Full Length Research Paper

Escherichia coli pathotypes among human immunodeficiency virus infected patients in the Limpopo Province

Samie A.[1]*, Nkgau T. F.[1], Bessong P. O.[1], Obi C. L.[2], Dillingham R.[3] and Guerrant R. L.[3]

[1]Department of Microbiology, University of Venda, Private Bag X5050, Thohoyandou 0950, Limpopo, South Africa.
[2]Academic and Research Division, Walter Sisulu University, Mthatha, South Africa.
[3]Centre for Global Health, School of Medicine, University of Virginia, Charlottesville, Va, USA.

In the present study, *Escherichia coli* strains isolated from clinical samples including stool, sputum and urine from HIV and AIDS patients as well as the isolates from water samples were tested for the presence of putative virulence genes in association with biofilm production, antibiotic resistance and beta lactamase production. From a total of 139 *E. coli* isolates, Entero aggregative *E. coli* was the most common pathotype in stools (52%) while Entero pathogenic *E. coli* was the most common in urine (24%) and water (39%). Isolates that were positive for *bfp*A and *eae* had the highest rates of biofilm production with 30.4% and 28.6% strong biofilm production rates respectively. *Agn*43 was found in about 44% of the isolates and biofilm formation was observed among 21.4% of the isolates positive for this gene. Enteroaggregative *E. coli* were more associated with multi drug resistance as well as beta lactamase production. The findings of this study suggest that water might be a source of infection for pathogenic *E. coli* UTIs in HIV patients and that Enteroaggregative *E. coli* remains a health hazard to HIV and AIDS patients in the Limpopo Province.

Key words: Antibiotic resistance, diarrhea, pneumonia, *Escherichia coli*, HIV and AIDS, epidemiology, Venda, South Africa.

INTRODUCTION

Infection with pathogenic strains of *Escherichia coli* can results in four main clinical syndromes such as enteric diarrheal diseases, urinary tract infections, sepsis and meningitis, of which diarrhea and urinary tract infections are the most common. With the advent of HIV and AIDS, pathogenic *E. coli* have become one of the most common opportunistic pathogens and previous studies in the Venda region have shown that Enteroaggregative *E. coli* is particularly common in stool samples of HIV positive patients compared to HIV negative patients, while the occurrence of other pathotypes of *E. coli* in this group of patients is not clearly defined (Samie et al., 2007). Furthermore, the mechanisms of pathogenicity of these strains have not been thoroughly investigated with particular reference to biofilm production.

Previous studies in rural areas of the Venda region have indicated high level of contamination of water sources used by the population (Obi et al., 2004) as well as weaning food consumed by children (Potgieter et al., 2005). However, the role of these organisms as opportunistic pathogens as well as their antibiotic resistance patterns is not well documented. *E. coli* strains harboring virulence genes are pathogenic and may cause diseases in humans and animals. Pathogenic and opportunistic *E. coli* have been recognized as disease causing agents since the early 1920s and are responsible

*Corresponding author. E-mail: samieamidou@yahoo.com, samie.amidou@univen.ac.za.Tel: +27159628186, +27760952916. Fax: 27 15 962 4749.

for 4 main clinical syndromes in humans. These includes; diarrhea (gastroenteritis), urinary tract infections, septicemia and neonatal meningitis (Nataro et al., 1998). Although generally rare, pathogenic *E. coli* can also cause hemolytic uremic syndrome (HUS) and pneumonia. Pathogenic *E. coli* strains are very versatile and pose different virulence factors such as adhesions, invasions, toxins and adverse defense mechanisms to the host response (Todar, 2009).

E. coli induced UTIs accounts for more than 90% of uncomplicated urinary tract infections in humans (Vagrali, 2009). The disease occurs worldwide and in most cases affects healthy individuals, majority being females within the age range of 18 to 24 (Marrs et al., 2005). Septicemia and neonatal meningitis have also been shown to be important emerging opportunistic infections in developing countries (Johnson and Russo, 2002). Over the past years, *E. coli* has been implicated as a causative agent of respiratory tract infections especially pneumonia. *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 Akhtar 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. 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; Bahadin et al., 2011). Clinically, *E. coli* induced respiratory tract infections is manifested by fever, shortness of breath, and increased respiratory secretions and may often appear as bronchopneumonia of the lower lobes (Wang et al., 2000). The present study was conducted to determine the different pathotypes of *E. coli* in urine, sputum, stool and water samples from HIV and AIDS patients in the Limpopo Province of South Africa in relation to biofilm formation and antibiotic susceptibility.

