African Journal of Biotechnology Vol. 11(34), pp. 8560-8570, 26 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2865
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Biofilm production and antibiotic susceptibility profile of *Escherichia coli* isolates from HIV and AIDS patients in the Limpopo Province

Samie, A. and Nkgau, T. F.

Department of Microbiology, University of Venda, Private Bag X5050, Thohoyandou 0950, Limpopo, South Africa.

Accepted 30 March, 2012

In the present study, *Escherichia coli* strains were isolated from water, stool, sputum and urine samples from HIV and AIDS patients attending treatment centers in the Limpopo Province, using standard microbiological procedures and identified by polymerase chain reaction (PCR). Biofilm assay was performed using the Congo red agar and the microtiter plate methods. Beta-lactamase production was tested using the iodometric method, while the antibiotic susceptibility profiles of the organisms were determined by the disc diffusion method. Of the 139 isolates tested, 58 (42%) were biofilm producers with 22 (16%) of these being strong biofilm producers. Antibiotic resistance was common but kanamycin, meropenem and lomefloxacin were the most active with 6.6, 5.8 and 4.3% resistance rates respectively. The rate of biofilm formation was higher among *E. coli* isolates from water (55.5% p-value=0.011). Antibiotic resistance was higher among biofilm producers compared to non producers particularly for penicillin (93.1%) and cefipime (50.0%). Lomefloxacin appeared to be the most active antibiotic against the biofilm producing strains with 1.7% resistance among the biofilm producers compared to 6.3% among the non-biofilm producers. Beta-lactamase production was higher among isolates from urine samples. This study suggests that *E. coli* strains that produce biofilm are common in water and urine samples. However, further studies are needed to determine the potential role of water in the production of urinary tract infections (UTIs) in HIV patients.

Key words: Co-infections, HIV and AIDS, epidemiology, Venda, South Africa.

INTRODUCTION

Human Immunodeficiency virus discovered in the 1980s is known to cause AIDS. From the discovery of AIDS to date, the pandemic has grown faster in the African continent compared to other areas of the world (Amico et al., 2010). The virus infects and destroys CD4 positive cells in humans, leaving the body defenseless. This increases the occurrence of opportunistic infections. Pathogenic *Escherichia coli* are responsible for a large number of infections including intestinal and extra intestinal infections, and recent studies have indicated that *E. coli* strains have emerged as important pathogens among HIV and AIDS patients (Avelino et al., 2010).

Although there have been reports on intestinal *E. coli* in South Africa, very few data exists on the occurrence and pathogenic mechanisms among extraintestinal *E. coli*. Such extra-intestinal *E. coli* include those responsible for urinary tract infections (UTIs) as well as those responsible for respiratory tract infections. Several studies have identified *E. coli* as the most common cause of UTIs (Vignesh et al., 2008).

Organisms that produce biofilm show much greater resistance to antibiotics than their free living counterparts. This increase in drug resistance is partly due to the penetration barrier that biofilm present to antimicrobials (Mohamed et al., 2007). Transmission of pathogenic *E. coli* occurs through fecal oral route and person to person contact. Food and water have also been shown to be common reservoirs of pathogenic *E. coli*. Outbreaks of *E.*

*Corresponding author. E-mail: samieamidou@yahoo.com or samie.amidou@univen.ac.za. Tel: +27159628186. Fax: 27 15 962 4749.

coli induced diarrhea involving contaminated water and food such as spinach, ground beef and dairy products have been documented (Vogt and Dippold, 2005). Cattles are the main reservoirs of *E. coli* 0157:H7 which is an enterohemorrhagic *E. coli* causing major food borne outbreaks in developed and developing countries (Buchanan and Doyle, 1997; Lim et al., 2010). Over the past years, *E. coli* has been implicated as a causative agent of respiratory tract infections/ pneumonia. Clinically, *E. coli* induced respiratory tract infections is manifested by fever, shortness of breath and increased respiratory secretions, and may often appear as broncho-pneumonia of the lower lobes (Wang et al., 2000). However, the disease may also be community acquired in individuals with diabetes mellitus, chronic obstructive pulmonary diseases and *E. coli* UTIs (Donnerberg and Nataro, 2000).

Although many antibiotics used in the treatment of *E. coli* infections are still effective as treatment, development of antibiotic resistance among different diarrheagenic and uropathogenic *E. coli* is fast becoming a problem (Boczek et al., 2007). Antibiotic resistance constitutes both a present and a future problem. This is because strains are acquiring resistance at a rate faster than the rates at which new drugs are developed. The present study was aimed to determine the capacity of *E. coli* strains isolated from HIV and AIDS patients to produce biofilm, beta lactamase and the antibiotic susceptibility profiles of the isolates.

MATERIALS AND METHODS

Ethical consideration

Ethical clearance of the study was obtained from the University of Venda Health, Safety and Ethics Committee. Authorization to conduct the study was obtained from the Department of Health, Limpopo in Polokwane. Ethical clearance and authorization was also obtained from the ethical committees of the Donald Fraser, Elim and Tshilidzini Hospitals. The objectives of the study were explained to the patients and their right to say no to participate in the study was explained to them. Once the patients had agreed to participate in the study, they were requested to sign a consent form. To preserve their privacy, the patients were given a code and were referred to by that code. The patients in the community were also requested to sign a consent form after the study has been explained to them. Whenever possible, different samples including sputum, urine, mouth wash and stools were collected.

Patients and sample collection

Clinical samples such as stools, sputum and urine were collected from HIV positive patients in different communities in the Limpopo province and water samples were collected from households of HIV positive individuals.

Bacterial isolates, cultures and maintenance

Collected samples were cultured on either eosine methylene blue (EMB) agar or MacConkey agar for 24 h at 37°C for the detection of *E. coli*. A single colony from positive samples was subcultured on nutrient agar for 24 h at 37°C. The cultures were kept in the freezer prior to the different tests and subcultured on a new nutrient agar when deemed necessary. The cultures were preliminary identified by the characteristic green metallic sheen on eosin-methylene blue (EMB) followed by standard biochemical tests. The final identification of the strains was made by polymerase chain reaction (PCR) as previously described by Juhna et al. (2007).

Biofilm testing

Biofilm formation was tested using two different methods, namely the microtiter plate (MT), and the Congo red agar (CRA) methods.

