

Antibiogram Profile of Extraintestinal Pathogenic *Escherichia Coli* Clinical Isolates From Patients with Urinary Tract Infections at Kericho District Hospital, Kenya

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Abstract: *Background:* *Escherichia coli* is the common cause of primary urinary tract infections (UTIs). UTIs are the most common non-intestinal infection in women worldwide. Antimicrobial susceptibility testing provides information that allows physicians to select the 21 most appropriate antimicrobial agents for treating a specific infection. *Methods:* A cross-sectional study was conducted at Kericho District Hospital in and out patient's clinic between January and June 2015. A total of 133 urine samples were collected and used to investigate the prevalence of UTIs and to determine the antimicrobial profile of extraintestinal *E. coli* clinical isolates among in and out patients. UTIs were diagnosed using mid-stream urine culture on standard media. The bacterial isolates recovered were tested against trimethoprim-sulfamethoxazole, gentamycin, amikacin, ceftriaxone, ciprofloxacin, ceftiofloxacin, cefepim, imipenem, ampicillin and amoxicillin-clavulanic acid using Kirby-Bauer disc diffusion technique. Data was entered in MS Excel spread sheet and analyzed by using SPSS version 20. *Results:* Among the 133 samples examined, 38% had *E. coli* isolates with 64% of them being from female patients compared to 36% from men. Approximately 73% of the isolates were resistant to trimethoprim-sulfamethoxazole and 91% resistant to amoxicillin clavulanic acid; 96% were susceptible to imipenem and 82% amikacin. *E. coli* showed high sensitivity to imipenem and amikacin but resistant to trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid. Chi-square analysis indicated no association between gender of patient and pathogens isolate ($p = 0.835$). 37 *Escherichia coli* was the most prevalent clinical isolate (p value = 0.905) although there was 38 no association between the age of patient and pathogens isolated in patients indicating a possibility of an equal chance of being infected irrespective of age. *Escherichia coli* was the most prevalent causative organism, showing multi drug resistance pattern. *Conclusion:* Considering the relatively high rates of UTIs and drug resistance observed in this study, continued local, regional, and national surveillance is warranted. Imipenem and amikacin should be considered as drug of choice for empirical treatment of community acquired uncomplicated UTIs in patients in Kericho district hospital.

Keywords: Urinary tract infection, resistance, susceptible, antibiotic, bacteriuria

1. Background

Extraintestinal pathogenic *E. coli* (ExPEC), especially the uropathogenic *E. coli* (UPEC) pathotype, is most commonly associated with human infections due to *E. coli* outside the intestinal tract [1] and display enhanced ability to cause infection outside the intestinal tract, carry specific genetic determinants or virulence factors that are clustered on different pathogenicity islands [2]. Urinary tract infection is the most common non-intestinal infection in women worldwide [3]. The pathogens causing UTI are consistent across the globe. Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infections [4]. Urinary tract infections (UTIs) are among the most common infections that affect humans [5]. It is accounting for approximately 35% of nosocomial infections and also due to the frequency, recurrence and difficulty in eradication [6, 7]. *Escherichia coli* is the most predominant pathogen causing community and nosocomially-acquired urinary tract infections [8, 9, 10]. It is the most common pathogen, accounting for 85% of community-acquired and 50% of hospital-acquired infections [11].

UTIs (if untreated) can lead to serious obstetric complications, poor maternal and perinatal outcomes for

example intrauterine growth restriction, caesarean delivery and preterm deliveries [12] in women. UTIs occur more frequently in developing countries among the low socio-economic population. In the United States, UTI account for 8.3 million out-patient visits and 1 million hospitalizations annually. Masinde *et al.*, [13] published a cross-sectional study from Tanzania in 2009 to determine the prevalence of UTI in both symptomatic and asymptomatic pregnant women. Of the 247 women in the 70 sample, 31.5% were symptomatic and of those women, 18% had bacteriuria. Interestingly, of 71 the 68.5% who were asymptomatic, 13% had bacteriuria as well [14].