MATERIALS AND METHODS

Ethical consideration and patients

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 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.

Sample collection and analysis

De-identified clinical samples including stools, sputum and urine were collected from HIV positive patients in different communities in the Limpopo province. Water samples were collected from households of HIV positive individuals in sterile plastic bottles using the cups found in the household and transported in a cooler box with ice to the Laboratory of Microbiology at the University of Venda. Collected samples were cultured on either Eosine Methylene Blue (EMB) agar or MacConkey agar (Oxoid, England) for 24 h at 37°C for the detection of *E. coli*. A single colony from each positive sample was subcultured on nutrient agar for 24 h at 37°C. The cultures were preserved in Brain Heart Infusion (Oxoid, England) and 25% glycerol and kept in the freezer prior to the different tests and subcultured on a new nutrient agar plate when deemed necessary.

Biofilm formation and antibiotic susceptibility profiles

Biofilm production was determined as previously described using the microtitration plate method (Mohammed et al., 2007). The antibiotic susceptibility profiles were determined using the disc diffusion method according to the CLSI recommendations (CLSI, 2010).

Detection of EHEC 0157: H7 using sorbitol MacConkey

Previous reports have indicated that EHEC 0157:H7 can be distinctively identified on Sorbitol MacConkey (Oxoid, England). Samples were subcultured on Sorbitol MacConkey agar for 24 h at 37°C. An isolate was considered positive if it appeared as clear colonies on the media while pink colonies were considered negative. *E. coli* 0157 was used as a positive control while *E. coli* ATCC 25922 was used as a negative control.

DNA isolation

About 10 colonies of the bacterial isolate, was resuspended in 1 ml of sterile distilled water followed by centrifugation for 5 min at 13400 rpm in an Eppendorf Microcentrifuge. The supernatant was decanted and the cells resuspended in 200 µl of sterile distilled water. Bacterial DNA was isolated by boiling the suspension for 15 min followed by centrifugation for 15 min at 13400 rpm (Eppendorf). The supernatant which contained the DNA was transferred into a new tube and kept at -20°C until further use.

Polymerase chain reaction (PCR) identification of *E. coli*

The isolates were further identified as *E. coli* by the amplification of portion of the 16S rRNA gene of *E. coli* (Juhna et al., 2007). The amplicons were then resolved in 1.5% agarose gels at a voltage of 80 for 45 min. The gel images were then taken using the gel documentation system (Vacutec).

DNA amplification for the detection of pathogenic genes

The isolates were tested for the presence of pathogenic genes including transcriptional activator of AAF/I (*agg*R), bundle forming pilus gene (*bfp*A), intimin (*eae*), verotoxin (*vt*), heat labile toxin gene

(*lt*), heat stable toxin gene (*st*), diffuse adherence structural subunit (*daa*E), virulence invasion factor (*Vir*F), invasion plasmid antigen (*Ipa*H), and antigen 43 (*Agn*43). The PCR amplifications were conducted in 2 different procedures using the primers as previously described by Gomez-duarte et al. (2008).

The first amplification of the pathogenic genes with the exception of *Agn*43 was done as described by Gomez-duarte et al. (2008). The reaction was performed in 25 µl final volume, containing the oligonucleotides (Inqaba biotec, Pretoria, SA), dream tag master mix (Fermentas, Burlington, Canada) and 5 µl of the template DNA. The primers were used in a multiplex PCR. The primers for *agg*R, *bfp*A, *VT* and *eae* were ran as multiplex 1 whereas LT, ST, daaE, VirF, IpaH were ran as multiplex 2. The multiplex PCR conditions were as follows; 2 minutes at 94°C for denaturing followed by 40 cycles of amplification at 92°C for 30 s, 59°C for 30 s and 72°C for 30 s and a final elongation step of 5 min at 72°C.

Amplification of agn43 was done as described as Merendez-aranchibia et al. (2008). The PCR amplification programs were as follows; 1 cycle of denaturation at 94°C for 5 min, 30 cycles of amplification at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min and final elongation step of 10 min at 72°C. The PCR products were analyzed on 2% agarose gel and stained with ethidium bromide.

Statistical analysis

All the results obtained were typed on an excel sheet for further 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 2 variables was considered significant if the p value was less than 0.05.

RESULTS

General characteristics of the study sample

In the present study, a total of 139 *E. coli* strains from as much patients were characterized for their capacity to produce biofilm, their antibiotic susceptibility profiles, their capacity to produce β-lactamase and their pathotypes. 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, 16 were from males and 1 was of unknown sex. Samples were collected from individuals aged between 4 and 79 years.