Microtiter plate (MT) method

The MT biofilm assay was carried out as described by Christensen et al. (1982) with a few modifications. Briefly, a fresh culture of the bacteria was prepared in 10 ml of brain heart infusion broth (BHIB). In brief, 198 µL of sterile brain heart infusion broth (1% sucrose) was added to each individual well of the sterile 96 well-U shaped microtiter plate, and then 2 µL of the bacterial suspension was added to each well containing the BHIB. Each assay was performed in duplicate and repeated on at least three different occasions. Enteroaggregative *E. coli* strain 042 previously shown to be a biofilm producer was used as a positive control and a well with only BHIB was used as a negative control. Based on the obtained OD values, the samples were classified as biofilm producers if their OD values exceeded the average plus two standards deviation of the negative controls (Mohamed et al., 2007). The samples were classified into strong producers (OD>1), moderate producers (OD ≥0.4≤1) and non producers (OD<0.4).

Congo red agar (CRA) method

The Congo red agar method was carried out as described by Freeman et al. (1989).

Beta lactamase testing

Two different methods based on the same principle were used to test for the production of beta lactamase, namely the iodometric agar method and the iodometric tube method. Both methods depend upon the ability of penicilloic acid (formed when the β-lactamase enzyme hydrolyses the amide bond in the β-lactam ring of penicillin analogues) to reduce iodine to iodide, resulting in a decoloration of the blue iodine-starch complex (Koneman et al., 1983). The two methods including the iodometric agar and the iodometric tube methods were used and compared.

Antibiotic susceptibility testing

The disc agar diffusion (DAD) method as described by Clinical and Laboratory standards institute (CLSI) was used to determine antibiotic susceptibility profile of *E. coli* isolates. The results were obtained by measuring the diameter of the inhibitory zone and analyzed according to the CLSI guidelines for Enterobacteriaceae (CSLI, 2008). A total of 16 antibiotics were used.

Statistical analysis

All the results obtained were typed on an excel sheet for further

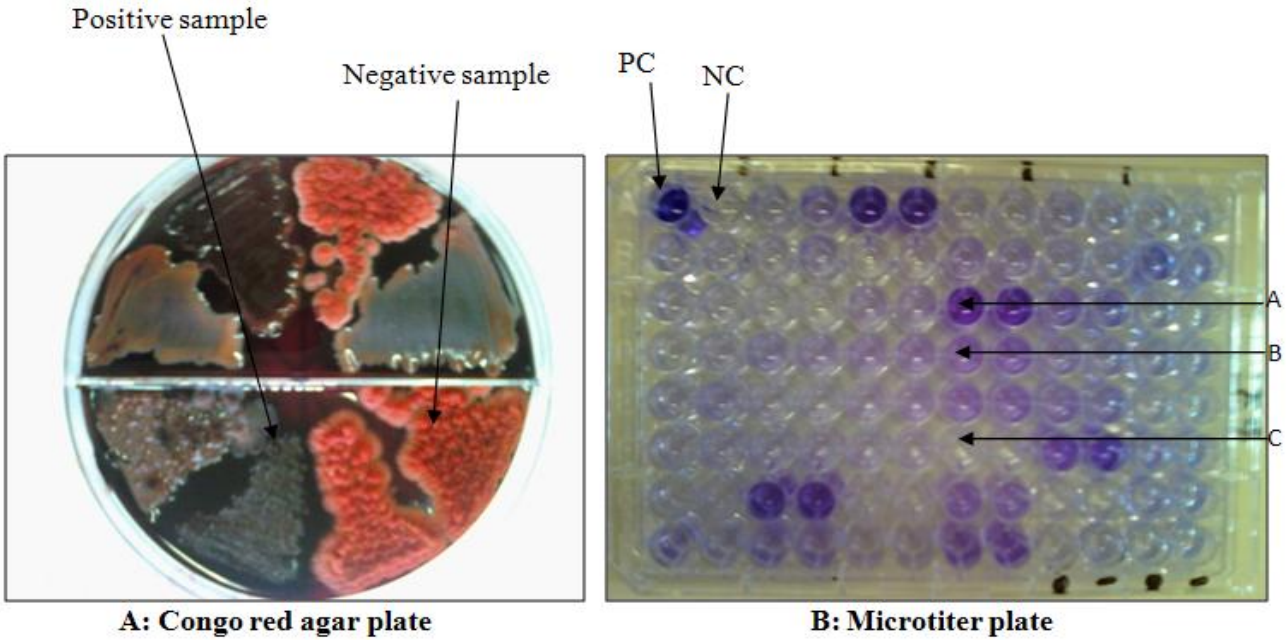

Figure 1. Pictures showing biofilm production on the CRA (A) and on the MT (B). On the CRA, black colonies indicate a positive biofilm producer whereas other colors constitute a negative result. For the microtitration method, a positive reaction was shown by the colour on the wells as indicated by arrows: A, strong producer; B, moderate producer; C, none producer. PC is the positive control (EAEC 042) and NC is the negative control.

analysis. Statistical analysis was conducted using the SPSS software (version 17.1). Different test performed on the software such as the Chi square, confidence interval and the Fishers exact tests were used to determine correlation between the different results obtained. The difference between two variables was considered significant if the p value was less than 0.05.

RESULTS

General characteristics of the study sample

In this study, a total of 139 *E. coli* strains were isolated and characterized for their capacity to produce biofilm, their antibiotic susceptibility profiles, their capacity to produce β-lactamase and their pathotypes. All the strains were confirmed by the PCR method to be *E. coli*. From these strains, 58(41.7%) were from stool samples, 45(32.4%) were from urine, 10 (7.2%) were from sputum and 26 (18.7%) were from water. Clinical samples were from HIV positive patients and water samples were collected from households of HIV patients from the Limpopo Province. From the stool samples collected, 41 were from females, while 17 were from males. Samples were collected from individuals aged between 4 and 79 years.

Biofilm production among *E. coli* strains from clinical and water samples from HIV patients

Two different methods were used to test for biofilm production, namely the microtiter plate (MT) and Congo red agar (CRA) methods. Considering both methods, a total of 72 (51.8%) strains were found to be biofilm producers. Figure 1 shows pictures of biofilm detection using the microtiter plate and the Congo red agar methods. In the microtitration method, the optical density (OD) was measured at 595 nm. The value for the negative control was 0.079 and the positive control was 3.877. The MT method showed that 58 (41.7%) strains were positive, while 56 (40.3%) were positive with the CRA method. Among all the positive strains, 42 (72.0%) were positive by both methods whereas 16 (11.5%) of the isolates were positive by the MT method and negative by the CRA method. Similarly, 14 (10.1%) of the isolates were positive by the CRA method while negative for the MT method.The sensitivity of the MT method was 81% (58/72) while that of the CRA method was 78% (56/72). Because of the higher sensitivity of the MT methods, its ease to perform and the fact that the strains could easily be classified as strong and moderate producers, this method was therefore considered for the analysis of the results. Furthermore, this is a method commonly used to detect biofilm production in *E. coli* strains. Biofilm producers were further classified as strong and moderate producers. Among the 58 (41.7%) biofilm producers, 22 (38.0%) were strong producers and 36 (62.0%) were moderate producers.