UTIs are mainly caused by gram-negative organisms that include *E. coli*, *Klebsiella*, *Proteus*, and *Pseudomonas* and gram-positive bacteria, group B *Streptococcus* and *Staphylococcus* species [15, 16, 17]. These organisms are mainly from the external genitalia, vagina, the genital tract, rectum, and gastro-intestinal tract. Most organisms causing UTIs originate from the bowel flora. UTIs are a result of interactions between the uropathogen and host. Successful infection of the urinary tract is determined in part by the virulence factor of the bacterium, the inoculum size, and the inadequacy of the host defense mechanisms. The pathogens causing UTs are almost always predictable, with *Escherichia*

coli being the primary etiologic agent among both outpatients and inpatients accounting for 75% to 90% of urinary tract infection isolates [18, 19].

The pathogenesis of urinary tract infection involves ascending infection with coliform bacteria colonizing the perineum in susceptible women (80–90% *Escherichia coli*, 5–10% *Staphylococcus saprophyticus* with the remainder caused by *Proteus* and other Gram negative rods) [20]. While not generally considered a cause of significant mortality, UTI do represent an important cause of morbidity. Treatment of UTI varies with the type but is usually empirical because of the common spectrum of uropathogens. Drug resistance among bacteria causing UTI has increased since introduction to UTI chemotherapy [21, 22, 23, 24]. Regular analysis of bacterial flora is important to formulate updated guidelines [25]. The magnitude of UTI among in and out patients in Kericho district hospital is not known. Hence this study was designed to ascertain the prevalence and antibiogram profile of *E. coli* causing UTI at Kericho District Hospital.

2. Methods

Study Design and Area

This study was a cross sectional conducted at Kericho district hospital in and out patient's clinic between January to June 2015. The hospital is located in Kericho town, Kericho County which is approximately 300 Km from Nairobi city, Figure 1. It is a major hospital in Kericho County. It has a population of 752,396 [26] and an area of 2,111 Km². According to Ministry of Health (MOH) report of 2013 the outpatient attending Kericho District Hospital per month is approximately 9,000 and inpatient per month is 1,350. Kericho District Hospital has a bed capacity of 250 patients.

Urine Sample Collection

A total of 133 midstream urine (MSU) samples were collected from study participants using a standard procedure. The midstream urine was collected using the techniques as was describe by Mwaka *et al.*, (2011). Midway during voiding, without stopping the urinary stream, the patients plunged with the right hand a sterile wide mouth screw cap plastic bottle to collect about 30 mls of MSU, and soon thereafter tightly close the bottles and hand them over to the study in the lab. The urine samples were placed in a cool box at 8 °C and transported to the microbiology laboratory within 2-4 hours of collection. All urine samples were turned up and down gently to allow proper mixing of urine and were analyzed immediately after they had been entered in a log book on arrival at the laboratory using dipstick (Mannheim GmbH, Germany) following manufacturer's instructions and culturing.

Bacterial culture

A standard calibrated wire loop with 4 mm internal diameter to remove 10µl of urine for inoculation onto Cystine Lactose Electrolyte Deficient (CLED) and MacConkey agar with crystal violet and 5% sheep blood agar (Oxoid N.Y., USA). The streak method was used to uniformly spread the urine onto the agar surface before aerobically incubating the plates at 37°C for 18 – 24 hours [27, 28]. Colonies were counted

after 24 hours of incubation. Plates with pure growth, and colonies >10⁴ CFU/ml were further subjected for identification and sensitivity testing. Cut off point of >10⁵ CFU/ml was used to define UTIs. Identification of the microorganisms was done following standard procedures, with use of biochemical tests which included triple sugar iron agar, Simmon's citrate agar, lysine decarboxylase, urease, and motility tests [27], and where these could not give conclusive results, API 20 method Beckton Dickson USA [29] was used to identify the isolates.

Anti-microbial susceptibility testing

Sensitivity tests were done using the Mueller – Hinton agar (Fluka, Sigma-Aldrich), following the commercial disc diffusion techniques of Kirby – Bauer [30], against a panel of 10 antimicrobial agents. The antibiotic discs and their concentrations per disc (mg): Trimethoprim-sulfamethoxazole (1.25/23.75), representative antibiotics of aminoglycosides such as gentamicin (10), amikacin (10); quinolones such as ciprofloxacin (5); various cephalosporins such as ceftriaxone (30), cefoxitin (30), cefepim (30); from carbapenem antibiotics such as imipenem (10); penicillin-like antibiotics such as ampicillin (10) and amoxicillin clavulanic acid (20/10) (Oxoid, England). *Escherichia coli* ATCC 25922 was used as a control strain. Direct measurements of diameters of zones of inhibition of bacterial growths on agar plates to 10 antibiotics were done with a calibrated ruler. The breakpoint for antimicrobial drugs was based on the guidelines provided by the CLSI [31].

