academicJournals

Vol. 8(43), pp. 3678-3686, 22 October, 2014
DOI: 10.5897/AJMR2014.7034
Article Number: DB74D3048269
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Virulence factors and antibiotic resistance patterns of uropathogenic *Escherichia coli*

Joshua Mbanga* and Raymond Mudzana

Department of Applied Biology and Biochemistry, National University of Science and Technology, P.O Box AC 939 Ascot, Bulawayo 00263, Zimbabwe.

Received 25 July, 2014; Accepted 29 September, 2014

Urinary tract infections (UTIs) are one of the most common infections in humans and the commonest cause is uropathogenic *Escherichia coli* (UPEC). UPEC possess various virulence factors which enable them to survive and grow in urine and other extra-intestinal environments. Similarly, avian pathogenic *E. coli* (APEC) are known for their ability to cause extra-intestinal diseases in birds. Since APEC and UPEC may encounter similar challenges when establishing infection in these locations, they may share a similar content of virulence genes and capacity to cause disease. In this study, 40 UPEC isolates were obtained from patients with suspected UTIs. Multiplex polymerase chain reaction (PCR) was then used to screen the 40 UPEC isolates for 12 virulence genes usually associated with APEC isolates. The *iutA* (35%), *fimH* (32.5%), *vat* (17.5%), *sitA* (17.5%), *sitD* (15%), *hlyF* (12.5%), *pstB* (10%) and *frz* (7.5%) genes were detected. None of the isolates had the *kpsM*, *ompT, uvrY* and *sopB* genes. Antibiotic resistance patterns were also determined for all 40 isolates. A high resistance to ampicillin (90%) and tetracycline (75%) accompanied by a high sensitivity to gentamycin (82.5%) and nitrofurantoin (62.5%) was observed. Eleven multi-drug resistance patterns were observed in 65% (26/40) of the isolates. The studied UPEC isolates were shown to possess APEC associated virulence genes at low percentage frequencies suggesting a slight overlap in virulence genotypes. Antibiotic resistance patterns suggest surveillance programs to monitor drug resistance should be put in place.

Key words: Uropathogenic *Escherichia coli*, virulence genes, multiplex polymerase chain reaction (PCR), antibiotic resistance, Zimbabwe.

INTRODUCTION

Pathogenic strains of *Escherichia coli* are responsible for urinary tract infections (UTIs) in humans. Despite the great wealth of knowledge on *E. coli*, it is still the commonest urinary tract pathogen causing 60–90% of infections (Cheesbrough, 2006; Barati et al., 2011; Perera et al., 2012).

In order to colonize and establish a UTI, uropathogenic *E. coli* strains take advantage of an assortment of virulence properties (Slavchev et al., 2009). By definition, virulence genes/factors (VFs) refer to the properties (gene products) that enable a microorganism to establish itself

*Corresponding author. E-mail: joshmbanga@gmail.com or joshua.mbanga@nust.ac.zw. Tel: +263774211463. Fax: +2639286390

on or within a host of a particular species and enhance it's potential to cause disease (Johnson, 1991). VFs include bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates and proteins that protect a bacterium and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium (Momtaz et al., 2013).

Recently, work on *E. coli* has sought to investigate the relationship between extra-intestinal pathogenic *E. coli* (ExPEC) strains (Ewers et al., 2007; Moulin- Schouleur et al., 2007; Johnson et al., 2008). These include mainly avian pathogenic *E. coli* (APEC) which causes colibacillosis in poultry, UPEC and neonatal meningitis *E. coli* (NMEC). Comparisons of these isolates have generally revealed that some overlaps exist in serogroups, virulence genotypes and abilities to cause disease in certain animal models (Rodriguez- Siek et al., 2005; Ewers et al., 2007; Moulin-Schouleur et al., 2007).

Work on virulence genes in the western world and most of Asia is on the rise, but in Africa very little information seems to be available (van der Westhuizen and Bragg, 2012; Randall et al., 2012). This study was undertaken to screen for avian-related virulence genes in UPEC. Use of the avian-related virulence genes was meant to allow us to compare virulence genes found in *E. coli* responsible for human UTIs to APEC in diseased chicken.

The 12 virulence genes that were used for molecular characterization of uropathogenic *E. coli* in the present study included the *frz* operon (Rouquet et al., 2009); vacuolating autotransporter toxin, *vat* (Parreira and Gyles, 2003); type 1 fimbrial adhesion gene, *fimH* (Mellata et al., 2003); capsule formation transporter gene, *kpsM* (Pavelka et al., 1991); the gene *ompT* (Cavard and Lazdunski, 1990); the *sitA* and *sitD* genes which are part of the sitABCD system (Runyen-Janecky et al., 2003); a putative avian haemolysin gene, *hlyF*; an aerobactin siderophore receptor gene, *iutA* (Williams and Warner, 1980; Morales et al., 2004); a transcriptional regulator of iron uptake gene in APEC, *uvrY* (Li et al., 2004); the gene *pstB* (Lamarche et al., 2005) and the plasmid partitioning protein encoded by *sopB* which is common in various plasmids.