Biofilm production and sample type

Biofilm production was mostly observed among

Table 1. Distribution of biofilm production by sample type.

Sample	None producer (n=81)	Moderate producer (n=36)	Strong producer (n=22)	P value
Stool (n=58)	43 (74.1%)	12 (20.7%)	3(5.2%)	0.008
Sputum (n=10)	6 (60.0%)	4 (40.0%)	0(0%)	0.008
Urine (n=45)	20 (44.4%)	13 (28.9%)	12 (26.7%)	0.008
Water (n=26)	12 (46.2%)	7 (26.9%)	7(26.9%)	0.008

Table 2. Antibiotic susceptibility profile of *Escherichia coli* isolated from stool, water, urine and sputum samples from HIV patients in the Limpopo province.

Group	Antibiotic (con)	Resistant (%)	Susceptible (%)	RBP (mm)
Penicillins	Penicillin G (10 µg)	127(91.4%)	12 (8.6%)	<14
	Amoxicillin (10 µg)	90 (64.7%)	49 (35.3%)	<13
Cephalosporins	Cefepime (30 µg)	62 (44.6%)	77 (55.4%)	<14
Carbapenems	Meropenem (10µg)	8 (5.8%)	131 (94.2%)	<13
Aminoglycosides	Amikacin (30µg)	22 (15.8%)	117 (84.2%)	<14
	Gentamycin (10µg)	20 (14.4%)	119 (85.6%)	<12
	Kanamycin (30µg)	9 (6.5%)	130 (93.5%)	<14
	Streptomycin (10µg)	62 (44.6%)	77 (55.4%)	<11
Quinolones	Nalidixic acid (30µg)	21(15.1%)	118 (84.9%)	<13
	Lomefloxacin (10µg)	6 (4.3%)	133 (95.7%)	<13
Tetracyclines	Tetracycline (30µg)	74 (53.2%)	65 (46.8%)	<14
Macrolides	Erythromycin (15µg)	119(85.6%)	20 (14.4%)	<13
Glycopeptide	Vancomycin (30µg)	118 (84.9%)	21 (15.1%)	<14
Phenicols	Chloramphenicol (30µg)	34 (24.5%)	105 (75.5%)	<12
Polymyxin	Polymyxin B (300µg)	80 (57.6%)	59 (42.4%)	<14
Others	Rifampicin (5µg)	110 (79.1%)	29 (20.9%)	<13

Con= antibiotic content of the disc; RBP=resistance break point.

organisms isolated from water and urine. 25 (55.0%) of the organisms from urine formed biofilm of which 13 (52.0%) were moderate and 12 (48.0%) were strong producers. 14 (53.8%) of the organisms from water formed biofilm of which 7(50.0%) were moderate and 7 (50.0%) were strong producers. 15 (25.9%) of the organism from stools formed biofilm of which 12(80.0%) were moderate and 3(20.0%) were strong producers. Four (40%) of the organism from the sputum formed biofilm and were all moderate producers. The difference was statistically significant (p value=0.008). Table 1 shows the distribution of biofilm production by sample type. Biofilm was detected more in females although the difference was not statistically significant (p value=0.110).

Antibiotic susceptibility profiles of *E. coli* isolates from clinical and water samples from HIV patients

The antibiotic susceptibility profile of *E. coli* to 16 different antibiotics was determined. A greater resistance was observed against penicillin (127, 91.4%), erythromycin (119, 85.6%) and rifampicin (110, 79.1%), while antibiotics such as cefipime (62, 44.6%) and streptomycin (74, 53.2%) had moderate resistance. The antibiotics lomefloxacin 6 (4.3%), meropenem 8 (5.8%) and kanamycin 9 (6.5%) were the most active. Table 2 shows the antibiotic susceptibility profiles of *E. coli* isolates to the different antibiotics used and the antibiotic content of the disc and the resistance breakpoint used.

Distribution of antibiotic resistance by sample type

Most water isolates showed higher resistance to amoxicillin, cefepime, meropenem, amikacin, gentamycin and nalidixic acid. However, resistance to erythromycin and rifampicin was higher among isolates from sputum. Resistance to lomefloxacin and polymyxinB was higher among isolates from stool, while isolates from urine were