EHEC 0157:H7 testing using sorbitiol MacConkey agar

Among the isolates tested using the SMAC test 15 (10.8%) were positive. Of these 15 isolates, 12 were from stool samples, while 1 was from urine sample and 2 were from water samples. Eleven isolates were from females while 1 isolate was from a male.

PCR detection of pathogenic genes

E. coli species confirmation by PCR

Although identified by culturing on eosine methylene blue agar (EMB) MacConkey agar (Oxoid, England), detection of the 16S rRNA was done to confirm whether the isolates were true *E. coli* strains. Among the 142 strains initially screened by EMB and MacConkey agar, 139 (98% strains tested positive by PCR and are the strains that were used in all results analysis.

Detection of pathogenic genes among the E. coli isolates

Among the *E. coli* strains studied for the various pathogenic genes, the most common genes were *AggR* with 31 (22.3% prevalence while 23 (16.6% were positive for bfpA (bundle forming pilus gene). Table 1 shows the distribution of the genes according to sample type. Among the 139 *E. coli* isolates 95 (66.3%) strains were positive for at least one of the virulence genes tested while 44 (31.7%) were negative for all the genes tested. Figures 1 and 2 show the pictures of the agarose gels of the PCR products for various pathogenic genes detected by PCR.

Distribution of pathogenic genes by sample type

AggR was detected mostly in isolates from stools 51.7% while only 3.9% was from water and none from sputum and urine. Amongst the stools samples 15 were diarrhea and 43 were non diarrhea. AggR was detected in 40% of the diarrheal stool samples and 55.8% of the non diarrhea stool samples. Other genes such as bfpA were found more in isolates from water 38.5% followed by urine 24.4% and stool 3.4%. The difference among the different sample types were statistically significant (p< 0.001). Genes such as eae, ST, LT, daaE and VirF were found mostly in isolates from urine however the difference was not statistically significant (P=0.218). The organisms from sputum were negative for all the genes tested (Table 1).

Distribution of pathogenic genes by sex

Among those strains isolated from the different samples, aggR was higher among males 39.1% compared to females 30.3%). Similarly *bfp*A was also detected more in males 13.0% than in females 10.6%) females. *eae* was detected only in females 4 (6.1% while none was detected in males. VT was detected in 2 (3% females and 1 (4.3% in males whereas LT was detected in 13 (19.7% females and 2 (8.7% males. ST was detected in 7 (10.6% females and 1 (4.3% males and 1 (4.2% in isolates of
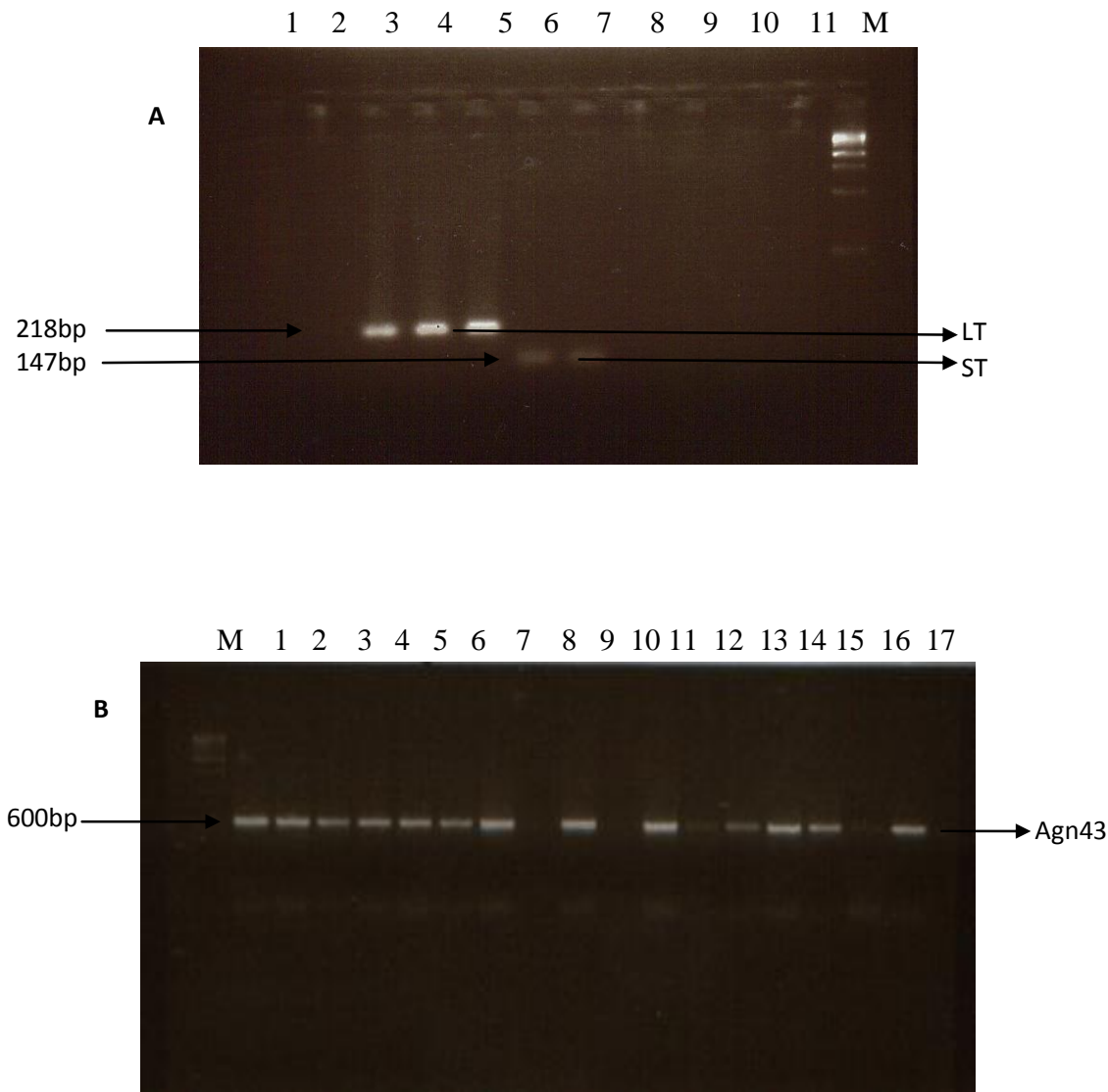
Table 1. Distribution of pathogenic genes by sample type. The percentages represent the occurrence of the specific gene in the type of samples.

