ORIGINAL ARTICLE

Vaginal Colonization by *Escherichia coli* in Pregnant Women at King Abdulaziz University Hospital, Jeddah, Saudi Arabia

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Submission:23 April 2017Accepted:28 May 2017

Citation

Jamal TY, Younus HA, Abduljabbar HS, Fatani AA, Makled AF. Vaginal colonization by *Escherichia coli* in pregnant women at King Abdulaziz University Hospital, Jeddah, Saudi Arabia. JKAU Med Sci 2017; 24 (3): 39-49. DOI: 10.4197/Med. 24.3.4

Abstract

Maternal and neonatal infections by Escherichia coli remain a challenging problem for obstetricians and pediatricians. This study aims to determine the prevalence of vaginal colonization by *Escherichia* coli among pregnant women attending the Antenatal Clinics at King Abdulaziz University Hospital in Jeddah- Saudi Arabia, and to investigate the susceptibility of isolated *Escherichia coli* to the most commonly used antimicrobials. In this study, 100 pregnant women in the last trimester and 25 non-pregnant women were screened for vaginal colonization of *Escherichia coli*. The suspected colonies were identified by conventional methods and were confirmed by automated technology "Vitek 2 System". Also, antimicrobial susceptibility tests were done by the same methods". Escherichia coli isolates were serotyped to detect the presence of K1 antigen. Out of 100 pregnant women in the last trimester; twenty-one (21%) were Escherichia coli positive. Also, out of 25 non-pregnant women; four (16%) were Escherichia coli-positive. All of the isolated Escherichia coli were susceptible to most antimicrobial agents. The percentage of vaginal Escherichia coli resistance to trimethoprim/sulfamethoxazole, ampicillin and piperacillin ranged from 38.1% to 42.9%. The virulence factor K1 antigen was demonstrated in 42.9% of Escherichia coli -positive pregnant women.

Keywords

Escherichia coli K1 strains; Vaginal colonization; Pregnant women; Saudi Arabia

Introduction

scherichia coli (*E. coli*) and group B streptococcus are the most common causative pathogens of neonatal sepsis and meningitis which result in neonatal morbidity and mortality^[1,2]. Escherichia coli is a common vaginal microflora of pregnant and nonpregnant women and has been identified as a vaginal colonizer in 24-31% of pregnant and 9-28% in nonpregnant women^[3]. Previous studies explained that the high prevalence of E. coli in vaginal colonization is due to the proximity of the vagina to anus, the large numbers of E. coli constantly shed in the female's own fecal flora and due to the functional changes that occur in pregnant females^[4,5]. Escherichia coli strains involved in neonatal infections originate from the vagina, which is colonized by the rectal source^[6]. Vaginal colonization by E. coli can cause ascending infections, usually after rupture of membranes, leading to preterm birth and other maternal and fetal morbidities^[3,7]. Many complications of E. coli infections can occur such as urinary tract infections, low birth weight neonates, early-onset neonatal sepsis and meningitis^[3,7]. According to the "National Neonatal Perinatal Data (NNPD) 2002-2003", the incidence of neonatal sepsis that caused by different microorganisms is 30/1000 live births^[8]. Some reports in Libya, Egypt, Jordan, and Iraq demonstrated that E. coli and other gram negative organisms were the causative pathogens in 65%-90% of all neonatal sepsis cases^[9]. Bassuni et al.^[10] found that E. coli is the predominant organism causing neonatal sepsis in the Asir region of Saudi Arabia. Escherichia coli vaginal colonization in pregnant women was found to be 10.8% in Nigeria and 15% in Spain; while it was found to be 13.7% in Pakistan, 9.2% in Nigeria and in 12% in Spain in non-pregnant women^[11-13]. The neonates acquire E. coli infections from the maternal birth canal before or during delivery leading to earlyonset neonatal septicemia within the first week of life^[1,14].

Approximately 80% of the cases of *E. coli* neonatal meningitis are caused by strains possessing K1 capsular polysaccharide^[15]. Some studies identified antimicrobial resistant vaginal *E. coli* strains in pregnant women; 85% of them were resistant to at least one drug, 30% were multi-drug resistant, 42.5% were extended spectrum beta-lactamase-producers. So, detection and proper management for these resistant strains during pregnancy can reduce the morbidity and mortality in neonates^[16].