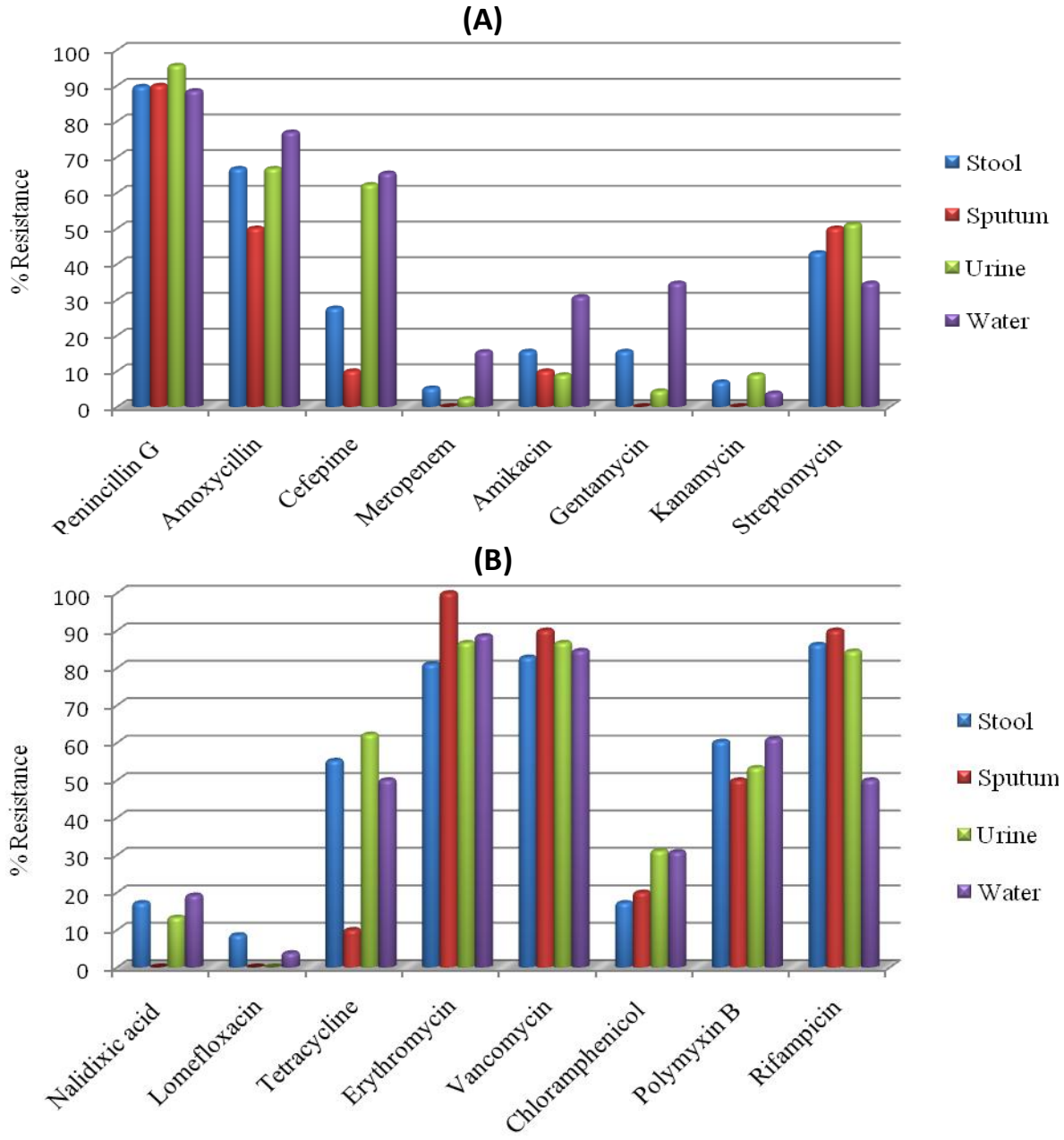

Figure 2. Comparative analysis of antibiotic resistance among *E. coli* isolates from different types of samples including stools, sputum, urine and water.

highly resistant to streptomycin and tetracycline. Figures 2A and B show the distribution of the antibiotic susceptibility profile of *E. coli* isolates according to the sample types.

Antibiotic resistance and sex

Resistance to penicillin and amoxicillin were much higher in females (92.4%; 63.4%) than in males (82.7%; 49.7%). Resistance to nalidixic acid and lomefloxacin were higher in males (21.8%; 8.7%) as compared to females (15.2%; 4.5%). Resistance to some antibiotics belonging to the group aminoglycosides such as kanamycin (9.1%) and streptomycin (51.2%) were higher in females than males (4.3%; 21.8%). Overall resistance was higher in females as compared to males. However the p values were not statistically significant and hence resistance to these antibiotics was not associated with gender with the exception of streptomycin (p=0.045) and tetracycline (p=0.031) in which the difference were statistically significant. Figure 3 shows the distribution of antibiotic

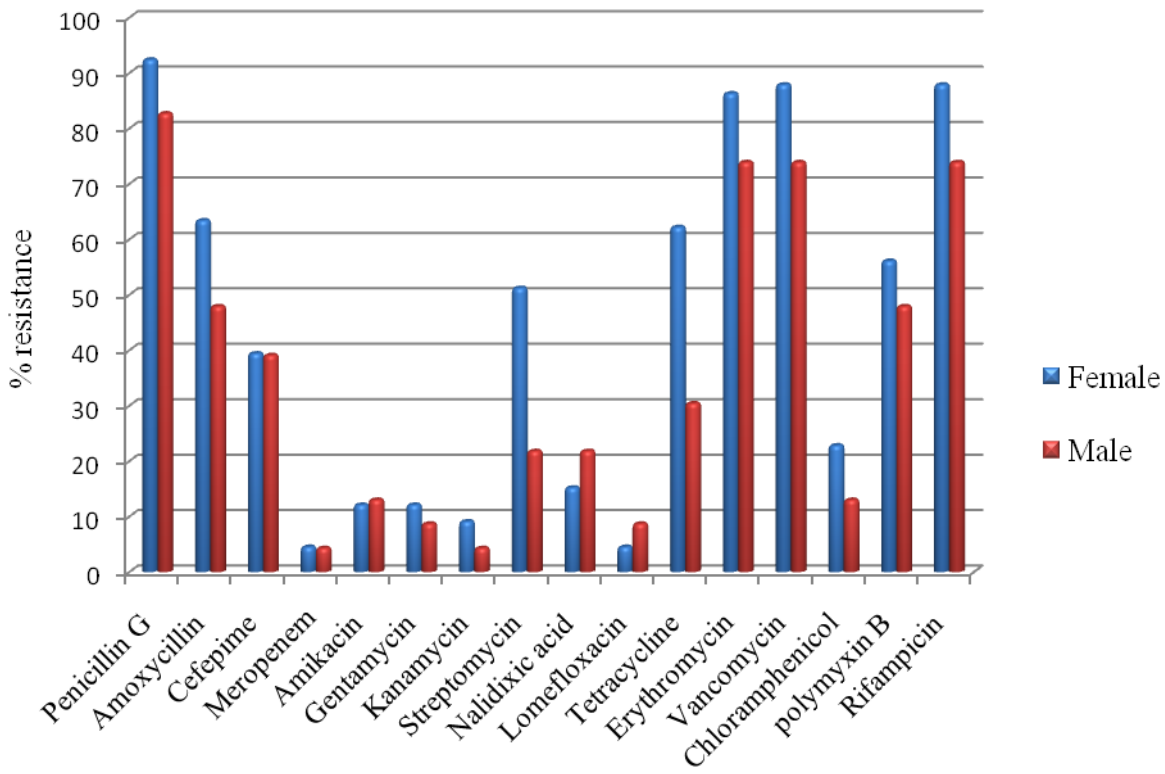

Figure 3. Comparative analysis of antibiotic resistance between isolates from males and females. Antibiotics such as streptomycin (p = 0.045) and tetracycline (p = 0.031) were associated with gender.

resistance by sex.

Multiple drug resistance profile of *E. coli* strains

Multiple drug resistance defined as resistance to at least three antibiotics was common among the organisms tested. Of the 139 isolates, none of the isolates were susceptible to all the 16 antibiotics, while only 2 (1.4%) were resistant to 1 and 2 antibiotics, respectively. The highest rate of multiple drug resistance was observed against 6 and 7 antibiotics at a time with 18.0% (25/139) and 15.1% (21/139), respectively, and the lowest multiple drug resistance rate was against 12 and 13 antibiotics (0.7%; 1/139). None of the isolates was resistant to more than 13 antibiotics. Figure 4 shows the rate of multiple drug resistance among the *E. coli* isolates.