One to 5 discrete colonies from pure *E. coli* plate was picked with a standard wire loop from a purity plate, and emulsified into 5 mls of sterile saline in a test tube. The saline was stirred with the loop to uniformly mix the colony in the saline. The turbidity of the saline was adjusted to match the standard McFarland 0.5, Biomerieux ®. A sterile cotton swab on a stick was dipped in the colony-saline mixture, excess saline was squeezed out by pressing the swab against the test tube, and then the cotton swab gently applied onto the surface of the Mueller-Hinton agar. Six antibiotic impregnated discs were aseptically and gently placed on the agar surface, and the plates incubated at 37°C aerobically for 18 - 24 hours. The zones of inhibition diameters around each disc were measured using a ruler, and compared against the zone diameter interpretative standards recommended by the Clinical Laboratory Standard Institute [31]. Results were reported as sensitive or resistant, for each antibiotic used.

Statistical analyses

Data entry was done on excel spread sheet, and then exported to SPSS version 20 software for statistical analysis. Chi square test was used for comparisons between significant and non-significant bacteriuria, as well as to demonstrate associations between categorical variables. P value of 0.05 was used to determine level of statistical significance.

Ethical Considerations

This research project was approved by Mount Kenya University Ethical Review Committee and also by National Council for Science, Technology and Innovation (NACOSTI). Informed consent was obtained from each

study participants before collecting urine specimens for laboratory analysis and confidentiality maintained.

3. Results

Prevalence of significant bacteriuria/urinary tract infections (UTI)

Of the 133 midstream urine samples cultured on CLED, 79.7% (106/133) of samples showed pure significant growth of bacteria, while 20.3% showed no bacterial growth after 72 hours of incubation.

Uropathogens Isolated by Culture

Five bacterial uropathogens were isolated from 106 midstream urine samples. Of these, up to 38 (35.8%) had *Escherichia coli*, while others included *Staphylococcus aureus* 27%, *Klebsiella pneumoniae* 16%, *P. auruginosa* and *Proteus* spp Figure 2, *E. coli* was the most prevalent.

Antimicrobial Sensitivity Test Profiles

A total of 38 uropathogenic *E. coli* isolates (n=22) from females were tested for susceptibility to ten antimicrobials. In females (Table 1), the isolates were sensitive to imipene 21 (95.5%) and amikacin 18 (81.8%). Resistance to amoxicillin-clavulanic acid 20 (90.9%), ampicillin 19 (86.4%), gentamycin, cefoxitin and ceftriaxone 12 (54.4%) respectively. There was a multidrug resistances observed in *E. coli* isolates which makes them unsuitable agents for empirical treatment for UTIs in the area of study. In males, a total of 16 isolates were tested against the ten antimicrobial agents. In males Table 2, imipenem 16 (100%) was sensitive followed by amikacin 14 (87.5%) and gentamicin 6 (37.4%) respectively. A resistance pattern was observed, amoxicillin-clavulanic acid and ampicillin 16 (100%), followed by trimethoprim-sulfamethoxazole 15 (93.75%), ciprofloxacin and cefoxitin 10 (62.5%) ceftriaxone and cefepin 9 (56.25%) which formed a tetramer resistance pattern. Imipenem and amikacin are sensitive to *E. coli* isolates either from females or males.

There was a high percentage of uropathogens in females 64.2% as compared to males 35.8% in the study area, there were more females than men. *E. coli* was still the most predominant uropathogen isolated in both females and males with 20.8% and 15.1% respectively and there was no association between gender of patient and pathogens isolated in patients and hence both genders can be infected and affected with pathogens, $p = 0.835$, Table 3.