Gene	Strain	Stool (n=58) (%)	Sputum (n=10) (%)	Urine (n=45) (%)	Water (n=26) (%)	Total	P value
agn43 (n=61)	-	32 (55.2)	0 (0)	17 (37.8)	12 (46.2)	61	0.241
aggR (n=31)	Entero aggregative *E. coli*	30 (51.7)	0 (0)	0 (0)	1 (3.8)	31	0.010
bfpA (n=23)	Enetro pthogenic *E. coli*	2 (3.4)	0 (0)	11 (24.4)	10 (38.5)	23	0.000
eae (n=7)	Enetro pthogenic *E. coli*/ Entero heamorrhagic *E. coli*	2 (3.4)	0 (0)	4 (8.9%)	1 (3.8)	7	0.511
VT (n=3)	Entero heamorrhagic *E. coli*	1 (1.7)	0 (0)	1 (2.2)	1(3.8)	3	0.918
LT (n= 17)	Entero toxigenic *E. coli*	7 (12.1)	0 (0)	10 (22.2)	0 (0)	17	0.102
ST (n=10)	Entero toxigenic *E. coli*	5 (8.6)	0 (0)	4 (8.9)	1 (3.8)	10	0.663
daaE (n=1)	Entero-Invasive *E. coli*	0 (0)	0 (0)	1 (2.2)	0 (0)	1	0.551
virF (n=1)	Diffusely adherent *E. coli*	0 (0)	0 (0)	1 (2.2)	0 (0)	1	0.551
Total		79	0.0	48	26	154	

Figure 1. Representative pictures of agarose gel electrophoresis of the PCR products of the genes; aggR, eae, bfpA and VT. A is a representative picture of the pathogenic gene aggR, Lane M is the molecular ladder, Lane N is the negative control, PC is the positive control (EAEC 042) and lanes 1 to 5 are positive samples. B is a representative picture of the pathogenic genes VT, bfpA and eae. Lane M is the molecular marker, Lane N is the negative control, lane 1-3 are positive VT samples, lane 5 is positive control, Lane 6-8 are positive samples for eae and lane 9-11 are positive samples for bfpA.

