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Full Length Research Paper

Antimicrobial susceptibility of *Escherichia coli* and other coliforms isolated from urine of asymptomatic students in Bayelsa State, Nigeria

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This study investigated the isolation rate and antimicrobial susceptibility of *Escherichia coli* and other coliforms from asymptomatic male and female students of Niger Delta University in Bayelsa State, Nigeria. *E. coli* and other coliforms from midstream clean-catch urine samples of asymptomatic male and female students were isolated and tested for their susceptibility to commonly used antimicrobial agents using the disk diffusion protocol described by the Clinical Laboratory Standards Institute (CLSI). Of the few subjects that harbored *E. coli*, more were males. Zone sizes for both isolate groups from males were higher than those from females. *E. coli* were more susceptible to the antimicrobials than the non - *E. coli* (unclassified coliform) isolates for both subjects, although the overall susceptibility of both isolate groups was poor. Gentamicin was the most active (64.5% for *E. coli* and 33.3% for unclassified coliforms) while tetracycline was the least (22.7% for *E. coli* and 0% for unclassified coliforms). The most common resistance phenotypes were "ATCtGSNa" (for *E. coli*) and "ATCtGSNaNC" (for unclassified coliforms); "ATCtGSNaNC" was observed in both isolate groups. Multiple antibiotic resistances were observed significantly in both *E. coli* (83.9%) and the unclassified coliforms (100%). As against 9.7% of the *E. coli* isolates, 40% of the unclassified coliforms were resistant to all the antimicrobials. MAR indices were very high (all above 0.2) in both isolate groups. Although asymptomatic male students of Niger Delta University harbored more *E. coli* than the female students, isolates from the female students pose greater risk of antimicrobial resistance owing to their lower susceptibility to antimicrobials compared with those from their male counterparts. The prior exposure of all the isolates to antibiotics as suggested by their high MAR indices provides justification for continuous monitoring of bacterial susceptibility to antibiotics before prescription in order to ensure adequate treatment of infections arising from urinary pathogens and reduction in the spread of bacteria resistant strain.

Key words: *Escherichia coli*, urine, asymptomatic, antimicrobial susceptibility.

INTRODUCTION

Urine contains a variety of fluids, salts and waste products; it usually does not have bacteria (Adult Health Advisor, 2005). When bacteria get into the bladder or kidney and multiply in the urine, they cause a urinary tract infection (UTI), the most common type being a bladder infection often called cystitis characterized by a syndrome involving dysuria, frequency, urgency and occasionally suprapubic tenderness (Akram et al., 2007). The presence of symptoms of lower tract without upper tract symptoms does not exclude upper tract infection, which is also often present (Sobel and Kaye, 2000). However,

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bacteria found in the urinary tract of older adults, without symptoms or associated consequences often referred to as asymptomatic bacteria, is also a well recognized phenomenon which may not require antibiotics. Asymptomatic bacteriuria occur reliably more frequently in females as compared with males and it is a major criterion of urinary tract infection (Nurullaev, 2004).

Bacterial infections of the urinary tract in humans are the most frequent bacterial disease, affecting outpatients, hospitalized patients and apparently healthy populations; and more common in females than males by virtue of the shortened urethra (Piatti et al., 2008; Todar, 2008; Sheffield and Cunningham, 2005; Olaitan, 2006). Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars (Gonzalez and Schaeffer, 1999). Risk factors for UTIs include diabetes, sickle cell disease, anatomical malformation of the urinary tract, poor toilet habits, pregnancy in women and prostrate enlargement in men (Wikipedia, 2009: http://en.wikipedia.org/wiki/).

UTIs are often treated with different broad-spectrum antibiotics even when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms. Fluoroquinolone are preferred as initial agents for empiric therapy of UTI in areas where resistance is likely to be of concern (Biswas et al., 2006; Schaeffer, 2002). This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance among most common uropathogens (Tankhiwale et al., 2004; Gupta et al., 2002; Goldstein, 2000).