This study aims to determine the prevalence of *E. coli* vaginal colonization among pregnant women attending the Antenatal Clinics at King Abdulaziz University Hospital (KAUH), to compare *E. coli* colonization in these pregnant and non-pregnant women and to investigate the susceptibility of isolated *E. coli* strains to the most commonly used antimicrobial agents.

Materials and Methods

This study was planned and designed as a casecontrol study and was carried out during the period from September 2012 to September 2013 at KAUH in Jeddah, Saudi Arabia. High vaginal swabs (HVSs) were collected from 100 pregnant women during the last trimester of the pregnancy (Group 1) and from 25 non-pregnant women (Group 2). Age of these women ranged from 18 to 45 years who attended the outpatient clinics of the Department of Obstetrics and Gynecology. Questionnaires regarding full history of these women were filled out. The collected vaginal swabs were transported to the Clinical and Molecular Microbiology Laboratory (at KAUH) in Stuart's transport media "(Nouva Aptaca S.r.l., Canelli, Italy)" immediately. These swabs were cultured on MacConkey's agar plates "(Saudi Prepared Media Laboratory Ltd. Co., Jeddah, Saudi Arabia)" and incubated for 24-48 hours at 37°C aerobically. The suspected E. coli colonies were identified by conventional methods^[17] including; colony appearance, Gram staining and series of standard bacteriological tests^[18]. "Vitek 2 (bioMérieux Inc., St. Louis, MO, USA)" was used for confirmation of the E. coli isolates colorimetrically. Also, antimicrobial susceptibility testing was done by "Vitek 2 using ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefuroxime, cefotaxime, cefepime, meropenem, amikacin, gentamicin, ciprofloxacin, nitrofurantoin and trimethoprim/ sulfamethoxazole as per Clinical Laboratory Standard Institute (CLSI) Standards and Guidelines" (CLSI., Wayne, PA USA). The E. coli isolates were serotyped by utilizing "BD Directigen™ Meningitis Latex Test System (Becton, Dickinson and Co., Franklin Lakes, NJ USA)" which is a presumptive" latex agglutination test" to detect K1 capsular polysaccharide antigen in the isolates.

This study was approved by the Research Ethics Committee of the Faculty of Medicine, King Abdulaziz University (Reference Number: 823-12). Data were collected, tabulated and statistically analyzed using SPSS for Windows, version 16 (SPSS Inc., Chicago, IL USA). Significance was made using *chi-square* test for non-parametric parameters and "student's" *t* test for parametric parameters.

Results

Out of 100 pregnant and 25 non-pregnant women; 21% and 16% were positive for *E. coli* vaginal colonization respectively with significant difference between them. The demographic and clinical data of pregnant and non-pregnant women are shown in Table 1 where the mean age of pregnant and non-pregnant women was 30.73 ± 5.93 and 32.36 ± 6.40 , respectively without significance difference (P = 0.229). There was a significant difference between pregnant and non-pregnant in both Saudi and non-Saudi women (P = 0.0001). All pregnant and non-pregnant women in this study were from urban areas. The most common co-morbidity was urinary tract infections in pregnant women (18%) and in non-pregnant women (16%). Further detailed analysis of the clinical data with the significance shown on Table 1

The most common co-morbidity in *E. coli*-positive and *E. coli*-negative pregnant women was urinary tract infections during the current study (28.6% and 15.2%, respectively) as shown in Figure 1 but without significant difference (P = 0.137). The other comorbidities such as *diabetes mellitus* and hypertension were higher in *E. coli*-positive than *E. coli*-negative pregnant women (Fig. 1). Further comparative analysis and significance shown on Table 2

The history of previous premature rupture of membranes (PROM) in pregnant women was 4.8% in *E. coli*-positive and 1.3% in *E. coli*-negative with no significant difference (P = 0.378) as shown in Figure 2. The history of previous neonatal deaths were 23.8% in *E. coli*-positive and 5.1% in *E. coli* negative pregnant women with significant difference (P = 0.019) as shown in Figure 3.

Table 3 shows the antimicrobial susceptibility patterns of the isolated *E. coli* strains in pregnant women. It was found that all *E. coli* isolates (100%) were susceptible to "amoxicillin- clavulanate, piperacillin-tazobactam, cefuroxime, cefotaxime, cefepime, meropenem, amikacin, gentamicin, ciprofloxacin and nitrofurantoin". Meanwhile, 12 (57.1%) of *E. coli* isolates were sensitive to ampicillin and piperacillin. Thirteen (61%) of the isolates were sensitive to trimethoprim\sulfamethoxazole.