Figure 2. Representative pictures of agaraose gel electrophoresis of the PCR products of the genes LT and ST (A) and agn43 (B). In A Lane M is the molecular marker, Lane 3-5 are samples positive for LT gene, lane 6-7 are samples positive for ST gene. In B Lane M is the molecular marker, Lane 1 to 17 are positive isolates with the exception of Lane 8 and 10 representing negative PCR products of the gene agn43.

unknown sex origin. Both daaE and VirF were detected in samples obtained from females. Overall, pathogenic genes were more common among females compared to males, however the differences were not statistically significant (p value all greater than 0.05).

Distribution of pathogenic genes amongst biofilm producers

The presence of pathogenic genes was compared among the non biofilm producers, moderate and strong biofilm producers. Among the strong biofilm producers, 59.1% of *agn*43 gene was detected followed by 31.8% of *bfp*A,

9.1% of *eae* and 13.6% of LT gene were more often detected amongst the strong biofilm producers while 13.9% of ST, 2.8% of VT were more often detected among the moderate producers. Among the non biofilm producers, 25.9% of *Agg*R, 1.2% 0f daaE and 1.2% of VirF were detected. However the differences were not statistically significant (Table 2).

Antibiotic resistance and pathogenic genes

In regard to antibiotic resistance 9.7% of the isolated displayed resistance to meropenem, 13.0% to lomefloxacin, 9.7% to kanamycin and 61.3% to

Table 2. Distribution of pathogenic genes amongst biofilm producers. The percentages represent the rate of biofilm production among isolates positive for the specific gene.

Gene	Non biofilm producer (n=81) (%)	Moderate biofilm producers (n=36) (%)	Strong biofilm producers (n=22) (%)	P value
AggR (31)	21 (67.7)	7 (22.6)	3 (9.6)	0.157
Ag43 (61)	31 (50.8)	17 (27.8)	13 (21.3)	0.195
bfpA (23)	10 (43.5)	6 (26.1)	7 (30.4)	0.067
Eae (7)	3 (42.8)	2 (28.6)	2 (28.6)	0.584
VT (3)	2 (66.7)	1 (33.3)	0 (0)	0.480
Lt (17)	10 (58.8)	4 (23.5)	3 (17.6)	0.959
St (10)	3 (30)	5 (50)	2 (20)	0.134
VirF (1)	1 (100)	0.0	0.0	0.697
daaE (1)	1 (100)	0.0	0.0	0.697

Table 3. Antibiotic resistance distribution among the different pathotypes of *E. coli*.

Strains / Antibiotics	EAEC AggR n=31 (%)	EPEC bfpA n=23(%)	EHEC/EPEC Eae n=7 (%)	VT n=3 (%)	ETEC LT n=17(%)	ST n=10(%)	DAEC daaE n=1(%)	EIEC virF n=1 (%)	Total resistance (%)
Penicillin G	71.0	82.6	100	100	94.1	100	100	100	91.4
Amoxycillin	58.1	52.2	57.1	100	58.8	60	100	100	64.7
Cefepime	22.6	78.3	71.4	0.0	41.2	40	0.0	100	44.6
Meropenem	9.7	13.0	0.0	0.0	5.9	20	0.0	0.0	5.8
Nalidixic acid	19.4	17.4	14.3	66.7	11.8	20	0.0	0.0	15.1
Lomefloxacin	13.0	8.7	0.0	0.0	5.9	20	0.0	0.0	4.3
Amikacin	16.1	13.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8
Gentamycin	16.1	13.0	14.3	33.3	0.0	0.0	100	0.0	14.4
Kanamycin	9.7	0.0	14.3	0.0	11.8	20	0.0	0.0	6.5
Streptomycin	45.2	30.4	28.6	33.3	35.3	30	100	100	44.6
Tetracycline	80.6	52.2	71.4	33.3	70.6	80	0.0	100	53.2
Chloraphenicol	61.3	17.4	14.3	0.0	28.4	20	0.0	100	24.5
Polymyxin B	61.3	60.9	57.1	66.7	29.4	30	100	100	57.6
Rifampicin	87.1	65.2	100	66.7	47.1	70	100	100	87.3

Table 4. Distribution of pathogenic genes among multiple drug resistant strains.