Antibiotic resistance/sensitivity patterns are important in the selection of antibiotics that can be used as combinations in the treatment of urinary tract infections. In a recent report by the World Health Organisation (WHO) it was mentioned how we are headed for a post-antibiotic era, in which common infections and minor injuries which have been treatable for decades will once again kill. The report draws on data from 114 countries, and focuses on antibiotic resistance in bacteria that cause common but serious diseases such as sepsis, diarrhea, pneumonia, urinary tract infections and gonorrhea (WHO, 2014). Because of the lack of an effective vaccine to combat UTIs, antimicrobial therapy remains crucial for the control of urinary tract infections. Another major objective of this study was to provide current antibiotic resistance patterns of UPEC isolates from Zimbabwe.

METHODOLOGY

Sample collection

Between the months of July and August 2013, 86 urine samples were obtained from patients visiting a leading diagnostic Medical Laboratory in Harare. These samples were from both symptomatic and asymptomatic patients being tested for UTIs. Of the 86 urines samples, only 13 were found to have *E. coli* as the cause of bacteriuria. Over a period of three months (October-December 2013) 27 *E. coli* isolates were obtained from a leading diagnostic Medical Laboratory in Bulawayo, these were also from suspected UTIs cases. A total of 40 *E. coli* isolates were used in this study, 13 from Harare and 27 from Bulawayo.

Isolation and identification of *E. coli*

Samples were cultured on cysteine lysine electrolyte deficient agar (CLED agar), blood agar and MacConkey agar (Oxoid, England) and then incubated aerobically at 37°C for 24 h. Biochemical tests were carried out including the Gram stain and the indole, citrate and methyl red test.

Antimicrobial susceptibility testing

The disc diffusion method was used to determine antibiotic susceptibility of the isolates on Mueller Hinton agar (Oxoid, UK). Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics: nitrofurantoin (50 µg), ampicillin (25 µg), nalidixic acid (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg) and gentamycin (10 µg). All antibiotic disks were from Oxoid, United Kingdom. The plates were incubated at 37°C for 24 h, and inhibition zones were measured. Interpretation of results followed criteria recommended by the Clinical Laboratory Standard Institute (CLSI, 2009).

DNA Isolation

Bacterial strains were subcultured at 37°C overnight in Luria-Bertani (LB) broth (Oxoid, Basingstoke, Hampshire, UK) and genomic DNA was extracted using a standard Phenol-Chloroform method (Sambrook and Russell, 2001). To check for purity, DNA was run along a 1% ethidium bromide stained agarose gel (Sigma-Aldrich, St Louis, USA) with a 1 kb DNA ladder (Thermo Scientific, USA) in TBE buffer for 1 h at 100 V and then viewed using a Uvipro-Silver Gel Documentation System (Uvitec, UK). The concentration of DNA was estimated by comparing the band light intensity to the band intensity on the 1 kb ladder on the Uvipro-Silver Gel Documentation System. DNA concentration of samples ranged from 75 ng/0.5 µg – 100 ng/0.5 µg.

Multiplex PCR for virulence genes

The presence of genes encoding virulence factors was detected using multiplex PCR amplification. Four multiplex PCR assays were used to detect 12 virulence genes (Table 1). The multiplex design was done according to that reported by van der Westhuizen and Bragg (2012) with slight changes in the primer and final magnesium chloride concentrations. The effected changes were done using primer concentrations of 0.5 µM for the *frz*, *sitD*, *fimH*, *ompT*, *iutA*, *pstB* and s*opB* genes and adjusting the final $MgCl_2$ concentration to

Table 1. Final primer concentrations used in the different multiplex PCRs.

Multiplex	Primer set	Conc (µM)	Primer set	Conc (µM)	Primer set	Conc. (µM)	Additional $MgCl_2$ (mM)	Final $MgCl_2$ (mM)
1	*frz*	0.5	*sitD*	0.5	*fimH*	0.5	1	3
2	*sitA*	2.0	*kpsM*	1.0	*vat*	0.5	1	3
3	*ompT*	0.5	*iutA*	0.5	*pstB*	0.5	1	3
4	*sopB*	0.5	*uvrY*	1.0	*hlyF*	0.5	1	3

Table 2. Frequency of 12 virulence genes in 40 uropathogenic *E. coli* isolates.