Figure 4 shows the results of screening for serotype *E. coli* K1 in pregnant women. The percentage of *E. coli* K1 strains in pregnant women was 42.9%. There was insignificant difference in pregnant and non-pregnant women regarding K1-positivity (P = 0.083). Further detailed analysis and significance on pregnant and nonpregnant shown on Table 4.

Discussion

Escherichia coli is one of the most common enteric microorganisms which is found frequently in the female genital tract^[12]. This microorganism possesses several virulence factors that allow it to cause vaginal colonization and has been implicated in different infections in pregnant women, mostly in urinary tract infections^[12]. Maternal and neonatal infections caused by *E. coli* remain a challenging problem for obstetricians and pediatricians because of the risk of early onset neonatal sepsis and meningitis caused by this organism which is obtained from the mother's birth canal before or during delivery^[14]. Therefore, it is useful to screen pregnant women for genital tract colonization by *E. coli* during the prenatal period^[19].

In this study, out of 100 pregnant women and 25 non-pregnant women (control group); 21 (21%) and 4 (16%) were E. coli-positive, respectively with significant difference between them (P = 0.001). Higher results of vaginal colonization by E. coli in pregnant women were recorded by Al-Mayahie^[20] in Irag and August^[21] in Tanzania (37.7% and 30%, respectively). Moreover, similar results of vaginal colonization by E. coli in pregnant women (19.9%) were reported by Barcaite et al.,^[22] in Lithuania. On the other hand, lower results were found by Al-Inany et al., [23] in Egypt (17%), Devi et al., ^[16] in India (16.2%), Hamedi et al., ^[24] in Iran (15%), Guiral et al.,^[12] in Barcelona, Spain (15%), Villar et al.,^[6] in Argentina (14.3%), Akerele et al.,^[25] in Nigeria (13.5%), Basu et al.,^[8] in India (11.2%), Zaria et al.,^[11] in Nigeria (10.8%), Agbakoba et al.,[26] in Nigeria (8.3%) and Ghartey et al.,^[27] in USA (10%). However, the lowest results were found in some studies that were done by Budisan and Ilie^[28] in Romania (5.9%) and by Obata-Yasuoka et al.,^[3] in Japan (3.4%) and by Wong^[29] in Hong Kong (2.4%). Rates of *E. coli* colonization in vagina may vary greatly according to the sample source, the culture medium used, the ethnic group, geographical location, socioeconomic status, immunological factors and the age of the population investigated^[30].