Number of antibiotics to which the organisms were resistant	Number of genes detected among the resistant strains						Total
	No gene	AggR	bfpA	Eae	LT/St	daaE	
3 (n=2)	1	0	1	0	0	0	2
4 (n=15)	4	0	7	3	1	0	15
5 (n=18)	16	0	0	0	1	1	18
6 (n=25)	10	3	8	2	2	0	25
7 (n=21)	4	13	1	2	1	0	21
8 (n=16)	7	9	0	0	0	0	16
9 (n=14)	7	5	1	0	1	0	14
10 (n=15)	7	1	4	2	1	0	15
11 (n=7)	5	0	0	0	2	0	7
12 (n=1)	0	0	0	0	1	0	1
13 (n=1)	1	0	0	0	0	0	1
Total	62	31	22	9	10	1	135

chloramphenicol were high among EAEC while resistance to penicillin was varied with 82.6% resistance and in cephalosporins, there was 78.3% resistance to cefepime and 13.0% resistance to meropenem among EPEC strains. Resistance to penicillin and vancomycin were high among EHEC strains while resistance to penicillin, amoxicillin, erythromycin and vancomycin were high among ETEC strains (Table 3).

Pathogenic genes and multiple drug resistance

Resistance to 3, 4 and 6 antibiotics at a time was higher among isolates positive for the *bfpA* gene (EPEC) while resistance to 7, 8 and 9 antibiotics were higher among strains positive for the *aagR* gene (EAEC). Resistance to 12 antibiotics at a time was found only in strains positive for the *lt* and *st* gene (ETEC). Majority of the strains resistant to 5 and 10 antibiotics were found in strains negative for the genes tested (Table 4).

Pathogenic genes and beta lactamase

From the 25 strains found to be beta-lactamase producers, aggR was detected in 24.0%, followed by *bfp*A that was detected in 12.0% while *eae, LT, ST* and daaE were found in 1(4%) each. VT, VirF and IpaH were not detected in any of the beta-lactamase producing 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). Studies on the prevalence as well as pathogenic mechanisms of diarrheagenic and extraintestinal *E. coli* isolated from human patients have received very little attention in Africa particularly in South Africa. Prevalence studies on the various *E. coli* pathotypes are important since it has been shown through various studies that the prevalence of diarrheagenic *E. coli* is region specific (Heine, 2007). This study was set out to determine the occurrence of the various *E. coli* pathotypes as well as their significance as intestinal and extraintestinal opportunistic pathogens in the Venda region of South Africa and to determine the effect of biofilm and beta lactamase production on the pathogenesis and antibiotic resistance profile amongst the isolates.

Over the past years biofilm production has been tested in various ways. In this study the organisms were screened for biofilm formation and then tested for the various pathogenic genes. *Agg*R commonly described as transcriptional activator in EAEC was common among the non biofilm producers as well as the biofilm producers with *ang*43 being the most detected gene among the biofilm producers, followed by bundle forming pilus and intimin coding for EPEC. Previous studies conducted by Mohamed et al. (2007) and Wakimoto et al. (2004) showed that *Agg*R was a major regulator among biofilm producers and as result biofilm production or PCR detection of *Agg*R has been used to identify EAEC. EAEC is not the only *E. coli* pathotypes capable of biofilm production. Moreira et al. (2006) showed that EPEC is capable of biofilm formation and that the genes involved in this mechanism are *bfpA* and *EspA*. This correlated well with our study findings as we also found EPEC being the second most common pathotypes forming biofilm.

Biofilm production by other pathotypes has received very little attention. Very few ETEC and EHEC were involved in biofilm formation whereas no EIEC and DAEC were shown to form biofilm. Similarly, a previous study by Chen et al. (2010) indicated that biofilm production was more associated with the genotype of *E. coli* while there was only a weak association between biofilm formation and antibiotic resistance.

In the present study, majority of the biofilm producers were found in isolates from water and urine samples. Several studies have also shown that biofilm production is becoming an important pathogenic mechanism among uropathogenic strains of *E. coli*. Suman et al. (2007) reported a high rate (92%) of biofilm producing uropathogenic *E. coli*. Biofilm production among UPEC is partially responsible for persistent and often chronic UTIs. Recent studies by Blango and Mulvey (2010) indicated that the persistence of UPEC within the bladder, regardless of antibiotic treatments, was likely facilitated by a combination of biofilm formation, entry of UPEC into a quiescent or semiquiescent state within host cells, and the stalwart permeability barrier function associated with the bladder urothelium. Although previous studies have not identified the origin of UPEC among patients, the present study demonstrated similarities among urinary *E. coli* isolates and water isolates. Further studies are needed to clarify the role of water in the transmission of uropathogenic *E. coli* among HIV and AIDS patients.