Name of gene	Primer name	Frequency (%)
Aerobactinsiderophore receptor	*iutA*	35
Type 1 fimbrial adhesin	*fimH*	32.5
Vacuolating autotransporter toxin	*vat*	17.5
SitABCD system	*sitA*	17.5
SitABCD system	*sitD*	15
Putative avian haemolysin	*hlyF*	12.5
PstSCAB system	*pstB*	10
frz operon	*frz*	7.5
APEC virulence regulator	*uvrY*	0
Capsule-protein transport of polysaccharides	*kpsM*	0
Episomal outer membrane protease	*ompT*	0
Plasmid partitioning protein	*sopB*	0

3 mM for all multiplex reactions. The primers used in our study are listed in Table S1 in the supplementary material. All primers used were obtained from Inqaba Biotech, South Africa. Three microliters of each of the DNA samples were mixed with all necessary components for amplification in a 0.2 ml PCR tube (Perkin-Elmer, USA) in a 25 µl reaction. The reaction mixture included 2.5 µl of ×10 PCR Dream Taq buffer (Thermo scientific, USA), 2 µl of dNTPs, 10 mM; 0.25 µl of Dream *Taq* polymerase (Thermoscientific, USA), 5 U/µl, nuclease free water to maintain a total volume of 25 µl. The appropriate primers were added to a maximum primer concentration of 2 µM and the $MgCl_2$ concentration was adjusted to a final concentration of 3 mM as shown in Table 1. Negative controls comprised of a water control. An Applied Biosystems GeneAmp® PCR System 9700 was used for the PCR thermal cycling conditions with an initial denaturation step at 94°C for 5 min, 35 cycles {denaturation 94°C for 30 s, annealing at 63°C for 45 s, extension 72°C for 1 min and 45 s and a final elongation step at 72°C for 10 min. The amplified products were then run along a 1% ethidium bromide stained agarose gel with a 100 bp DNA ladder (Thermo scientific, USA) in TBE buffer for 1 h at 100 V and then viewed using a Uvipro-Silver Gel Documentation System (Uvitec, UK).

The multiplex PCRs described were used to screen for the presence of 12 virulence genes in the UPEC isolates in duplicate. Prevalence of each virulence gene was then calculated (Table 2).

RESULTS

All 40 isolates obtained from the two leading diagnostic laboratories in Harare and Bulawayo were positively identified and confirmed to be *E. coli* through culturing and biochemical tests. After successful DNA isolation and quantification, the DNA of each of the 40 UPEC isolates was subjected to 4 different multiplex PCRs. Each multiplex reaction amplified three virulence gene regions. This was done in order to screen the UPEC isolates for 12 APEC associated virulence genes. The percentage frequency of each gene was then determined and the results are shown in Table 2. The *iutA* gene had the highest presence rate of 35%; followed by *fimH* (32.5%), *vat* (17.5%) *sitA* (17.5%) and *sitD* (15%). The data obtained from electrophoresis agarose gels was used to assign virulence gene profiles to each UPEC isolate, this is summarized in Table 3.

Antibiotic susceptibility testing was done for all 40 UPEC isolates. The isolates were assayed against a panel of six antibiotics. The results shown in Table 4 suggest a high resistance of UPEC to ampicillin (90%) and tetracycline (75%) whilst a high sensitivity to gentamycin (82.5%) and nitrofurantoin (62.5%) was observed. The prevalence of antibiotic resistance phenotypes of all *E. coli* isolates is presented in Table 5. Sixty five percent of the isolates showed resistance to at least 3 antibiotics, and 11 different antibiotic resistance patterns were observed. The most common resistance pattern, exhibited by 10 isolates, was resistance to ampicillin, nalidixic acid, ciprofloxacin and tetracycline (pattern D). This study was sanctioned by the NUST ethical committee and no names were recorded during the study.

Table 3. Presence or absence of expected amplicons and virulence profiles of UPEC isolates