Escherichia coli is recognized as one of the most frequently isolated bacteria in asymptomatic bacteriuria and UTIs (Stamm, 1994; Todar, 2008). *E. coli* is the predominant facultative anaerobe of the human colonic microflora; most *E*. *coli* strains are harmless to humans, but pathogenic strains can cause gastroenteritis, urinary tract infections and neonatal meningitis; and in rare cases, hemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pneumonia (Todar, 2008). Uropathogenic *E*. *coli* (UPEC) cause 90% of the urinary tract infections (UTIs) in anatomically-normal, unobstructed urinary tracts; the bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder (Todar, 2008). A typical patient with uncomplicated cystitis is a sexually-active female who was first colonized in the intestine with an uropathogenic *E*. *coli* strain that was later propelled into the bladder from the periurethral region during sexual intercourse.

No report is available on the isolation frequency and antimicrobial susceptibility of *E*. *coli* from the student population: a sexually active age group, of the Niger Delta University in Bayelsa State, Nigeria. It was therefore thought necessary to investigate the isolation frequency of *E*. *coli* and other coliforms in asymptomatic male and female undergraduate students of the University; and also to study the effects on the bacteria isolated of commonly used antimicrobial agents in the area.

MATERIALS AND METHODS

Sample collection

A total of 240 midstream clean-catch urine samples (120 each from male and female) were collected between June and October 2008 into sterile containers from asymptomatic students of Niger Delta University in Bayelsa State, Nigeria at the two campuses (College of Health Sciences and the Main Campus). Only students of age 18 - 35 years (males) and 15 - 30 years (females) and who were not on antimicrobial therapy as at sample collection or had not taken antimicrobial two weeks prior to sampling time were included in this study.

Isolation and identification of *E. coli* and other coliforms from urine

Urine samples were immediately (or within 6 h of collection) inoculated on MacConkey agar (Fluka Biochemical, Germany) prepared according to the manufacturer's instruction and incubated aerobically at 37°C for 24 h. Pink colonies from the MacConkey agar were further sub-cultured on eosin methylene blue (EMB) agar (International Diagnostics Group, UK) prepared according to the manufacturers instruction and incubated at 37°C for 24 h. Colonies that had metallic sheen on EMB were presumptively taken as *E*. *coli* and further characterized microscopically (as gram- negative rod) and biochemically (as Indole $^{(+)}$, Citrate $^{(-)}$ and Urea $^{(-)}$). The pink colonies from MacConkey agar that did not produce metallic sheen on EMB were designated as unclassified coliforms. Well isolated organisms were maintained on nutrient agar (Fluka Biochemical, Germany) prior to antimicrobial susceptibility testing.

Antimicrobial susceptibility test

Antimicrobial susceptibilities of the isolates to eight common antimicrobial agents were determined by the disc diffusion method for rapidly growing aerobic organisms in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI), formerly National Committee on Clinical Laboratory Standards (NCCLS) (CLSI, 2006). Briefly, four well isolated colonies from 24-h nutrient agar culture were transferred to tubes containing Mueller Hinton broth (Fluka Biochemical, Germany) and incubated at 37°C for 24 h. The bacterial suspension was adjusted using sterile saline (0.85% w/v NaCl: Scharlau, Brazil) to the turbidity of 0.5 McFarland standard (prepared by adding 0.5 ml of a 1.175% (w/v) of barium chloride dehydrate ($BaCl_2.2H_2O$: BDH Chemical Ltd, Poole, England) to 99.5 ml of 1% (v/v) sulphuric acid (H_2SO_4: Fluka Biochemical, Germany). The surfaces of Mueller Hinton agar (Fluka Biochemical, Germany) were streaked with the adjusted suspensions within five minutes of adjusting turbidity; and the inoculums were allowed to dry for five minutes. Multo disks (Abidec Company, England) containing ampicillin (25 µg), tetracycline (25 µg), Cotrimoxazole (25 µg), gentamicin (10 µg), streptomycin (25 µg), nalidixic acid (30 µg), nitrofurantoin (200 µg) and colistin (25 µg) were placed on the inoculated agar surfaces (in triplicates), allowed to stand for 15 min and then incubated in inverted position at 37°C for 24 h. The zones of inhibition were finally measured, including the diameter of the disk using a ruler to the nearest millimeter and recorded. A control organism such as *E*. *coli* ATCC 9637 (instead of ATCC 25922 due to its unavailability in the laboratory) was used to validate the accuracy of the antimicrobial susceptibility tests. Isolates were classified as "Resistant", "Intermediate susceptible" or "Susceptible" based on the standard

Table 1. Isolation rate of *E. coli* and unclassified coliforms.