Enteroaggregative *E. coli* described about 2 decades earlier is an emerging diarrheagenic agent particularly among HIV and AIDS patients (Nataro et al., 1987; Gassama-sow et al., 2004). In the present study EAEC was found to be the most prevalent pathotypes from stool samples (51%) and only 1(strain was found in isolates from water. This indicates that EAEC is an important diarrheagenic pathogen among HIV and AIDS patients in the Limpopo Province. Similar findings have been described here (Samie et al., 2007) and elsewhere (Gassama-sow et al., 2004). However, Obi et al. (2004) found that EAEC was not as common in stools and water samples. In a study conducted in Costa Rica, enteropathogenic *E. coli* and enteroinvasive *E. coli* pathotypes were the most prevalent (21 and 19%, respectively) in children population (Pérez et al., 2010). This difference might be due to the variation in the type of patients as well as the sources of the different samples collected. Poor personal hygiene, contaminated food and water might be a source of infection with EAEC in these patients. Several studies have described that contaminated food and water hygiene are the main vehicles of transmission with EAEC (Huang et al., 2006).

EPEC, one of the first pathotype to be implicated as causative agents of diarrhea was the second most common pathotypes isolated and was found mainly in samples from water (38.5%) and urine (24.4%). Obi et al. (2004) also found that EPEC (34.1%) was also the most prevalent pathotype isolated from water samples

collected from various rivers in Venda region. The high prevalence of EPEC in water may be due to fecal contamination of water used by the population. Some of these patients are from poverty stricken communities and do not have access to safe clean drinking water neither can they afford purification reagents. The high prevalence of EPEC in water may be a source of infection with uropathogenic *E. coli* in HIV and AIDS patients in the Limpopo Province. Very few ETEC (7.2%), EHEC (6.5%), DAEC (0.7%) and EIEC (0.7%) were isolated from both the clinical and water samples. Similarly in a study conducted by Okeke et al. (2003) very few ETEC, EHEC and no EIEC were found, however their prevalence of DAEC was higher (15.4%) in their study.

Bacteriuria is common in HIV patients and a recent study had estimated that about the most common bacterial organism was *E. coli* representing about 31% of the coliforms (Njunda et al., 2009). Although several studies have described the occurrence of *E. coli* organisms in Urinary samples from HIV, very few have determined their pathogenic characteristics in terms of pathogenic genes as well as biofilm formation. 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%, that 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. In the present study the most common pathotypes of *E. coli* found in urine samples was EPEC which was similar to the most common type found in water. Further studies are needed in order to determine the potential mode of transmission of these organisms in water samples as well as different risk factors associated with the occurrence of these *E. coli* organisms in urine samples.

Conclusion

EAEC was confirmed as the most common diarrheagenic *E. coli* strain among HIV and AIDS patients while EPEC might be the most common cause of *E. coli* UTIs among these patients. The isolation of EPEC from urine samples and water samples at higher rates may indicate that, water might be a source of infection of HIV and AIDS patients with uropathogenic strains of *E. coli* in the Limpopo Province. Biofilm production and the presence of putative virulence genes may be responsible for the diarrheal symptoms as well as recurrent urinary tract infections among HIV and AIDS patients. The high prevalence of EPEC strains in water is also of concern as it poses a health hazard to those individuals using the water for everyday life. Further characterization of these pathogenic strains will improve their detection and

possibly the design of protective substances such as vaccines and will constitute a milestone in the control of opportunistic infections among HIV and AIDS patients in this part of the world and elsewhere.

ACKNOWLEDGEMENTS

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.

Akhtar N, Khan, Noor-us-Saba, Samad A, Abbas A, Qazilbash A (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.

Bahadin J, Teo SS, Mathew S (2011). Aetiology of community-acquired urinary tract infection and antimicrobial susceptibility patterns of uropathogens isolated. Singapore Med. J. 52(6):415-20.

Blango MG, Mulvey MA (2010). Persistence of uropathogenic *Escherichia coli* in the face of multiple antibiotics. Antimicrob. Agents Chemother. 54(5):1855-63.

Chen C, Liao X, Jiang H, Zhu H, Yue L, Li S, Fang B, Liu Y (2010). Characteristics of *Escherichia coli* biofilm production, genetic typing, drug resistance pattern and gene expression under aminoglycoside pressures. Environ. Toxicol. Pharmacol. 30(1):5-10.

Donnerberg MS, Nataro JP (2000). Chapter 3. 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. pp. 87-115.

Gassama-Sow, Sow PS, Gueye M, Gueye N, Perret JL, Mboup S, Aıdara-Kanel A (2004). Characterisation of pathogenic *E. coli* in Human immunodeficiency Virus- related diarrhea in Senegal. J. Infect. Dis. 189:75-78.