4. Discussions

Antibiotic resistance is a major clinical problem in treating infections caused by *E. coli*. The resistance to the antimicrobials has increased over the years and normal intestinal microbial flora became a reservoir for resistant genes. Though UTIs are common in both men and women, there is a large difference in UTI prevalence between them due to variety of factors [32]. Kebira *et al.*, [33] opined that female had high UTIs than men caused by *E. coli* with 24% in Thika Level 5 Hospital, this was in agreement with our findings. Women are more susceptible to UTIs and have a 50% chance of experiencing at least one episode of UTI during their lifetime [3, 34]. *E. coli* strains were the most

common isolates. Similar findings have been reported in northern Tanzania with 202 68.4%. [35]. A similar high proportion of *E. coli* was also reported in Sudan with 791.9% [36] and Yemen [37] studies. In the present study, the predominant bacterial isolates were gram negative which 205 was similar to other findings [38] and Addis Ababa, Ethiopia [15]. Though *E. coli* is considered the most uropathogenic due to its virulence factors for colonization and invasion of the urinary epithelium [39], *S. aureus* can be also the most important uropathogen in the study area but it needs further study on its epidemiology and risk factors. Certain virulence factors like haemolysin production and presence of fimbriae in the *E. coli* may be associated with urovirulence [40].

Prescribing and giving antibiotics without testing antibiotic resistance pattern in developing countries including Kenya is a common problem for the development of drug resistance. In the present study, *E. coli* isolates were highly resistant to trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid in both genders. A similar finding was also reported in other studies [41, 42]. This high antibiotic resistance might be associated with previous exposure of the bacterial isolates to these antibiotics. In addition, in rural settings including the study area, use of antibiotics without proper prescription with health professionals is also a common practice. All these factors may be used as a risk factor for the development of antibiotic resistance especially for the commonly used ones. Furthermore, all of the *E. coli* isolates in this study were multidrug resistant. This may be due to the fact that the easy availability and indiscriminate use of commonly used drugs such as ampicillin, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole may lead to an increase to resistance. Yismaw [19] and Astal *et al.*, [43] have also reported resistance of *E. coli* to gentamicin, ciprofloxacin and ceftriaxone in the Gaza Strip respectively which is consistent with this study.

Kebira *et al.*, [33] also stated that *E. coli* was 100% resistant to trimethoprim-sulfamethoxazole in UTI cases in Thika, Kenya which was also consistent with the findings at Kericho District Hospital. *Escherichia coli* with its multidrug resistant strains has been found to be the commonest cause of UTI [44]. Imipenem was found to be the best effective antimicrobial in treatment of UTI in Kericho District hospital. *Escherichia coli* has been documented as the most important pathogen associated with urinary tract infections in many countries [45]. UTI in males, though rare, occurs in much less frequency [46] this too is consistent with our findings in this study area.

5. Conclusion

Based on our study findings, *E. coli* was found to be more prevalent in females than males. As reported previously, our study confirmed *Escherichia coli* to be a major uropathogen. The study further detected increasing resistance of *Escherichia coli* strains to trimethoprim-sulfamethoxazole. The results of this study highlight the need for continued surveillance of antimicrobial resistance among uropathogens causing UTI, so as to increase positive outcomes of clinical interventions. Imipenem and amikacin should be considered as drug of choice for empirical treatment of

community acquired uncomplicated UTI in patients in Kericho district hospital.

Competing Interests: There is no conflict of interest.

6. Author's Contribution

DC: Conceived the study and participated in the design of the study, experimental setups, acquisition of data, data storage and management. Drafting of the manuscript.

JHOO: Participated in the study design, statistical analysis and interpretation, drafting of the manuscript and revising it critically.

OWL: Participated in the study design, critic the manuscript for intellectual content.

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Figure 1: Map of Kenya Showing Kericho County and the Sites of study, GIS -0.37159, 35.2801