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Parameters	Pregnant	Non-Pregnant	Significance	
	(No. = 100)	(No. = 25)	(P. value)	
Age (Years)	30.73 ± 5.93 (18.00-42.00) 32.36 ± 6.40 (23.00-45.00)		0.229	
Nationality			0.283	
Saudi	48 (48.00%)	48 (48.00%) 15 (60%)		
Non-Saudi	52 (52%)	10 (40%)	0.0001	
Residency				
Urban	100 (100%)	25 (100%)	1.000	
Rural	U	0	-	
Co-Morbidity	7 (70/)	1		
Diabetes mellitus	/ (/%)	-	-	
Urinary Tract Infections	18 (18%)	4 (16%)	0.814	
Smoking	1 (1%)	2 (8%)	0.041	
Hypertension	/ (/%)	-		
Previous Pre-eclampsia	2 (2%)	1 (4%)	0.559	
Cancer	1 (1%)	1 (4%)	0.285	
Immunodeficiency	2 (2%)	1 (4%)	0.559	
Contraceptive uses	/9 (/9%)	13 (52%)	0.048	
Loop	10 (10%)	2 (8%)	0.021	
Pills	29 (29%)	6 (24%)	0.0001	
Others (Condoms)	40 (40%)	6 (24%)	0.0001	
Antibiotics Usage	13 (13%)	4 (16%)	0.696	
Types of Antibiotic				
Amoxicillin/Clavulanate	11 (11%)	2 (8%)	0.257	
Azithromycin	-	1 (4%)	-	
Clindamycin	-	1 (4%)	-	
Amoxicillin	2 (2%)	-	-	
Duration of Antibiotic (days)	3.92 ± 2.75 (1.00-7.00)	15.25 ± 11.12 (3.00-30.00)	0.003	
Menstrual Cycles			0.061	
Regular	90 (90%)	19 (76%)	0.0001	
Irregular	10 (10%)	6 (24%)	0.371	
Obstetric History				
Gravidity	3.77 ± 2.63 (1.00-14.00)	3.08 ± 2.55 (0.00-9.00)	0.240	
Parity	2.35 ± 2.26 (0.00-13.00)	2.48 ± 1.98 (0.00-7.00)	0.793	
Abortion	0.47 ± 0.90 (0.00- 5.00)	0.68 ± 1.03 (0.00-4.00)	0.314	
Premature Rupture of Membranes	2 (2%)	2 (8%)	0.127	
Weeks of Gestation (weeks)	33.67 ± 3.88 (25.00-40.00)	-	-	
Mode of Previous Deliveries			0.295	
Normal Vaginal Delivery	58 (58%)	16 (64%)	0.0001	
Cesarean Section	17 (17%)	1 (4%)	0.0001	
Normal Vaginal Delivery and Cesarean	9 (9%)	4 (16%)		
Section	16 (16%)	4 (16%)		
Primigravida				
Previous Neonates			0.057	
Full lerm	95 (95%)	21 (84%)	0.0001	
Preterm	5 (5%)	4 (16%)	0.527	
Neonatal Deaths	9 (9%)		0.119	
Previous Neonatal Diseases			0.073	
Septicemia	-	1 (4%)	-	
Low Birth Weight	1 (1%)	1 (4%)	1.000	
Escherichia coli Isolation			0.576	
Positive	21 (21%)	4 (16%)	0.001	
Negative	79 (79%)	21(84%)	0.0001	

Table 1. Demographic and clinical data of all participants (pregnant and non-pregnant women).

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Figure 1. The distribution of *diabetes mellitus*, urinary tract infections and hypertension in *Escherichia coli* - positive and *Escherichia coli* - negative pregnant women. (*E. coli: Escherichia coli*).

 Table 2. Co-morbidity of vaginal Escherichia coli - positive and negative in pregnant women).

Daramotors	E. coli-positive	E. coli-negative	Significance
Parameters	(No.= 21)	(No.= 79)	(P. value)
Co-morbidity:			
Diabetes mellitus	2 (9.5%)	5 (6.3%)	0.454
Urinary tract infections	6 (28.6%)	12 (15.2%)	0.137
Smoking	-	1 (1.3%)	-
Cancer	-	1 (1.3%)	-
Previous Pre-eclampsia	1 (4.8%)	1 (1.3%)	0.378
Hypertension	2 (9.5%)	5 (6.3%)	0.454
Immunodeficiency	-	2 (2.5%)	-
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E. coli: Escherichia coli



Figure 2. The distribution of history of previous premature rupture of membranes in vaginal *Escherichia coli*- positive and negative pregnant women. (*E. coli: Escherichia coli*).

Antimizzahiel Azent	<i>Escherichia coli</i> Isolates (No. = 21)			
Antimicrobial Agent	Susceptible (S)		Resistant (R)	
	No.	(%)	No.	(%)
Beta-lactam Drugs:				
Ampicillin	12	57.1	9	42.9
Piperacillin	12	57.1	9	42.9
Meropenem	21	100	0	0
Cefuroxime	21	100	0	0
Cefotaxime	21	100	0	0
Cefepime	21	100	0	0
Beta-lactam drugs/Beta-lactamase Inhibitors:				
Amoxicillin/Clavulanate	21	100	0	0
Piperacillin/Tazobactam	21	100	0	0
Aminoglycosides:				
Amikacin	21	100	0	0
Gentamicin	21	100	0	0
Quinolones:				
Ciprofloxacin	21	100	0	0
Nitrofurantoin	21	100	0	0
Trimethoprim/Sulfamethoxazole	13	61	8	38.1

Table 3. Antimicrobial susceptibility pattern of Escherichia coli strains isolated from pregnant women.



Figure 3. The distribution of history of previous neonatal deaths in vaginal *Escherichia coli*- positive and negative pregnant women. (*E. coli: Escherichia coli*).