Gomez-Duarte OG, Bai J, Newell E (2008). Detection of *Escherichia coli, Salmonella spp, Shigella spp, Yersinia Enterolitica, Vibrio cholera, and Campylobacter spp.* Enteropathogens by a 3-reaction multiplex Polymerase chain reaction. J. Diag. Microbiol. Infect. Dis. 63:1-9.

Heine L (2007). Optimization of culture methods for the selective isolation of diarrhoeagenic *Escherichia coli* from water. Master's thesis. University of Johannesburg. pp.1-108. (Available from htt://ujdigspaceuj.ac.za:8080/dspace/ bitsream/) [Retrieved 3 December 2009].

Huang PB, Mohanty A, Dupont HL, Okhuysen PC, Chiang T (2006). A review of an emerging enteric pathogen Enteroaggregative *Escherichia coli*. J. Med. Microbiol. 55:1301-1311.

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.

Johnson JR, Russo TA (2002). Extraintestinal *Escherichia coli*: "The other bad *E. coli*". J. Lab. Clin. Med. 139:155-162.

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.

Marrs CF, Zhang L, Foxman B (2005). *Escherichia coli* mediated Urinary tract infections: Are there distinct uropathogenic *E. coli* (UPEC) pathotypes? FEMS Microbiol. Let. 252:183-190.

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.

Moreira CG, Palmer K, Whiteley M, Sircili MC, Trabulsi LR, Castro AFP, Sperandio V (2006). Bundle-Forming Pili and EspA Are Involved in Biofilm Formation by Enteropathogenic *Escherichia coli*. J. Bacteriol. 188:3952-3961.

Nataro JP, Kaper JB, Robin-Brocono R, Prudo V, Vial P, Levine MM (1987). Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. J. Paediat. Infect. Dis. 6:829-831.

Nataro JP, Steiner T, Guerant RL (1998). Enteroaggregative *Escherichia coli*. J. Emerg. Infect. Dis. 4:251-261.

Njunda L, Kamga HL, Palle JN, Mokube JA, Assob Nguedia JC, Achidi EA, Akuro S, Mbuagbaw J, Ndumbe PM (2009). Coliforms Associated With Bacteriuria in Human Immunodeficiency Virus (HIV) Positive Patients Attending The Bamenda Provincial hospital. Internet. J. Infect. Dis. 7(2).

Obi CL, Green E, Bessong PO, de Viliers B, Hoosen AA, Igumbor EO, Potgieter N (2004). Gene encoding virulence markers among *Escherichia coli* isolates from diarrhea stool samples and river sources in rural Venda communities of South Africa. Water SA 30:37-42.

Okeke IN, Ojo O, Lamikanra A, Kaper JB (2003). Etiology of acute diarrhea in adults in Southwestern Nigeria. J. Clin. Microbiol. 41:4525-4530.

Pérez C, Gómez-Duarte OG, Arias ML (2010). Diarrheagenic *Escherichia coli* in children from Costa Rica. Am. J. Trop. Med. Hyg. 83(2):292-7.

Potgieter N, Obi CL, Bessong PO, Igumbor EO, Samie A, Nengobela R (2005). Bacterial contamination of vhuswa- A local weaning food and stored drinking water in impoverished households in the Venda region of South Africa. J. Health. Pop. Nutr. 23:150-155.

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. S. Paulo. 51:59-65.

Samie A, Obi CL, Dillingham R, Pinkerton RC, Guerrant RL (2007). Enteroaggregative *Escherichia coli* in Venda, South Africa: Distribution of virulence-related genes by Multiplex Polymerase Chain Reaction in stool samples of Human Immunodeficiency Virus (HIV)-positive and HIV-Negative individuals and primary school children. Am. J. Trop. Med. Hyg. 77:142-150.

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.

Todar K (2009). Online textbook of bacteriology. University of Wisconsin department of bacteriology. http://www.textbookofbacteriology.net/e.coli ./html [Retrieved 01 April 2009].

Vagrali MA (2009). Sideosphores production by uropathogenic *Escherichia coli*. Indian J. Pathol. Microbiol. *52*:126-127.

Wakimoto N, Nishi J, Sheikh J, Nataro JP, Sarantuya J, Iwashita M, Manago K, Tokuda K, Yoshinaga M, Kawano Y (2004). Quantitative biofilm assay using a microtiter plate to screen for enteroaggregative *Escherichia coli*. Am. J. Trop. Med. Hyg. 71:687-690.

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.