Isolate	Multiplex 1			Multiplex 2			Multiplex 3			Multiplex 4			Total/12	Virulence profile
	frz	*sitD*	*fimH*	*sitA*	*kpsM*	*vat*	*ompT*	*iutA*	*pstB*	*sop B*	*uvrY*	*hlyF*		
1	+	+	+	+	-	+	-	+	-	-	-	-	6	C
2	-	+	+	-	-	-	-	-	-	-	-	-	2	A
3	-	+	+	-	-	-	-	-	-	-	-	-	2	A
4	+	+	+	-	-	+	-	+	-	-	-	-	5	B
5	-	-	-	-	-	-	-	-	-	-	-	-	0	A
6	+	+	+	-	-	-	-	+	-	-	-	-	4	B
7	-	-	-	-	-	-	-	-	-	-	-	-	0	A
8	-	-	-	-	-	-	-	-	-	-	-	-	0	A
9	-	-	-	-	-	-	-	-	-	-	-	-	0	A
10	-	+	+	-	-	-	-	-	-	-	-	-	2	A
11	-	-	-	-	-	-	-	+	-	-	-	-	1	A
12	-	-	-	-	-	-	-	-	-	-	-	-	0	A
13	-	-	-	-	-	-	-	+	-	-	-	-	1	A
14	-	-	-	-	-	-	-	-	-	-	-	-	0	A
15	-	-	-	-	-	-	-	-	-	-	-	-	0	A
16	-	-	-	-	-	-	-	-	-	-	-	-	0	A
17	-	-	+	-	-	-	-	-	-	-	-	-	1	A
18	-	-	+	-	-	-	-	+	-	-	-	-	2	A
19	-	-	-	-	-	-	-	-	-	-	-	-	0	A
20	-	-	-	-	-	-	-	-	-	-	-	-	0	A
21	-	-	-	-	-	-	-	-	-	-	-	-	0	A
22	-	-	-	-	-	-	-	-	-	-	-	-	0	A
23	-	-	-	-	-	+	-	+	-	-	-	-	2	A
24	-	-	-	-	-	-	-	-	-	-	-	+	1	A
25	-	-	-	-	-	-	-	-	-	-	-	-	0	A
26	-	-	-	-	-	-	-	+	-	-	-	+	2	A
27	-	-	-	-	-	-	-	-	-	-	-	-	0	B
28	-	-	-	-	-	-	-	-	-	-	-	-	0	A
29	-	-	-	-	-	-	-	-	-	-	-	-	0	A
30	-	-	+	+	-	+	-	-	+	-	-	+	5	B
31	-	-	+	-	-	-	-	+	-	-	-	-	2	A
32	-	-	+	+	-	-	-	+	-	-	-	-	3	B
33	-	-	-	+	-	-	-	-	-	-	-	-	1	A
34	-	-	-	-	-	-	-	+	-	-	-	-	1	A
35	-	-	+	+	-	+	-	-	+	-	-	-	4	B
36	-	-	-	+	-	-	-	+	+	-	-	-	3	B
37	-	-	-	-	-	+	-	-	+	-	-	+	3	B
38	-	-	-	+	-	+	-	+	-	-	-	+	4	B
39	-	-	-	-	-	-	-	+	-	-	-	-	1	A
40	-	-	+	-	-	-	-	-	-	-	-	-	1	A

Presence (+) or absence (-) of the expected amplicons during the four different multiplex PCRs. Last column indicates virulence profiles assigned to each isolate. A indicates presence of between 0 and 2 virulence genes, B between 3 and 5 genes and C indicate 6 or more virulence genes.

DISCUSSION

Virulence gene profiles

In this study 40 *E. coli* isolates were obtained from patients with suspected UTIs and screened for 12 virulence genes, some of which have been well characterized in previous studies (Karimian et al., 2012; Guiral et al., 2012; Farshad et al., 2012). The *uvrY* gene has not been used to screen for UPEC in previous studies using multiplex PCR tech niques.

Table 4. Antibiotic susceptibility profiles of 40 uropathogenic *E. coli* isolates.

Antibiotic	Concentration (µg)	Resistant no. (%)	Intermediate no. (%)	Susceptible no. (%)
Gentamycin	10	4 (10)	3 (7.5)	33 (82.5)
Nitrofurantoin	50	13 (32.5)	2 (5)	25 (62.5)
Ciprofloxacin	5	19 (47.5)	2 (5)	19 (47.5)
Nalidixic Acid	30	23 (57.5)	1 (2.5)	16 (40)
Tetracycline	30	30 (75)	2 (5)	8 (20)
Ampicillin	25	36 (90)	1 (2.5)	3 (7.5)

Table 5. Antimicrobial resistance patterns of the 40 uropathogenic *E. coli* isolates.

Pattern	No. of isolates	Resistance pattern*
A	2	Amp, Nit, Nal, Cip, Tet, Gen
B	2	Amp, Nit, Nal, Cip, Tet
C	1	Amp, Nal, Cip, Tet, Gen
D	10	Amp, Nal, Cip, Tet
E	1	Amp, Nal, Cip, Gen
F	2	Amp, Nit, Nal, Tet
G	3	Amp, Nit, Tet
H	2	Amp, Nal, Tet
I	1	Amp, Nal, Cip
J	1	Amp, Nit, Nal
K	1	Amp, Cip, Tet

*Amp- ampicillin, Nit- nitrofurantoin, Nal- nalidixic acid, Tet- tetracycline, Cip- ciprofloxacin, Gen- gentamycin.