Distribution of multiple drug resistance by sample type

Resistance to 3 antibiotics was found only in strains from stools and water. Majority of the strains resistant to 4 antibiotics were found in sputum (50.0%) and stool (13.8%), while only 7.7% from water and none from urine were resistant to 4 antibiotics at a time. Figure 5 shows the distribution of multiple drug resistant strains by sample type.

Distribution of antibiotic resistance among biofilm producers

The antibiotic susceptibility profiles of *E. coli* were compared between the none, the moderate and the strong biofilm producers. Resistance to antibiotics such as cefepime (63.6%), Kanamycin (13.6%) and chloramphenicol was higher among the strong biofilm producers, while resistance to antibiotics such as nalidixic acid was higher among the moderate biofilm producers. Resistance to antibiotics such as erythromycin was higher among the non biofilm producers. Overall resistance was higher among the strong biofilm producers when compared to both the non producers and the moderate producers. However, the differences were not statistically significant with the exception of resistance to chloramphenicol (p value=0.024). Table 3 shows the results of the antibiotic susceptibility profile among the none and biofilm producers.

Beta lactamase production using the iodometric agar and tube method

Beta lactamase production was tested using two methods

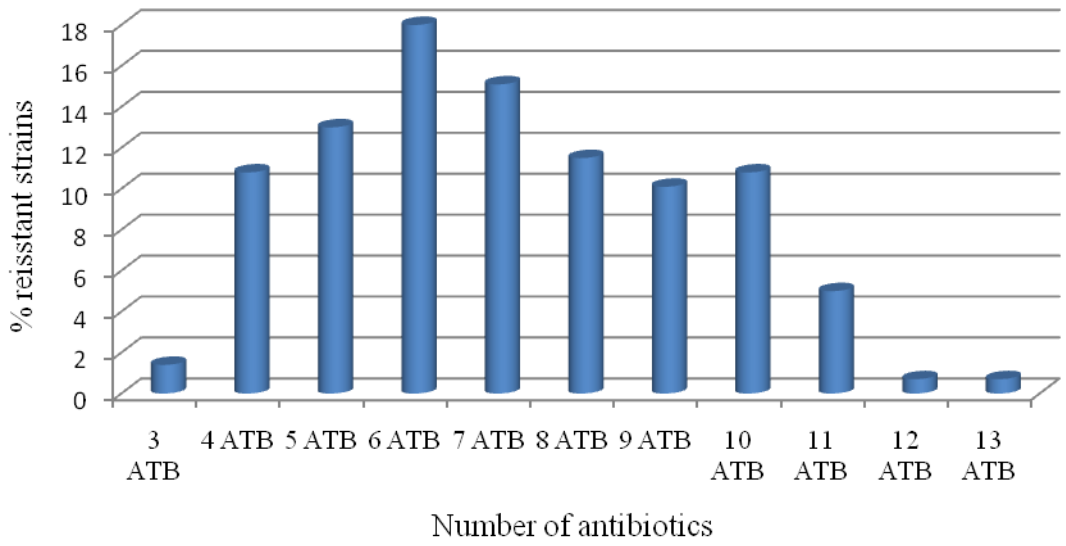

Figure 4. Multiple drug resistance among *E. coli* strains.

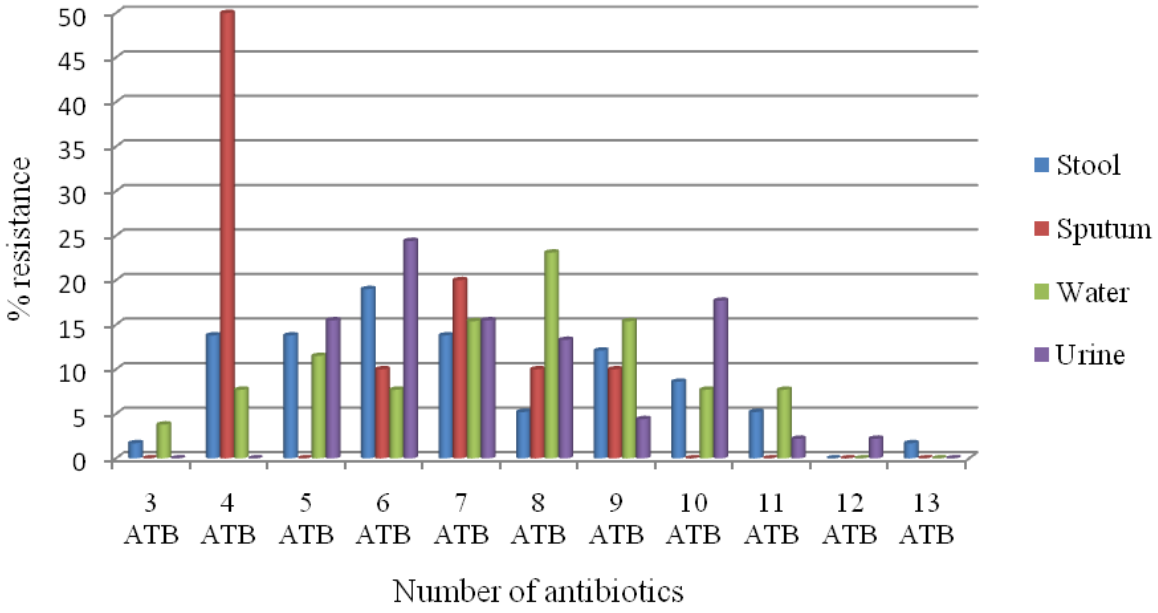

Figure 5. Comparative analysis of multiple drug resistance among *E. coli* isolates from different type of samples including stools, sputum, urine and water.

based on the same principle; namely the iodometric agar and iodometric tube method. When considering both methods, a total of 35 (25.2%) strains were found to be beta lactamase producers. With the tube method 25 (18.0%) strains tested positive, while 24 (17.3%) tested positive with the agar method. Among the positive strains, 18(13.0%) were positive for both methods, while 7 (5.0%) of the isolates were positive for the tube method and negative for the agar method and 6 (4.3%) of the isolates were positive for agar method while negative for the tube method. The tube method was chosen for further analysis as it was found to be faster, easier to conduct and the interpretation of the results was easier.

Amongst the different samples tested, urine isolates had the highest rate of beta lactamase production (22%) followed by isolates from stools (20.7%), and then water (7.7%). None of the isolates from sputum produced the beta lactamase enzyme. Among clinical samples, the rate of beta-lactamase production was higher in *E. coli* isolates from males (39.1%) compared to females (12.0%), however, the difference was statistically not significant (p=0.187).

Table 3. Distribution of antibiotic resistance among biofilm producers.