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Parameters	Pregnant Women (No. = 21)		Non-Pregnant Women (No. = 4)		Significance (P. value)
	No.	(%)	No.	(%)	
Escherichia coli K1- positive	9	42.9%	3	75%	0.083
Escherichia coli K1- negative	12	57.1%	1	25%	0.002

Table 4. The serotyping of Escherichia coli K1 in pregnant and non-pregnant women.



Figure 4. The distribution of vaginal Escherichia coli K1 and non-K1 Escherichia coli in pregnant women. (E. coli: Escherichia coli).

Escherichia coli is the most common cause of urinary tract infections (UTIs) among pregnant women, and UTIs are most frequent during pregnancy and can cause maternal-fetal morbidities including premature rupture of membranes, preterm birth, low birth weight, neonatal sepsis and perinatal deaths^[31]. The present study revealed that the percentage of UTIs in *E. coli*-positive pregnant women (28.6%) was more than in *E. coli*-negative pregnant women (15.2%) with insignificant difference (P = 0.137).

Vaginal colonization by *E. coli* during pregnancy is strongly associated with PROM and is the most prevalent pathogen of neonatal sepsis in the neonates born after PROM^[32,33]. In this study, the percentage of the history of previous premature rupture of membranes in *E. coli*-positive pregnant women (4.8%) was higher than in *E. coli*-negative pregnant women (1.3%) without significant difference (P = 0.378).

Vaginal colonization by *E. coli* is a common causative agent for life-threatening neonatal infections and it was detected in 21% of fetuses that have died

during the third trimester of pregnancy. Thus, *E. coli* infections have been identified as the most common risk factor of death in neonates^[34]. In this study, the prevalence of history of previous neonatal deaths in *E. coli*-positive pregnant women (23.8%) was higher than in *E. coli*-negative pregnant women (5.1%) with significant difference (P = 0.019); this could explain the relationship between vaginal *E. coli* colonization and neonatal deaths.

In this study, antimicrobial susceptibility tests were done by "VITEK 2" system where they showed that all the *E. coli* isolates (100%) were susceptible to "meropenem, cefuroxime, cefotaxime, cefepime, amoxicillin/clavulanate, piperacillin/tazobactam, amikacin, gentamicin, ciprofloxacin and nitrofurantoin". Meanwhile, 42.9% of *E. coli* isolates were resistant to "ampicillin and piperacillin" and 38.1% were resistant to "trimethoprim\ sulfamethoxazole". Some investigators reported same result as: Akerele *et al.*,^[25] who found that all *E. coli* isolates (100%) were sensitive to ciprofloxacin; Barcaite *et al.*,^[22] in Lithuania found that all *E. coli* isolates (100%) were susceptible to" cefuroxime,

cefotaxime, amikacin and ciprofloxacin". In agreement with this result, Al-Mayahie^[20] in Iraq recorded that all E. coli isolates (100%) were susceptible to meropenem and 31.5% of them were resistant to trimethoprim/ sulfamethoxazole. On the other hand, Al-Mayahie^[20] also reported different results that all E. coli isolates (100%) were resistant to "amoxicillin/clavulanate and cefotaxime" and 36.8%, 31.5% of the isolates were resistant to "gentamicin and ciprofloxacin" respectively. In partial agreement with the present results, Barcaite et al.,^[22] in Lithuania found that all of the E. coli isolates (100%) were susceptible to "cefuroxime, cefotaxime, amikacin, and ciprofloxacin"; while they observed resistance to "ampicillin (25.9%), piperacillin (16.6%), ampicillin/sulbactam (7.8%), gentamicin (2.6%) and piperacillin/tazobactam (1.0%)". The present findings were in the same ranges as that reported by Zai et al.,^[35] in Pakistan who found that 77.7% of the isolates were susceptible to "gentamicin and 14.8% were susceptible to ampicillin". Some experts consider ciprofloxacin contraindicated during pregnancy especially during the first trimester^[36].

Escherichia coli strains that possess K1 capsular polysaccharide antigen can cause 40% of neonatal sepsis and 80% of neonatal meningitis^[37]. The virulence of *E. coli* K1 strains is referred to the ability of the K1 capsular polysaccharide to repel phagocytic cells, inactivate complement system, resist antibody-independent serum, cross the blood–brain barrier (BBB) and invade the brain microvascular endothelial cells^[37]. In this study, vaginal *E. coli* K1strains isolated from pregnant women was 42.9%. Also, a similar result by Obata-Yasuoka *et al.*,^[3] in Japan who found that *E. coli* K1 in pregnant women was 40%. However, a lower result was detected in a study done by Kaczmarek *et al.*,^[37] in Poland who found that 20.9% of pregnant women were *E. coli* K1-positive.

