial susceptibility testing of Enterobacteriaceae

The API 20 E system (bioMérieux, Marcy l'Étoile) was used for the biochemical identification of all Enterobacteriaceae.

Antimicrobial susceptibility testing of all isolates was performed using the Kirby–Bauer disk diffusion method. The minimal inhibitory concentration (MIC) of each isolate was determined using the Epsilometer test (AB Biodisk, Solna, Sweden).

For phenotypic detection of ESBL, an overnight culture suspension of the test isolate, adjusted to 0.5 McFarland's standard, was inoculated onto the surface of a Mueller Hinton agar plate. Cefotaxime (30 μg) and cefotaxime-clavulanic acid (30 μg/10 μg) disks were placed 20 mm apart on the agar. Similarly, ceftazidime (30 μg) and ceftazidime-clavulanic acid (30 μg/10 μg) disks were placed 20 mm apart. Plates were incubated for 24 hours at 37°C, and an increase of 5 mm or more in the zone diameter for an antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was interpreted as a positive result for ESBL production.[5]

Phenotypic detection of ESBLs in isolates harboring AmpC beta-lactamases was detected using the diffusion synergy test with cefepime (30 μg) and amoxicillin-clavulanic acid (20 μg/10 μg).[6]

Strains hyperproducing AmpC beta-lactamases were suspected when a MIC ⩾16 μg/ml for cefoxitin, a MIC ⩾16 μg/ml for cefotaxime, and a negative ESBL test were observed.

Results

Of the 259 vaginal samples analysed, 48 (18.5%) yielded Enterobacteriaceae strains, 37 (14.3%) of which were *E. coli*, seven (2.7%) were *Proteus mirabilis*, three (1.2%) were *Klebsiella pneumoniae*, and one (0.4%) was *Enterobacter cloacae*. The resistance rate of *E. coli* to ampicillin and gentamicin was 48.6 and 10.8%, respectively. Resistance to third generation cephalosporins was observed in two *E. coli* isolates (0.8% of all vaginal specimens) and this was due to the presence of ESBLs.

Of the 259 rectal samples, 27 yielded Gram-negative bacilli on CHROMagar ESBL plates. Overall, 19 samples (7.3% of all perianal specimens) yielded Enterobacteriaceae resistant to third generation cephalosporins. Species distribution, MIC values, and phenotypic characterization of beta-lactamases are shown in Table 1. *E. coli* represented the majority (78.9%) of the isolates recovered. A total of 14 *E. coli* ESBL-producers (from 5.4% of the pregnant women) were isolated and all of them had

Table 1 Characteristics of 19 Enterobacteriaceae strains recovered from CHROMagar ESBL plates that are resistant to third generation cephalosporins

		MIC (μg/ml)				Detection of ESBL		
Isolate		CTX	CAZ	FEP	Hyperproduction of AmpC	CTXCL/CTX	FEP-AMC	Resistance to antibiotics other than beta-lactams
VI/PI 1	*E. coli*	>256	4	8	−	+	+	NA-CIP-SXT-TET-CHL
VI/PI 2	*E. coli*	64	1	4	−	+	+	NA-SXT-TET
PI 3	*E. coli*	64	0.25	2	−	+	+	NA-CIP
PI 4	*E. coli*	>256	4	16	−	+	+	NA-CIP-SXT-TET-CHL
PI 5	*E. coli*	>256	2	16	−	+	+	TET
PI 6	*E. coli*	128	1	4	−	+	+	NA-TET
PI 7	*E. coli*	128	1	2	−	+	+	TET-CHL
PI 8	*E. coli*	>256	2	8	−	+	+	SXT-TET
PI 9	*E. coli*	32	1	2	−	+	+	
PI 10	*E. coli*	32	1	2	−	+	+	SXT-TET-CHL
PI 11	*E. coli*	>256	1	8	−	+	+	NA-SXT-TET
PI 12	*E. coli*	16	16	0.125	+	−	−	NA-SXT-TET
PI 13	*E. coli*	32	0.5	2	−	+	+	
PI 14	*E. coli*	64	1	2	−	+	+	NA-SXT-TET
PI 15	*E. coli*	128	1	2	−	+	+	
PI 16	*E. cloacae*	16	4	1	+	−	−	
PI 17	*E. cloacae*	>256	>256	16	+	−	+	NA-CIP-SXT-TET-CHL-GM
PI 18	*C. freundii*	>256	>256	2	+	−	−	
PI 19	*C. koseri*	>256	0.5	0.5	−	+	+	

CAZ, ceftazidime; CHL,chloranfenicol; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; GM, gentamicin; NA, nalidixic acid; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.
VI/PI,vaginal and perianal isolate; PI, perianal isolate.
CTXCL/CTX, ≥5-mm increase in the zone diameter for cefotaxime which were tested in combination with clavulanic acid vs. its zone when tested alone; FEP-AMC, synergy test with cefepime and amoxicilina clavulanic acid.
Hyperproduction of AmpC, MIC≥16 μg/ml for cefoxitin, and cefotaxime with a negative ESBL test.