Similarly, the *frz* and *pstB* genes have been shown to contribute to virulence but are new in the diagnostic context (Li et al., 2004; Lamarche et al., 2005; Rouquet et al., 2009). The multiplex PCRs of this study have an advantage over previous studies as they include these recently discovered virulence genes.

An aerobactinsiderophore receptor gene, *iutA*, which is known to contribute to iron uptake (Williams and Warner, 1980; Morales et al., 2004) was present in 35% of UPEC isolates (Table 2). Presence of the gene in UPEC agrees with other studies (Johnson, 1991; Guiral et al., 2012) that have shown that the bacterial sidephore “aerobactin” is associated with *E. coli* strains which cause pyelonephritis and cystitis. It is an iron sequestration and transport system which enables *E. coli* to grow in iron poor environments such as dilute urine (Johnson et al., 2008).

The type 1 fimbrial adhesin gene, *fimH*, contributes to protection from host heterophils (Mellata et al., 2003). The gene had the second highest frequency (32.5%) and may be useful by UPEC for adhesion to uroepithelial cells. *FimH* is the adhesin protein known to be responsible for binding to mannosylated glycoproteins and is located at the distal tip of the heteropolymeric type I pilus rod (Johnson, 1991; Slavchev et al., 2009). Detection of the gene in the present study agrees with most studies (Tiba et al., 2008; Johnson, 1991; Karimian et al., 2012), however values as high as 79.67% have been reported in other studies (Karimian et al., 2012).

The *sitA* and *sitD* genes which are part of the SitABCD system, and are classified as a bacterial iron transporter (Runyen-Janecky et al., 2003) were also found in 17.5 and 15% of the UPEC isolates respectively. Apart from mediating iron and manganese transport, the SitABCD operon has also been suggested to confer resistance to oxidative stress possibly required during interaction with phagocytes (Sabri et al., 2008). The presence of these genes agrees with other studies (Schouler et al., 2004; Snyder et al., 2004; Rodriguez et al., 2005) who believe that in *E. coli*, SitABCD-encoding genes are associated with clinical strains isolated from extra-intestinal infections from poultry and human UTIs.

The vacuolating autotransporter toxin, *vat* gene which has been shown to induce cytotoxic effects in host cells (Parreira and Gyles, 2003) was present in 17.5% of our isolates. This disagrees with a study by Johnson et al. (2008) who found a prevalence of 62.3% for the *vat* gene in UPEC isolates. A putative avian haemolysin gene, *hlyF*, responsible for iron uptake (Williams and Warner, 1980; Morales et al., 2004) was also amplified in 12.5% of the isolates. A 50.4% presence for *hly A* was observed by Karimian et al. (2012), but our findings agree with recent studies by Farshad et al. (2012) who obtained a 15.62% for hemolysin (*hly*) in UPEC from children in Iran. In another study on 531 UPEC isolates, Johnson et al. (2008) found a prevalence of 5.6% for the *hlyF* gene. Also, according to Johnson (1991) the percentage frequency of genes from the *hly* operon varies with the patient’s condition. *HlyF* and *vat* have been well documented in chickens suffering from colibacillosis (van der Westhuizen and Bragg, 2012) and very little seems to have been published on the genes in UPEC.

The *pstB* gene which is part of the pstSCAB operon, has been shown to increase resistance to polymyxin, rabbit serum and acid shock (Lamarche et al., 2005). We detected it in 10% of the studied UPEC isolates. Some studies (Surin et al., 1985; Rao and Torriani, 1990) suggest that the gene may be responsible for mediating the uptake of phosphate from the outside to the inside of the cell. The *frz* operon was present in 7.5% of the UPEC

isolates and work by Rouquet et al. (2009) suggests that the gene products from the *frz* operon are used by *E. coli* to promote growth in serum during oxygen-restricted conditions. Urine, like serum is also oxygen-restricted, therefore UPEC may use this gene for their survival. Very little has been published on the *pstB* and *frz* operon in UPEC.

Four (*uvrY, sopB, kpsM* and *ompT*) of the twelve genes were not detected in all the 40 *E. coli* isolates (Table 2). Transcriptional regulator of iron uptake genes, *uvrY* (Li et al., 2008) together with the plasmid partitioning protein encoded by *sopB* known to be common in various plasmids were both absent. If a virulence gene in APEC does not have a homologue in UPEC the gene region cannot be amplified except in cases of a zoonotic infection. APEC virulence regulator (*uvrY*) is a VF particularly found in *E. coli* associated with chicken suffering from colibacillosis. Therefore, the gene's absence in all UPEC isolates studied supports work done by Randall et al. (2012), who suggested that *E. coli* strains in diseased chickens are generally different from those causing disease in humans.