Number of urine specimens screened	Number (%) positive for *E. coli*		Number (%) positive for Coliforms		Total isolation rate (%)	
	Male (n = 120)	Female (n = 120)	Male (n = 120)	Female (n = 120)	*Escherichia coli* (n = 240)	Coliforms (n = 240)
240	21 (17.5)	10 (8.3)	2 (1.7)	13 (10.8)	12.9	6.3

interpretation chart updated according to the current CLSI (formerly NCCLS) standard.

RESULTS

Bacteria isolated from urine

A total number of 31 (12.9%) *E*. *coli* and 15 (6.3%) unclassified coliforms was obtained from the 240 urine specimens screened (Table 1). As against generally held opinion, the isolation rate of *E*. *coli* obtained from our study was rather low; and more from male specimens. However, unclassified coliforms were more in the female urine samples. The precise identity of the unclassified coliforms was not further determined due to unavailability of confirmatory tests at the laboratory where the bench work was carried out. These isolates were gram-negative rods and lactose fermenting on MacConkey agar, yielding pink-colored colonies; some were large and mucoid. However, these isolates did not produce metallic sheen on EMB agar (and were therefore not *E*. *coli* species).

Antimicrobial susceptibility of the isolates

The measured inhibition zone diameters (in millimeters) and interpretation are as given in Table 2 (for *E*. *coli*) and Table 3 (for unclassified coliforms) . Zone sizes obtained for the isolates from male subjects were generally higher than those from female isolates for both isolate groups. The percentage susceptibilities (shown in Table 4) indicate that the *E*. *coli* were generally more susceptible to the antimicrobials tested than the non - *E*. *coli* (unclassified coliform) isolates, although the overall susceptibility of both isolate groups was poor. For the *E*. *coli*, susceptibility was in the order: gentamicin > streptomycin > nitrofurantoin > colistin > nalidixic acid > cotrimoxazole > ampicillin > tetracycline; and for the unclassified coliforms, the order of susceptibility was: gentamicin > nitrofurantoin and colistin > streptomycin and nalidixic acid > cotrimoxazole > ampicillin > tetracycline.

Distribution of resistance phenotypes in the isolates

The most common resistance phenotypes were “ATCtGSNa” (for *E*. *coli*) and “ATCtGSNaNC” (for unclassified coliforms) (Table 5). Resistance phenotypes observed only in *E*. *coli* were: “Na”, “AT”, “AC”, “CtN”, “ATN”, “TGNa”, “TNaC”, “NaNC”, “ATCtS, “ANaNC”, “ATCtSC”, “ATCtNaN”, “ATNaNC”, “TCtGNC”, “ATCtGSNa”, “ATCtSNC”, “ATCtSNaC”, “ATCtNaNC” and “TCtGSNaN”; those observed only in the unclassified coliforms were: “ATCtC”, “TNaNC”, “ATCtSNa”, “ATCtGSNaC”, “ATCtGSNaN” and “ATCtSNaNC”; and those shared by both isolate groups were: “ATC”, “ATCtGSN” and “ATCtGSNaNC”.

Multiple antibiotic resistance (MAR) in the isolates and MAR indices

Multiple antibiotic resistances (Table 6), defined here as resistance to at least three antibiotics, were observed significantly in both *E*. *coli* (83.9%) and the unclassified coliforms (100%). As against 9.7% of the *E*. *coli* isolates, 40% of the unclassified coliforms were jointly resistant to all the antimicrobials tested. MAR indices (Table 7) were very high, all above 0.2 in both isolate groups.

DISCUSSION

The very low isolation rate of *E*. *coli* obtained from our study is not in agreement with some previous reports that suggest *E*. *coli* as the most frequently isolated bacteria from urine in UTIs (Stamm, 1994; Todar, 2008).