Figure 2: Bacterial uropathogens isolated from 106 in and out patients

Figures

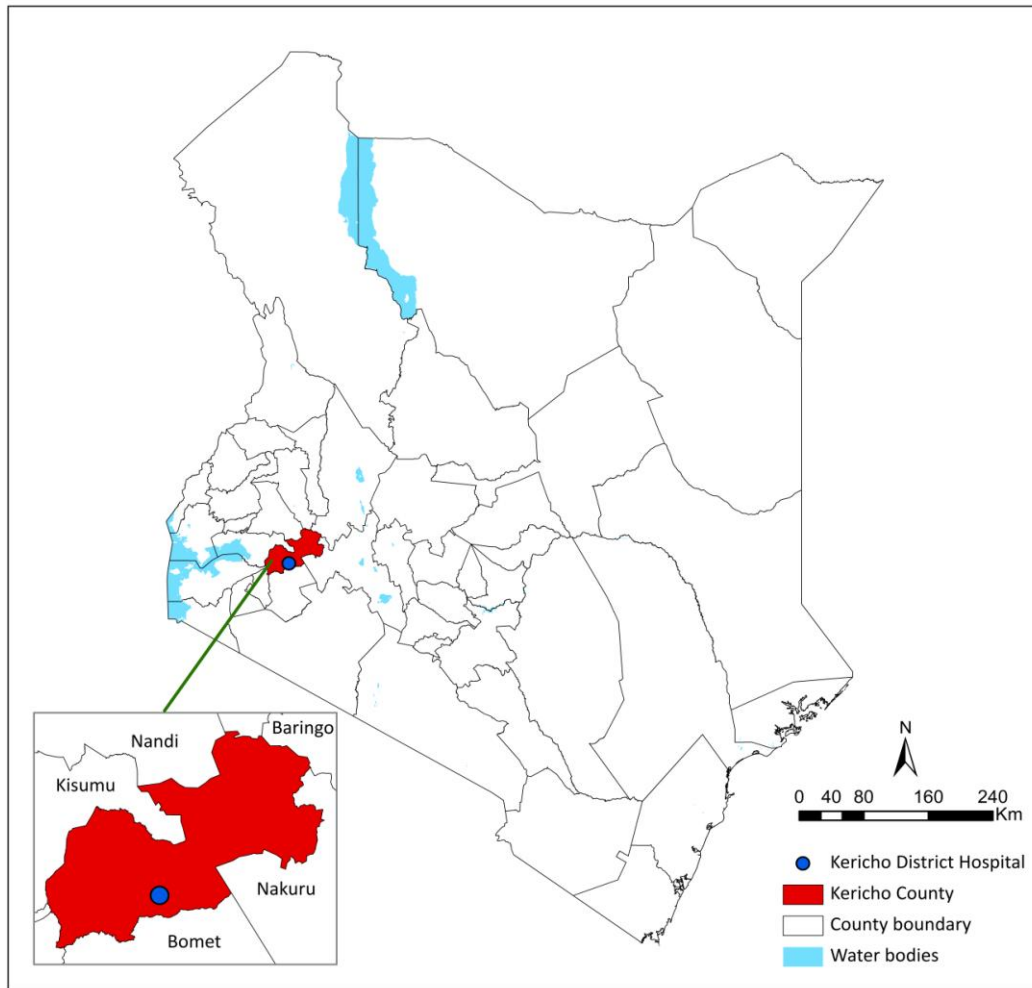


Figure 1

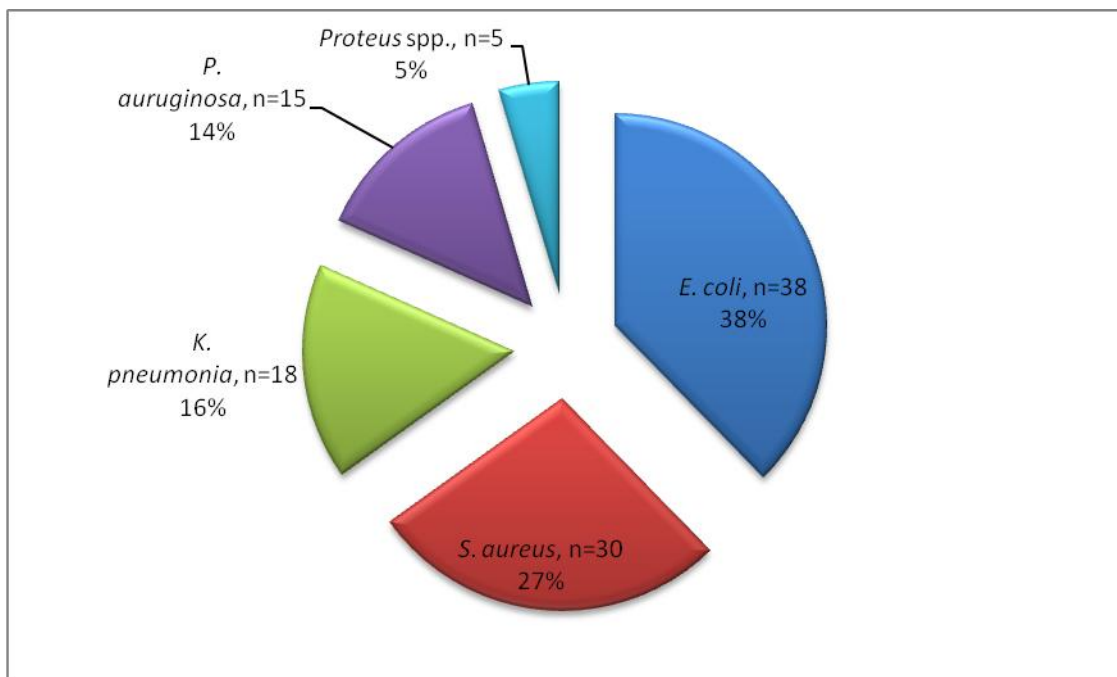


Figure 2

Tables

Table 1: Antimicrobial Susceptibility of *E. coli* Clinical Isolates (n=22) by Disc Diffusion on Females with UTI from Kericho District Hospital

Antimicrobial Agent tested	Zone of diameter nearest whole mm			Resistance	Intermediate	Sensitive
	R	I	S			
Trimethoprin-sulfamethoxazole	<10	11-15	>16	16 (72.7%)	3 (13.6%)	3 (13.6%)
Gentamicin	<12	13-14	>15	12 (54.4%)	3 (13.6%)	7 (32%)
Amikacin	<14	15-16	>17	2 (9.1%)	2 (9.1%)	18 (81.8%)
Ceftriaxone	<19	20-22	>23	12 (54.4%)	4 (18.2%)	6 (27.3%)
Ciprofloxacin	<10	21-30	≥31	9 (40.9%)	6 (27.3%)	7 (31.8%)
Cefoxitin	<14	15-17	>18	12 (54.4%)	3 (13.6%)	7 (31.8%)
Cefepim	<14	15-17	>18	10 (45.5%)	5 (22.7%)	7 (31.8%)
Imipenem	<19	20-22	>23	1 (4.5%)	0	21 (95.5%)
Ampicillin	<13	14-16	>17	19 (86.4%)	1 (4.5%)	2 (9.1%)
Amoxicillin-clavulanic acid	<13	14-17	≥18	20 (90.9%)	0	2 (9.1%)

Key: R- Resistance; I- Intermediate; S- Sensitive

Table 2: Antimicrobial Susceptibility Profile of *E. coli* Clinical Isolates (n=16) by Disc Diffusion on Males with UTI from Kericho District Hospital