Another gene that was absent was the capsule formation transporter gene *kps M* (Pavelka et al., 1991). This finding differs from some previous studies by Momtaz et al. (2013) and Tiba et al. (2008) who indicated presence of the *kpsMT* gene in UPEC. The gene *ompT* which encodes for the episomal outer membrane protease that cleaves colicins (Cavard and Lazdunski, 1990) was also absent in the samples. These findings agree with other recent studies by Karimian et al. (2012) and Momtaz et al. (2013).

Virulence profiles were generated for each *E. coli* isolate used in our study (Table 3).The UPEC isolates were profiled as being 75% profile A, 22.5% profile B and 2.5% profile C. None of the isolates had more than 6 virulence genes. Generally, the UPEC isolates did not possess most of the APEC virulence genes assayed for (Table 3). This suggests that UPEC isolates may have different virulence genotypes than those reported for APEC (Johnson et al., 2008). A potential bias and limitation in our study could have been the use of *E. coli* isolates from both symptomatic and asymptomatic patients and failure to test each isolate using *in vivo* animal models to determine actual virulence.

We also report that *E. coli* isolates which were resistant to all of the six antibiotics had none or only one of the VFs studied. This may suggest that some of the isolates were related, but it however also supports recent studies in Turkey by Giray et al. (2012) who found that *E. coli* strains with low numbers of virulence genes exhibit a high antibiotic resistance. A larger sample size as well as more VFs should be studied to fully investigate this relationship.

Antibiotic susceptibility profiles

The studied UPEC isolates showed a high resistance to ampicillin (90%) and tetracycline (75%) whilst showing a high sensitivity to gentamycin (82.5%) and nitrofurantoin (62.5%) (Table 4). The results agree with previous studies done in the country by Mbanga et al. (2010) who found that UPEC showed the highest resistance to ampi-cillin (84%) whilst showing a low resistance to nitro-furantoin (16%). Our findings are also similar to previous studies from Mexico City (Molina-Lopez et al., 2011), Sri Lanka (Perera et al., 2012), Nigeria (Okonko et al., 2009) India (Mandal et al., 2012) and Iran (Barati et al., 2011) which found a low resistance to nitrofurantoin and a high resistance to ampicillin.

UPEC strains tend to be resistant to drugs that are frequently used. However, gentamycin is inappropriate for frequent use, rather it is commonly used for severe UTIs. Our findings show that most isolates were susceptible to gentamycin (82.5%) but this differs from a study done in Iraq by Chateen et al. (2007), who found UPEC to be highly resistant to gentamycin.

E. coli isolates clearly demonstrated high resistance to most examined antibiotics (Table 5) and this has been reported in other studies (Dromigny et al., 2005). Five percent of UPEC isolates were resistant to all six antibiotics and none of the isolates were susceptible to all the antibiotics. All *E. coli* showed resistance to at least one or more antibiotics. Sixty five percent of the *E. coli* isolates were responsible for the 11 different multi-drug resistance (MDR) patterns (UPEC showing resistance to ≥3 antibiotics) as shown in Table 5. This agrees with recent findings in Iran by Farshad et al. (2012), but differs from other studies were lower levels of MDR isolates have been reported (Linder et al., 2005; Rijavec et al., 2006). The most common pattern was pattern D followed by pattern G (Table 5).

Conclusion

Low percentage frequencies of the studied VFs were detected in UPEC causing UTIs. Half of all UPEC isolates in the present study possessed none, or only one, of the VFs characterized and, as such, it is reasonable to assume that UPEC isolates have different virulence factors than those reported for APEC. However, to fully investigate zoonosis between chicken and humans, virulence genes exclusive to chicken isolates should be used. The antibiotic resistance results show that antibiotic resistance is on the rise and nitrofurantoin should be the drug of choice in Zimbabwe.

Conflict of interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Special thanks go to the International Network for the

Availability of Scientific Publications (INASP) for their assistance through AuthorAID. This study was supported by the National University of Science and Technology (NUST) Research Board.

REFERENCES

Barati L, Ghezelsofla F, Azarhoush R, Heidari F, Noora M (2011). Antibiotic sensitivity of isolated *E. coli* from pregnant women's urine. J. Gorgan. Univ. Med. Sci. 13(3):101-107.

Cavard D, Lazdunski C (1990). Colicin cleavage by *ompT* protease during both entry into and release from *Escherichia coli* cells. J. Bacteriol. 172:648-652.

Chateen EA, Sheelan AA, Sinan BI, Sajida K (2007). Study of urinary tract infection among pregnant women in Kirkuk. Tikrit Med. J. 13(2):39-43.