Many urinary tract bacteria are capable of expressing resistance in one form or another. While colistin sulphate may be ineffective because of cross-resistance (Mordi and Erah, 2006), the higher resistances observed in the present study to the orally available and cheap drugs namely ampicillin, tetracycline, cotrimoxazole, nalidixic acid and nitrofurantoin, could be due to their free access, misuse and abuse. Some studies (Ehinmidu, 2003; Inabo and Obanibi, 2005; Mordi and Erah, 2006) have reported similar observation. The -lactam group of antibiotics is the most commonly used worldwide in human and veterinary medicine (Sanders and Sanders, 1992; Livermore, 1996), and this explains the many reported cases of ampicillin resistance in *E*. *coli* worldwide (Gruneberg, 1984; Lamikanra and Ndep, 1989; Manges et al., 2001; Ehinmidu, 2003; Xiao et al., 2005). The widespread and inappropriate use of antibiotics is re-cognized as a significant contributing factor to the spread

Table 2. Susceptibilities of *E. coli* to common antibiotics.

Isolates	Source	Antibiotic inhibition zone diameter (mm) and interpretation*																													
		Amp			Tet			Cot			Gen			Str			Nal			Nit			Col									
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
EC1	Male			18			22	0						26			24	0					17			15					
EC2	Male	2			2			2						16	0				18			16		6							
EC3	Male			18	14			9			12						20			14	12			8							
EC4	Male	12					16			20			14				18			14		16		8							
EC5	Male		16		12					17	11						15	13					20			10					
EC6	Male	1			1			2			2			6				1			16					10					
EC7	Male			18			17			16				18			19	11		14				8							
EC8	Male	1			2			2						19			16	7		0						10					
EC9	Male	3			2			1			3			4				1		14				8							
EC10	Male		16		1			1			2			2				1		1											12
EC11	Male	1			2			1						18	8				14			16		8							
EC12	Male	1			12				14					16		14		10		3				8							
EC13	Male	7					18			18				18			20	8		11				8							
EC14	Male	0			2			2			0			4				2					18			12					
EC15	Male	1			2			1						16	8				14			16				10					
EC17	Male	8			12			0						15			18	13		14				0							
EC18	Male	2			2			1			2			4				2			16										11
EC19	Male			22			17			18				16			21	12					17								18
EC20	Male	2			2			2			0			4				2					18			12					
EC21	Male		16				16	2						22			16		18	2											14
EC24	Male	10			12			10						18			16	12		14				8							
EC22b	Female	13			8				13			14					15		16	10						10					
EC23	Female	11			11					17				15		13			15				17								11
EC25	Female	9			11					19		13					17		15		15			8							
EC26	Female			19	11					17				17		13		11			15			7							
EC27	Female	5			2			0				14			5				15	0				7							
EC28	Female	0			0			0			5				0			0		0				0							
EC29	Female	0			0			0			0				1			0		0				7							
EC30	Female	9			12			0			12				9				15	13											11
EC31	Female	0			0			0				14			0			10					21	4							
EC32	Female		15				15			16				15			16	11		13				7							

*Interpretation was based on the standard interpretation chart updated according to the M2-A9 (9th editon) CLSI (formerly NCCLS) Standard; EC- *E. coli*; R- Resistance to the drug; I- Intermediate susceptibility to the drug; S- Susceptibility to the drug; 0- No inhibition zone of inhibition around the antibiotic disk; Amp- ampicillin; Tet- tetracycline; Cot- cotrimoxazole; Gen- gentamicin; Str- streptomycin; Nal- nalidixic acid; Nit- nitrofurantoin; Col- colistin.

of bacterial resistance and the development of resistance to antimicrobial agents (Mincey and Parkulo, 2001). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance (Granizo et al., 2000).

Over 50% susceptibility of *E. coli* to gentamicin and streptomycin observed in this study might be due to their requirement for parenteral administration which hinder their misuse and abuse. Resistance to the aminoglycosides by *E. coli* is also not new (Ngwai et al., 2005; Mordi and Erah, 2006; Olaitan, 2006). The observation that some isolates were resistant to streptomycin but not to gentamicin could be explained by the fact that gentamicin, in addition to binding to a specific S12 protein in the 30S ribosome, also binds to the L6 protein of the 50S ribosome to inhibit protein synthesis (Tripathi, 2003). Hence, a possible alteration of the S12 protein target alone in the streptomycin-resistant isolates is incapable of affecting its action.