Antibiotic	None producers (n=81)	Moderate (n=36)	Strong (n=22)	P value
Penicillin G	73 (90.1%)	33 (91.7%)	21 (95.6%)	0.730
Amoxicillin	52 (64.2%)	23 (63.9%)	16 (72.8%)	0.737
Cefepime	(33 (40.7%)	15 (41.7%)	14 (63.6%)	0.147
Meropenem	5 (6.2%)	2 (5.6%)	1 (4.5%)	0.957
Nalidixic acid	11 (13.6%)	9 (25%)	1 (4.5%)	0.090
Lomefloxacin	5 (6.2%)	1 (2.8%)	0 (0%)	0.392
Amikacin	14 (17.3%)	6 (16.7%)	2 (9.1%)	0.638
Gentamycin	14 (17.3%)	5 (13.9%)	1 (4.5%)	0.318
Kanamycin	5 (6.2%)	1 (2.8%)	3 (13.6%)	0.261
Tetracycline	45 (55.6%)	16 (44.4%)	13 (59.1%)	0.450
Erythromycin	71 (87.7%)	30 (83.3%)	18 (81.8%)	0.711
Vancomycin	69 (85.2%)	30 (83.3%)	19 (86.4%)	0.946
Chloramphenicol	19 (23.5%)	5 (13.9%)	10 (45.6%)	0.024
Polymyxin B	48 (59.3%)	18 (50.0%)	14 (63.6%)	0.530
Streptomycin	38 (46.9%)	17 (47.2%)	7 (32.8%)	0.421
Rifampicin	69 (85.2%)	24 (66.7%)	17 (77.2%)	0.049

Distribution of beta-lactamase production amongst biofilm producers

From the 58 biofilm producers, 9 (15.6%) were found to be beta-lactamase producers, of which 6 (10.4%) were among the moderate biofilm producers and 3 (5.2%) among the strong biofilm producers. However, the difference was not statistically significant (p=0.521).

Association between beta lactamase and resistance to antibiotics

The antibiotic susceptibility profiles of β-lactamase producing stains were compared with non β-lactamase producing strains. Resistance to antibiotics such as penicillin (92.0%), amoxicillin (76%), cefepime (48%) was higher among the β-lactamase producing strains, although the difference was not significant (p value> 0.05). Resistance to antibiotic such as nalidixic acid, streptomycin and erythromycin were higher amongst the non β-lactamase producing strains; the difference was also not significant.

Multiple drug resistance among beta-lactamase positive strains

The rate of multiple drug resistance was compared between the β-lactamase positive and negative strains. Resistance to 3, 4, 5, 6 and 7 antibiotics was higher among the β-lactamase negative strains, while resistance to 8, 9, 10 and 11 antibiotics was higher amongst the β-lactamase positive strains with greater resistance to 8 antibiotics. Strains resistant to 12 and 13 antibiotics were found only among the β-lactamase negative stains. Figure 6 shows the rate of multiple drug resistance of β-lactamase positive and negative strains.

DISCUSSION

Several studies have implicated *E. coli* as an important etiological agent of diarrhea and as an emerging opportunistic pathogen particularly among HIV positive patients in developing countries (Abong et al., 2008; Rossit et al., 2009). This study determined the antibiotic resistance profiles in association with biofilm-producing capacities as well as beta lactamase production of *E. coli* isolates obtained from HIV and AIDS patients in the Limpopo Province in South Africa.

Biofilm formation has been described as an important pathogenic feature presented by different types of organisms including bacteria and fungi; the methods used for the detection vary between studies and include mainly the MT and the CRA methods. A study conducted by Mathur et al. (2006) showed that there was no correlation between the CRA and the MT plate methods and that the MT plate method was the most sensitive (97.1% compared to 6.8% CRA), accurate (97.2% compared to 40.9% CRA) and the most reproducible method for screening for biofilm formation. In the present study, both methods were used and compared for sensitivity and ease of application. Most studies conducted for biofilm formation among *E. coli* strains have used the microtiter plate method in combination with the detection of various virulence genes (Rijavec et al., 2008). Hence, the MT method remains the method of choice in terms of accuracy, sensitivity and ease of application for detecting biofilm formation among *E. coli* strains.

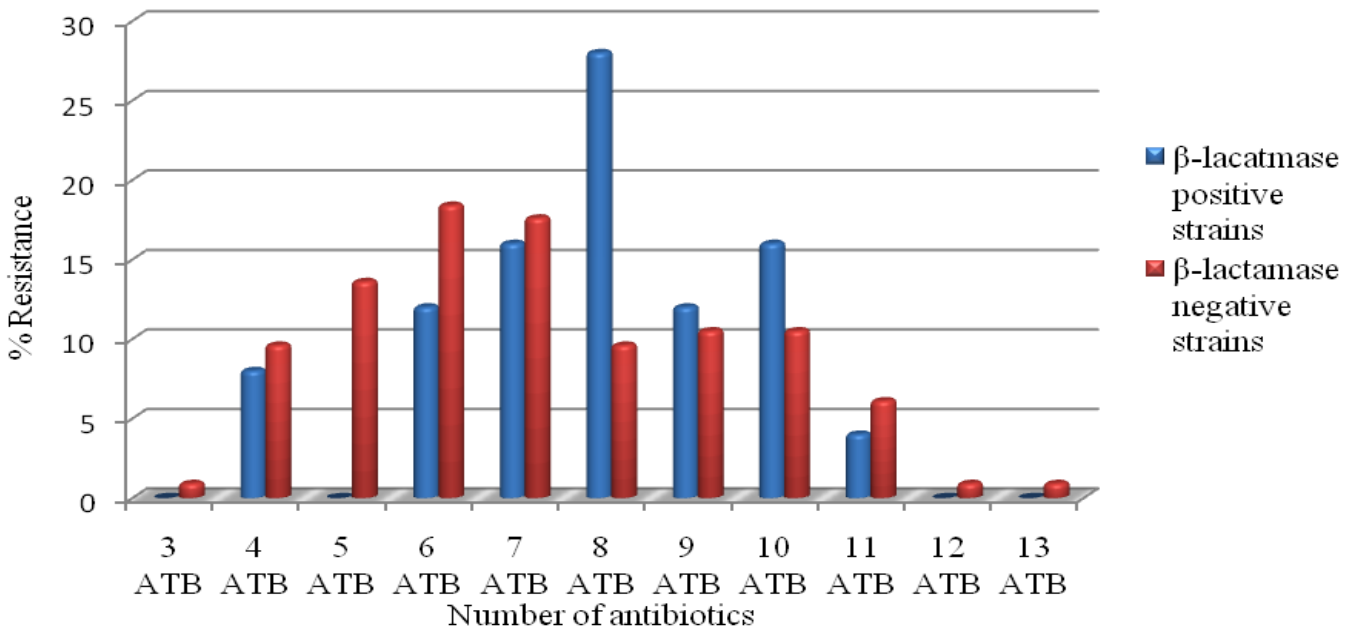

Figure 6. Comparative analysis of multiple drug resistance between β-lactamase positive and negative strains.