Antimicrobial Agent tested	Zone of diameter nearest whole mm			Resistance	Intermediate	Sensitive
	R	I	S			
Trimethoprin-sulfamethoxazole	<10	11-15	>16	15 (93.75%)	0	1 (6.25%)
Gentamicin	<12	13-14	>15	8 (50%)	2 (12.5%)	6 (37.5%)
Amikacin	<14	15-16	>17	0	2 (12.5%)	14 (87.5%)
Ciprofloxacin	<19	20-22	>23	10 (62.5%)	3 (18.75%)	3 (18.75%)
Ceftriaxone	<10	21-30	≥31	9 (56.25%)	3 (18.75%)	4 (25%)
Cefoxitin	<14	15-17	>18	10 (62.5%)	2 (12.5%)	4 (25%)
Cefepim	<14	15-17	>18	9 (56.25%)	2 (12.5%)	5 (31.25%)
Imipenem	<19	20-22	>23	0	0	16 (100%)
Ampicillin	<13	14-16	>17	16 (100%)	0	0
Amoxicillin-clavulanic acid	<13	14-17	≥18	16 (100%)	0	0

Key: R- Resistance; I- Intermediate; S- Sensitive

Table 3: Gender of Patient in Relation to Pathogenic Microorganism Isolated

Bacterial isolates	Gender of Patients		Total
	Female	Male	
<i>E. coli</i>	22(20.8%)	16(15.1%)	38(38.8%)
<i>S. aureus</i>	20(18.9%)	11(10.4%)	31(29.2%)
<i>K. pneumoniae</i>	12(11.3%)	6(5.7%)	18(17.0%)
<i>P. aeruginosa</i>	11(10.4%)	4(3.8%)	15(14.2%)
<i>P. mirabilis</i>	3(2.8%)	1(0.9%)	4(3.8%)
Total	68(64.2%)	38(35.8%)	106(100%)