Conclusion

The prevalence of the vaginal colonization by *E. coli* among pregnant women during the third trimester at KAUH in Jeddah, Saudi Arabia was 21%. Out of them, 28.6% had urinary tract infections during their current pregnancies. Risk factors such as a history of previous premature rupture of membranes and previous neonatal deaths in these pregnant women were found to be 4.8% and 23.8% respectively. The virulent *E. coli* K1 strains were demonstrated in 42.9% of *E. coli*-

positive pregnant women. The percentage of vaginal *E. coli* resistance to trimethoprim/sulfamethoxazole, ampicillin and piperacillin ranged from 38.1% to 42.9%.

Recommendations

- 1. All women with a history of risk factors (such as urinary tract infections, premature rupture of membranes, neonatal sepsis, neonatal meningitis, and neonatal deaths) during their previous pregnancies should be screened for vaginal *E. coli* colonization.
- 2. All pregnant women should be screened for asymptomatic UTIs to decrease the risk of preterm labor and other complications.
- 3. Intrapartum antimicrobial prophylaxis can be given to pregnant women with vaginal colonization by *E. coli* in some high-risk cases.
- 4. Further studies should be carried out to investigate the transmission of vaginal *E. coli* from the pregnant women to their neonates.

Conflict of Interest

The authors have no conflict of interest.

Disclosure

None of the authors received any type of commercial support either in forms of compensation or financial for this study. They have no financial interest in any of the products or devices, or drugs mentioned in this article.

Ethical Approval

Obtained.

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استعمار الإشريشية القولونية للمهبل عند النساء الحوامل في مستشفى جامعة الملك عبد العزيز بجدة، المملكة العربية السعودية

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المستخلص. الإلتهابات الإشريشية القولونية لدى الأمهات الحوامل والمواليد تمثل مشكلة لأطباء الأطفال والنساء والتوليد وذلك لما يمكن أن تسببه هذه البكتيربا من الإنتان الجرثومي والتهاب السحايا للمواليد. تهدف هذه الدراسة إلى استقصاء وجود استعمار الإشريشية القولونية للمهبل في الأثلوث الأخير من الحمل، وتحديد العقاقير المضادة للميكروبات بإجراء اختبارات حساسية البكتيريا للمضادات الحيوية. تَم التحري عن استعمار الإشريشية القولونية للمهبل عند ١٠٠ امراً وحامل في الأثلوث الثالث من الحمل و٢٢ امراً هَ غَيْرَ حامل، تَمَّ الحصول عن استعمار الإشريشية القولونية للمهبل عند ١٠٠ امراً وحامل في الأثلوث الثالث من الحمل و٢٢ امراً هَ غَيْرَ حامل، تَمَّ الحصول عن استعمار الإشريشية القولونية للمهبل عند ١٠٠ امراً وحامل الإشريشية القولونية. وقد تم تحديد الأنماط المصالية بالكشف عن وجود المستَّضد ٢١ في العزلات البكتيرية. كان مُعدَّل التشريشية القولونية. وقد تم تحديد الأنماط المصالية بالكشف عن وجود المستَّضد ٢١ في الوزلات البكتيرية. كان مُعدَل التشريشين المتعمار الإشريشية للمولونية للمهبل ٢١٪ عِند الحوامل و٦١٪ عند غير الحوامل. وورجد في هذه الدراسة أن جميع المهريشينية القولونية. وقد تم تحديد الأنماط المصالية بالكشف عن وجود المستَضد ٢١ في العزلات البكتيرية. كان مُعدَل التشار استعمار الإشريشية القولونية للمهبل ٢١٪ عِند الحوامل و٢١٠ عند عنه ومعند المتعمان وورجد في هذه الدراسة أن عميع العزلات كانت حساسية ليميع المصادات الحيوية للميكروبات الشائعة الاستعمال (مثل الميروبينيم، والسيفوتكسيم) ما عد العرولية المهبيلين، والبيبير اسيلين، والتريميثوبر يم/السلفاميثوكسازول. ووجد أن مُعدَل إنتشار العزلات من النوع ٢٨