The high level multiple resistances observed is probable indication of an earlier exposure of the MAR isolates to these drugs. This is suggested by the high MAR indices observed. An MAR index (a tool that reveals the spread of bacterial resistance in a given population)

Table 3. Susceptibilities of the unclassified coliforms to common antibiotics.

Isolate	Source	Antibiotic inhibition zone diameters (mm)																											
		Amp			Tet			Cot			Gen			Str			Nal			Nit			Col							
		R	I			S	R	I	S	R	I	S	R	I	S				R	I	S	R	I	S	R	I	S	R	I	S
C2	Male	1			3		2			10			10			2						18	8							
C3	Male			21	4			14			18			17		3			10				2						
C4	Female	1			1		1					17	3			0						17			9				
C5	Female	3			9		0			14					17		15				14		0						
C6	Female	0			0		0			9			0			0			11				8						
C7	Female	0			0		0			8			8			0			3				8						
C8	Female	0			0		0			14			0			9			0				1						
C9	Female	0			0		0			9			0				18		0					10					
C10	Female	10			0		0			11			0			12			0				8						
C11	Female	0			3		0			6			0			0			0				7						
C12	Female	0			5		0			0			0			10			0										11
C13	Female	0			1		0			11			0			0			5				8						
C14	Female	0			0		0			2			6			7			9										11
C15	Female	7			11			11			13			17				19			18		5						
C16	Female	1			0		0			9			0			11			0				8						

*Interpretation was based on the standard interpretation chart updated according to the M2-A9 (9th edition) CLSI (formerly NCCLS) Standard; C- unclassified coliforms (pink colonies from MacConkey agar which did not produce metallic sheen growth on eosin methylene blue [EMB] agar); R- Resistance to the drug; I- Intermediate susceptibility to the drug; S- Susceptibility to the drug; 0- No inhibition zone of inhibition around the antibiotic disk; Amp- ampicillin; Tet- tetracycline; Cot- cotrimoxazole; Gen- gentamicin; Str- streptomycin; Nal- nalidixic acid; Nit- nitrofurantoin; Col- colistin.

Table 4. Percentage susceptibilities of *E. coli* and unclassified coliforms to antibiotics.

Antibiotics	Disk content (µg)	Number (%) susceptible to drugs	
		***E. coli* (n = 31)**	**Coliforms (n = 15)**
Ampicillin (Amp)	25	9 (29)	1 (6.7)
Tetracycline (Tet)	25	7 (22.6)	0 (0)
Cotrimoxazole (Cot)	25	11 (35.5)	2 (13.3)
Gentamicin (Gen)	10	20 (64.5)	5 (33.3)
Streptomycin (Str)	25	17 (54.8)	3 (20)
Nalidixic acid (Nal)	30	13 (41.9)	3 (20)
Nitrofurantoin (Nit)	200	15 (48.4)	4 (26.7)
Colistin (Col)	25	14 (45.2)	4 (26.7)

Table 5. Distribution of the *E. coli* and unclassified coliforms into resistance phenotypes.

Resistance phenotypes	Number (%) of isolates with corresponding phenotypes	
	***E. coli* (n = 31)**	**Coliforms (n = 15)**
Na	1 (3.2)	0 (0)
AT	1 (3.2	0 (0)
AC	1 (3.2)	0 (0)
CtN	1 (3.2)	0 (0)
ATC	1 (3.2)	1 (6.7)
ATN	1 (3.2)	0 (0)
TGNa	1 (3.2)	0 (0)
TNaC	1 (3.2)	0 (0)
NaNC	2 (6.5)	0 (0)
ATCtS	1 (3.2)	0 (0)
ATCtC	0 (0)	1 (6.7)

Table 5. cont.