The prevalence and detection of biofilm producers have been showed to be dependent upon various factors such as the method, media and incubation period used (Mathur et al., 2006). In the present study, we found that the prevalence of biofilm producers was lower (41.7%) as compared to the findings by others such as Mohamed et al. (2007) (53.0%) who used Luria broth incubated for 18 h and fixed the bacteria with Bouin fixative prior to the rinsing step with phosphate buffered saline (PBS) and Merendez-arancibia et al. (2008) (78%) who used minimal glucose medium (M63) and incubated for 24 h. This could be because these studies were focused on one *E. coli* pathotypes, particularly the entero-aggregative *E. coli* (EAEC) previously known to be a biofilm producer. In addition, samples tested for biofilm were often collected from diarrheagenic stool samples and water samples whereas in this study water, stools, urine and sputum samples were used and could have resulted in lowering the overall percentages of biofilm producers. Biofilm producers were common among isolates from water and urine and among the clinical isolates, the prevalence was higher among female individuals. These findings are in agreement with the studies conducted by Sharma et al. (2009) who found a rate of 67.5% and Suman et al. (2007) who found a rate of 92.0%, which shows that there is an increased prevalence of biofilm formation by uropathogenic *E. coli* and females are in most cases at higher risks of acquiring *E. coli* induced UTIs.

Several studies conducted on the antibiotic susceptibility profiles of *E. coli* have shown a steady increase of antibiotic resistance amongst clinical isolates which remains a growing problem (Boczek et al., 2007). In the present study, higher resistance was observed against penicillin (~90%), followed by erythromycin, vancomycin, rifampicin (~80%) and amoxicillin (~60%), whereas lomefoxacin, kanamycin and meropenem (all less than 10%) were the most effective antibiotics against the isolates. These findings are in agreement with studies conducted by obi et al. (2004b, 2007) that showed that most *E. coli* strains isolated were highly resistant to penicillin (~90%) and amoxicillin (~60%) and susceptible to meropenem (~8%) and gentamycin (~10%). However, in this study, we found that there was a slight increase in resistance to the antibiotic gentamycin (14.4% compared to 10% found by obi et al. (2007). Majority of the resistant strains were isolated from stool followed by urine and water. Isolates from sputum had the least resistance. These findings are also in agreement with the study conducted by Obire et al. (2009) that showed that there was a slight increase in drug resistance in isolates from stool and urine (30.95%) and Walia et al. (2005) that showed an increase in drug resistance among isolates from water (92%) and urine (53%).

E. coli induced pneumonia is less common than enteric and urinary tract infections, and in some cases, the organism is rarely isolated from sputum samples. In a study conducted by Khan et al. (2002), 22 sputum samples were collected from which no *E. coli* was isolated. The pathogenicity of *E. coli* induced pneumonia is poorly understood (Jeyaseelan et al., 2007). The disease results from micro aspiration of the upper airway secretions that have been colonized with the organisms in severely immunocompromised patients, thus making it a common cause of nosocomial pneumonia.

Multiple drug resistance defined as resistance to three

or more antibiotics was also a common phenomenon among the isolates. In this study, it was found that 97.1% of the strains were resistant to multiple antibiotics. Although generally lower than our findings, Obi et al. (2007) also showed that close to 50% of the E. coli isolated were resistant to multiple antibiotics. A study conducted by Obire et al. (2009) also showed that multiple drug resistance was common among their isolates with most strains resistant to 7 followed by 6 and 4 antibiotics, similar to the results obtained in this study.

Study on the production of the enzyme beta-lactamase and extended spectrum beta-lactamase is important as it has been shown through several studies that strains that produce these enzyme show greater resistance to beta-lactam antibiotics rendering many penicillins and cephalosporins ineffective as therapy (Ruppe et al., 2009). In this study, beta-lactamase production was tested using two methods based on the same principle to compare for sensitivity and ease of application. The tube method was found to be the better method as it is was rapid, accurate and more sensitive and hence is recommended for testing for beta lactamase production if the iodometric method is used. The prevalence of beta lactamase producing strains (18%) was found to be lower than that obtained by Shibi (1995) who found a rate of 64% in a study conducted in Saudi Arabia. The antibiotic resistance profile of B-lactamase positive strains was also analyzed to determine whether these strains had a higher rate of resistance within the beta-lactam antibiotics as well as other classes of antibiotics. It was found that majority of beta lactamase producing strains were resistant mainly to gentamycin, amikacin and polymyxin B. Multiple drug resistance to 8, 9, 10 and 11 antibiotics was found to be higher among the beta lactamase positive strains. These findings are in agreement with the study conducted by Bristianou et al. (2008) who noted that this enzyme might be responsible for multiple drug resistant E. coli strains.

This study has for the first time described the capacity of local E. coli isolates to produce biofilm in relation to antibiotic susceptibility among E. coli isolates in the Limpopo Province. Increased drug resistance to some antibiotics as well as multiple drug resistance which may partially be due to production of the enzyme β-lactamase is of concern. Therefore, regular monitoring and further studies on multiple drug resistant E. coli strains is recommended to unveil the evolving nature of antibiotic resistance.

ACKNOWLEDGEMENTS

The authors are grateful to the staff and patients from Thsilidzini, Donald Fraser and ELIM hospitals. We are also grateful to the Department of Health in Polokwane. The present study was partially funded by a grant from the National Research Foundation to Dr Samie and other funding came from the University of Venda Research and Publication committee.

REFERENCES

Abong BO, Mwambakana TN, Momba MNB, Malakalo VK (2008). Prevalence of Escherichia coli 0157: H7 among diarrhoeic HIV/AIDS patients in the eastern cape province-South Africa. Pak. J. Biol. Sci. 118: 1066-1075.

Khan AN, Saba N, Samad A, Qazilbash AA (2002). Incidence and Antibiogram Patterns of Escherichia coli Isolated from Various Clinical Samples from Patients at N.I.H. Islamabad. Pak. J. Biol. Sci. 5: 111-113.

Amico P, Aran C, Avila C (2010). HIV spending as a share of total health expenditure: an analysis of regional variation in a multi-country study. PLoS One. 5(9): e12997.