Cheesbrough M (2006).Microbiological tests. In: District Laboratory Practice in Tropical Countries Part 2, 2nd edn, New York: Cambridge University Press. pp. 105:133-136

Clinical Laboratories Standards Institute (CLSI). (2009). Performance of standards for antimicrobial disk susceptibility tests; approved standards 10th edn, 29:M02-A10. Wayne, PA.

Dromigny AJ, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude DJ (2005). Risk factors for antibiotic-resistant *Escherichia coli* isolated from community-acquired urinary tract infections in Dakar, Senegal. J. Antimicrob. Chemother. 56:236-239

Ewers C, Li G, Wilking H, Kießling S, Alt K, Antáo EM, Laturnus C, Diehl I, Glodde S, Homeier T, Bo¨hnke U, Steinru¨ck H, Philipp HC, Wieler LH (2007). Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: How closely related are they? Int. J. Med. Microbiol. 297:163-176.

Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M. Mohammadzadegan R (2012). Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic *Escherichia coli* in children in Jahrom, Iran. Arch. Iran Med. 15(5):312-316.

Giray B, Ucar BF, Aydemir SS (2012). Characterization of uropathogenic *Escherichia coli* strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir. Turk. J. Med. Sci. 42:1328-1337.

Guiral E, Bosch J, Vila J, Soto SM (2011). Prevalence of *Escherichia coli* among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbiol. Lett. 314:170-173

Johnson JR (1991). Virulence factors in *Escherichia coli* Urinary Tract Infection. Clin. Microbial. Rev. 4(1):80-128

Johnson TJ, Wannemuehler Y, Doetkott C, Johnson SJ, Rosenberger SC, Nolan LK (2008). Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J. Clin. Microbiol. 46:3987-3996.

Karimian A, Momtaz H, Madani M (2012). Detection of uropathogenic *Escherichia coli* virulence factors in patients with urinary tract infections in Iran. Afr. J. Microbiol. Res. 6(39):6811-6816

Lamarche MG, Dozois CM, Daigle F, Caza M, Curtiss R III, Dubreuil JD, Harel J 2005. Inactivation of pst system reduces the virulence of an avian pathogenic *Escherichia coli* O78 strain. Infect. Immun. 73:4138-4145.

Li G, Ewers C, Laturnus C, Diehl I, Alt K, Dai J, Antáo EM, Schnetz K, Wieler LH (2008). Characterization of *ayjjQ* mutant of avian pathogenic *Escherichia coli* (APEC). Microbiology 154: 1082-1093.

Li X, Lockatell, CV, Johnson DE, Lane MC, Warren JW, Mobley HL (2004). Development of an intranasal vaccine to prevent urinary tract infection by *Proteus mirabilis*. Infect. Immun.72:66-75

Linder JA, Huang ES, Steinman, MA, Gonzales R, Stafford RS (2005). Fluoroquinolone prescribing in the United States. Am. J. Med. 118: 259-268.

Mandal J, Acharya NS, Buddhapriya D, Parija CS (2012). Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli.* Indian J. Med. Res. 136: 842-849.

Mbanga J, Dube S, Manyanduki H (2010). Prevalence and drug resistance in bacteria of the urinary tract infections in Bulawayo Province, Zimbabwe. East Afr. J. Public Health 7(3):229-32.

Mellata M, Dho-Moulin M, Dozois CM, Curtiss III R, Brown PK, Arné P, Brée A, Desautels C Fairbrother JM (2003). Role of virulence factors in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. Infect. Immun. 71:536-540.

Molina-López J, Aparicio-Ozores G, Ribas-Aparicio MR, Gavilanes-Parra S, Chávez-Berrocal EM, Hernández-Castro R, Manjarrez-Hernández AH (2011). Drug resistance, serotypes, and phylogenetic groups among uropathogenic *Escherichia coli* including O25-ST131 in Mexico City. J. Infect. Dev. Ctries. 5(12):840-849.

Momtaz H, Karimian A, Madani M, Dehkordi SF, Ranjbar R, Sarshar M, Souod N (2013). Uropathogenic *Escherichia coli* in Iran: Serogroup distributions, virulence factors and antimicrobial resistance properties. Ann. Clin. Microbiol. Antimicrob. 12: 10.1186/1476-0711-12-8.

Morales C, Lee MD, Hofacre C, Maurer JJ (2004). Detection of a novel virulence gene and a *Salmonella* virulence homologue among *Escherichia coli* isolated from broiler chickens. Foodborne Pathog. Dis. 1:160-165.

Moulin-Schouleur MM, Reperant S, Laurent A, Bree S, Mignon-Grasteau P, Germon, Rasschaert D, Schouler C (2007). Extraintestinal pathogenic *Escherichia coli* strains of avian and human origin: link between phylogenetic relationships and common virulence patterns. J. Clin. Microbiol. 45:3366-3376.