Resistance phenotypes	Number (%) of isolates with corresponding phenotypes	
	***E. coli* (n = 31)**	**Coliforms (n = 15)**
ANaNC	1 (3.2)	0 (0)
ATCtSC	2 (6.4)	0 (0)
TNaNC	0 (0.0)	1 (6.7)
ATCtNaN	1 (3.2)	0 (0)
ATCtSNa	0 (0)	1 (6.7)
ATNaNC	1 (3.2)	0 (0)
TCtGNC	1 (3.2)	0 (0)
ATCtGSNa	4 (12.9)	0 (0)
ATCtGSN	1 (3.2)	1 (6.7)
ATCtSNC	1 (3.2)	0 (0)
ATCtSNaC	1 (3.2)	0 (0)
ATCtNaNC	2 (6.5)	0 (0)
TCtGSNaN	1 (3.2)	0 (0)
ATCtGSNaC	0 (0)	1 (6.7)
ATCtGSNaN	0 (0)	2 (13.3)
ATCtSNaNC	0 (0)	1 (6.7)
ATCtGSNaNC	3 (9.7)	6 (40.0)

A = ampicillin; T = tetracycline; Ct = co-trimoxazole; G = gentamicin; S = streptomycin; Na = nalidixic acid; N = nitrofurantoin; C = colistin.

Table 6. Multiple antibiotic resistance (MAR) in *E. coli* and unclassified coliforms.

Number of antimicrobial agents isolate is resistant to	Number (%) of isolates with multiple resistance	
	***E. coli* (n = 31)**	**Coliforms (n = 15)**
3	6 (19.4)	1 (6.7)
4	2 (6.5)	2 (13.3)
5	5 (16.1)	1 (6.7)
6	10 (32.3)	1 (6.7)
7	0 (0)	4 (26.7)
8	3 (9.7)	6 (40)

above 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are used (Krumpermann, 1983).

Conclusion

Although in a small sample size, *E. coli* appears to be more prevalent in the asymptomatic male than female student population of Niger Delta University investigated. In addition, isolates from the female students pose greater risk of antimicrobial resistance owing to their lower susceptibility to antimicrobials compared with those from their male counterparts. The prior exposure of isolates to antimicrobial agents as suggested by their high MAR indices provides justification for continuous monitoring of bacterial susceptibility to antibiotics before prescription in order to ensure adequate treatment of infections arising from urinary pathogens and reduction in the spread of bacteria resistant strain. The emergence and spread of antimicrobial resistance is an important public health issue.

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Table 7. Multiple antibiotic resistance index (MAR-I) of *E. coli* and unclassified coliforms.

Isolates	Source	Number of antibiotics isolate is resistant to (a)	Number of antibiotics tested (b)	MAR-I (a/b)
EC2	Male	5	8	0.63
EC3	Male	5	8	0.63
EC5	Male	3	8	0.38
EC6	Male	6	8	0.75
EC7	Male	3	8	0.38
EC8	Male	5	8	0.63
EC9	Male	8	8	1.00
EC10	Male	6	8	0.75
EC11	Male	5	8	0.63
EC12	Male	5	8	0.63
EC13	Male	4	8	0.50
EC14	Male	6	8	0.75
EC15	Male	4	8	0.50
EC17	Male	6	8	0.75
EC18	Male	6	8	0.75
EC20	Male	6	8	0.75
EC24	Male	6	8	0.75
EC22b	Female	3	8	0.38
EC25	Female	3	8	0.38
EC26	Female	3	8	0.38
EC27	Female	6	8	0.75
EC28	Female	8	8	1.00
EC29	Female	8	8	1.00
EC30	Female	6	8	0.75
EC31	Female	6	8	0.75
EC32	Female	3	8	0.38
C2	Male	7	8	0.88
C3	Male	4	8	0.50
C4	Female	5	8	0.63
C5	Female	4	8	0.50
C6	Female	8	8	1.00
C7	Female	8	8	1.00
C8	Female	7	8	0.88
C9	Female	6	8	0.75
C10	Female	8	8	1.00
C11	Female	8	8	1.00
C12	Female	7	8	0.88
C13	Female	8	8	1.00
C14	Female	7	8	0.88
C15	Female	3	8	0.38
C16	Female	8	8	1.00

EC- *E. coli*; C- unclassified coliforms.

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