MIC values for cefotaxime at least 32 times higher than those for ceftazidime, suggesting that they expressed a cefotaximase-type enzyme. The frequency of co-resistance among the *E. coli* ESBL-producing isolates was as follows: 71.4% were resistant to tetracycline; 50.0% were resistant to nalidixic acid; 21.4% were resistant to ciprofloxacin; 50.0% were resistant to trimethoprim sulfamethoxazole; 28.6% were resistant to chloramphenicol; and none were resistant to gentamicin and nitrofurantoin. Notably, three out of the 14 ESBL-producing *E. coli* were fully susceptible to non-beta-lactam antibiotics. An ESBL-producing *E. cloacae* strain was detected using the diffusion synergy test with cefepime and amoxicillin-clavulanic acid, and an ESBL-producing *Citrobacter koseri* strain with a similar phenotype to *E. coli* was recovered.

Discussion

The prevalence of vaginal *E. coli* colonization during pregnancy in the present study was 14.3%. The rate of colonization was similar in other studies and ranged from 7 to 19.9%.[7–10] Perinatal antibiotic usage was not associated with changes in resistance rates of *E. coli* and other pathogens.[11] The high rate of resistance to ampicillin (48.3%) and gentamicin (10.8%) in vaginal *E. coli* isolates likely reflects the increasing prevalence of *E. coli* resistant to antibiotics in Argentina, where the levels of resistance of ambulatory isolates to ampicillin and gentamicin ranged from 53 to 75% and from 7 to 18% , respectively.[12]

In the present study, a significant number of pregnant women were colonized with Enterobacteriaceae resistant to third generation cephalosporins (7.3%). ESBL-producing *E. coli* (found in 5.4% of the pregnant women) was the most prevalent organism. To our knowledge, no studies have determined the prevalence of vaginal or perianal colonization with multidrug-resistant bacteria in this population.

In 2008, Boyer-Mariotte *et al.*[13] reported a case of fatal neonatal meningitis caused by a CTX-M-15-producing *E. coli* strain. Meningitis caused by ESBL-producing *E. coli* via mother-to-neonate transmission was confirmed.[14] In addition, the introduction of community-acquired ESBL-producing strains into hospitals has been demonstrated.[15,16] Molecular characterization of the enzymes expressed by these bacteria was not performed in our study. However, the MIC values for cefotaxime and ceftazidime suggest that these strains produced a cefotaximase-type enzyme.

It is clear that a general screening of pregnant women for *S. agalactiae* is required. However, there are no recommendations for the screening of *E. coli*, probably because it is assumed that *E. coli* strains within community settings are fully susceptible to third generation cephalosphorins. The prevalence of neonatal meningitis in Argentina caused by third-generation-cephalosporin-resistant *E. coli* is unknown. High resistance rates to third-generation cephalosporins were detected among Gram-negative bacilli isolated from Latin American medical centers enrolled in the SENTRY Antimicrobial Surveillance Programs. ESBL rates were 18.1% among *E. coli* and 60.4% among *Klebsiella* spp. from Argentina.[17] Further studies are necessary to identify risk factors for the acquisition of third-generation-cephalosporin-resistant Enterobacteriaceae in pregnant women. A recent study of healthy participants attending an infection control symposium showed that pet animals and foreign travel are risk factors for colonization with ESBL-producing *E. coli*.[18] Screening strategies designed to monitor for ESBL-producing *E. coli* are not recommended in all pregnant women. In spite of this, these strategies could be useful in endemic areas to prevent perinatal transmission and the introduction of multiresistant strains to the maternity ward. In conclusion, the results of this study indicate that colonization with cefotaxime-resistant *E. coli* is significant among pregnant women in Argentina. We believe that collecting and collating local epidemiological data is important if we are to track and monitor the spread of resistant strains in community settings.

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