Avelino F, Saldaña Z, Islam S, Monteiro-Neto V, Dall'Agnol M, Eslava CA, Girón JA (2010). The majority of enteroaggregative Escherichia coli strains produce the E. coli common pilus when adhering to cultured epithelial cells. Int. J. Med. Microbiol. 300(7): 440-8.

Boczek LA, Rice EW, Johnston B, Johnson JR (2007). Occurrence of antibiotic resistant Uropathogenic Escherichia coli clonal group A in wastewater effluents. J. Appl. Environ. Microbiol. 73: 4180-4184.

Bristianou M, Panagou C, Adamisa T, Raftogiannis M, Antonopoulou A, Chrisofos C, Galani I, Kanellakopoulou K, Tsaganos T, Giamarellos-Bourboulis EJ (2008). The impact of multidrug resistance on the pathogenicity of Escherichia coli: an experimental study. Int. J. Antimicrob. Ag. 31: 261-223.

Buchanan RL, Doyle MP (1997). Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic E. coli. A Scientific Status Summary of the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition, Chicago, Ill., Food Technol. 51(10): 69-76.

Christensen GD, Simpson WA, Bisno AL, Beachery EH (1982). Adherence of slime producing strains of Staphylococcus epidermidis to smooth surface. J. Infect. Dis. Immunol. 37: 318-426.

Clinical and Laboratory Standards Institute CLSI (2008). Performance standards for antimicrobial susceptibility testing: 18th informational supplement (M100-S18), CLSI, Wayne, PA.

Donnerberg MS, Nataro JP (2000). The molecular Epidemiology of Escherichai coli infections. Microbial foodborne diseases: Pathogenesis and toxin synthesis. Cary JW, Linz JE, Bhatnagar D (Editors). Technomic publishing company. New Holland. 3: 87-115.

Freeman DJ, Falkiner FR, Keane CT (1989). New method for detecting slime production by coagulase negative staphylococci. J. Clin. Pathol. 42: 872-874.

Jeyaseelan S, Young SK, Fessler MB, Liu Y, Malcom KC, Yamamoto M, Akira S, Worthen GS (2007). Toll/IL-1 receptor domain containing adaptor inducing IFN-β (TRIF)-mediated signaling contributes to inate immune responses in the lung during *Escherichia coli* pneumonia. J. Immunol. 178: 3153-3160.

Juhna T, Birzniece P, Larsson S, Zulenkovs D, Shapiro A, Azeredo NF, Menard-Szczebara F, Castaganet S, Feliers C, Keevil CW (2007). Detection of Escherichia coli in biofilm from pipe samples and coupons in drinking water distribution networks. J. Appl. Environ. Microbiol. 73: 7456-7464.

Koneman EW, Allen SD, Dowell VR, Somers HN (1983). Colour atlas and textbook of diagnostic microbiology 2nd edition. JB lippin cott company USA.

Lim JY, Yoon J, Hovde CJ (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. J. Microbiol. Biotechnol. 20(1): 5-14.

Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A (2006). Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian J. Med. Microbiol. 24: 25-29.

Mohamed JA, Huang DB, Jiang Z, DuPont HL, Nataro JP, Belkind-Gerson J, Okhuysen PC (2007). Association of putative Enteroaggregative Escherichia coli virulence genes and biofilm production in isolates from travelers to developing countries. J. Clin. Microbiol. 45: 121-126.

Obi CL, Bessong PO, Momba MNB, Potgieter N, Samie A, Igumbor EO (2004). Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities, South Africa. Water SA. 4: 519-520.

Obi CL, Ramalivhana J, Momba MNB, Onabolu B, Igumbor EO, Lukoto M, Mulaudzi TB, Bessong PO, Jansen van Rensburg EL, Green E, Ndou S (2007). Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhoea and their household drinking water in rural communities in Limpopo Province South Africa. Afr. J. Biotechnol. 6: 1035-1047.

Obire O, Dumka G, Putheti R (2009). Antibiotic resistance in *Escherichia coli* isolated from patients. Drug Invent. Today, 1(2): 140-145.

Rijavec M, Muller-premru M, Zakdnik B, Zgur-Bertok D (2008). Virulence factors and biofilm production among *Escherichia coli* strains causing bactereamia of urinary tract origin. J. Med. Microbiol. 57: 1329-1334.

Rossit AB, Goncalves AC, Franco C, Machado RL (2009). Etiological agents of diarrhea in patients infected by the Human Immunodeficiency Virus-1. Rev. Inst. Med. Trop. Sao Paulo. 51: 59-65.

Ruppe E, Sophea H, Sovannarith L, Valerieh GA (2009). CTX-M(beta)-lactamses in *Escherichia coli* from community-acquired urinary tract infections, Cambodia. *Emerging infectious Diseases.* The free library 2009. Available from http://www.thefreelibrary .com/ CTX-M(beta)-lactamses in *Escherichia coli* from community-acquired….90201212073. [Retrieved 10 February 2010].

Sharma M, Aparna, Yadav S, Chaudhary U (2009). Biofilm production in uropathogenic *Escherichia coli*. Indian J. Pathol. Microbiol. 52: 294-294.

Shibi AM (1995). Incidence of beta-lactamase production and antibiotic susceptibilities among clinical isolates in Saudi Arabian hospitals. Curr. Ther. Res. 56: 407-414.

Suman E, Jose J, Varghese S, Kotian MS (2007). Study of biofilm production in *Escherichia coli* causing urinary tract infection. Indian J. Med. Microbiol. 25: 305-306.

Vignesh R, Shankar EM, Murugavel KG, Kumarasamy N, Sekar R, Irene P, Solomon S, Balakrishnan P (2008). Urinary infections due to multi-drug-resistant *Escherichia coli* among persons with HIV disease at a tertiary AIDS care centre in South India. Nephron. Clin. Pract. 110(1): c55-7.

Vogt RL, Dippold L (2005). *Escherichia coli* 0157:H7 outbreak associated with consumption of ground beef, June-July 2002. Public Health Rep. 120: 174-178.

Walia SK, Kaiser A, Parkash M, Shaundry GR (2005). Self transmissible and antibiotic resistance to ampicillin, streptomycin and tetracycline in *Escherichia coli* from contaminated drinking water. J. Environ. Sci. Health [A]. 31: 651-662.

Wang J, Hseuh PR, Wang JT, Lee LA, Yang PC, Luh KT (2000). Recurrent infections and chronic colonisation by an *Escherichia coli* clone in the respiratory tract of a patient with severe cystic bronchiectasis. J. Clin. Microbiol. 38: 2766-2767.