Okonko OI, Donbraye-Emmanuel BO, Ijandipe AL, Ogun AA, Adedeji OA, Udeze OA (2009). Antibiotics sensitivity and resistance patterns of uropathogens to nitrofurantoin and nalidixic acid in pregnant women with urinary tract infections in Ibadan, Nigeria. MEJSR 4(2): 105-109.

Parreira RV Gyles LC (2003). A novel pathogenicity island integrated adjacent to the *thrW*tRNA gene of avian pathogenic *Escherichia coli* encodes a vacuolating autotransporter toxin. Infect. Immun. 71(9): 5087-5096

Pavelka MS Jr, Wright LF, Silver RP (1991). Identification of two genes, *kpsM* and *kpsT*, in region 3 of the polysialic acid gene cluster of *Escherichia coli* K1. J. Bacteriol. 173:4603-4610.

Perera J, Randeniya C, Perera P, Gamhewage N, Jayalathaarachchi, R (2012). Research article: Asymptomatic bacteriuria in pregnancy: prevalence, risk factors and causative organisms. Sri Lankan J. Infect. Dis. 1(2):42-46.

Randall L, Wua G, Phillips N, Coldham N, Mevius D, Teale C (2012). Virulence genes in *blaCTX-M Escherichia coli* isolates from chickens and humans. Res. Vet. Sci. 93:23-27

Rao NN, Torriani A (1990). Molecular aspects of phosphate transport in *Escherichia coli*. Mol. Microbiol. 4:1083-1090.

Rijavec M, Starcic-Ergivec M, Ambrozic Augustin J, Reissbrodt R, Fruth A, Krizan-Hergouth V (2006). High prevalence of multidrug resistance and random distribution of mobile genetic elements among uropathogenic*Escherichia coli* (UPEC) of the four major phylogenetic groups. Curr. Microbiol. 53:158-162.

Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Fakhr MK, Nolan LK (2005). Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology 151:2097-2110.

Rouquet G, Porcheron G, Barra C, Re´pe´rant M, Chanteloup KN, Schouler C, Gilot P (2009). A metabolic operon in extraintestinal pathogenic *Escherichia coli* promotes fitness under stressful conditions and invasion of eukaryotic cells. J. Bacteriol. 191(13): 4427-4440.

Runyen-Janecky LJ, Reeves SA, Gonzales EG, Payne SM (2003). Contribution of the *Shigellaflexneri Sit, Iuc,* and *Feo* iron acquisition systems to iron acquisition *in vitro* and in cultured cells. Infect. Immun. 71:1919-1928.

Sabri M, Caza M, Proulx J, Lymberopoulos MH, Bre´e A, Moulin-Schouleur M, Curtiss R, Dozois CM (2008). Contribution of the SitABCD, MntH, and FeoB metal transporters to the virulence of avian pathogenic *Escherichia coli* O78 strain x7122. Infect. Immun. 76:601-611.

Sambrook J, Rusell DW (2001). Molecular Cloning: A Laboratory Manual, 3rd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Schouler C, Koffmann F, Amory C, Leroy-Setrin S, Moulin-Schouleur M (2004). Genomic subtraction for the identification of putative new virulence factors of an avian pathogenic *Escherichia coli* strain of O2 serogroup. Microbiology 150: 2973-2984.

Slavchev G, Pisareva E, Markova N (2009). Virulence of uropathogenic *Escherichia coli.* J. Cult. Collect. 6:3-9.

Snyder JA, Haugen BJ, Buckles EL, Lockatell CV, Johnson DE, Donnenberg MS, Welch RA, Mobley HL (2004). Transcriptome of uropathogenic *Escherichia coli* during urinary tract infection. Infect. Immun. 72:6373-6381.

Surin BP, Rosenberg H, Cox BG (1985). Phosphate-specific transport system of *Escherichia coli*: nucleotide sequence and gene-polypeptide relationships. J. Bacteriol. 161:189-198.

Tiba RM, Yano T, Leite SD (2008). Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis. Rev. Inst. Med. Trop. S Paulo 50(5):255-260.

van der Westhuizen AW, Bragg RR (2012). Multiplex polymerase chain reaction for screening avian pathogenic *Escherichia coli* for virulence genes. Avian Pathol. 41(1):33-40.

Williams PH, Warner PJ (1980). ColV plasmid-mediated, colicin Vindependent iron uptake system of invasive strains of *Escherichia coli*. Infect. Immun. 29:411-416.

World Health Organisation (WHO). (2014). Antimicrobial resistance: global report on surveillance. http://www.who.int/drugresistance/documents/surveillancereport/en/ (accessed 23/07/14).

Table S1. Primers used for amplifying regions in APEC virulence genes.

Primer name	Name of targeted region/gene	Primer Sequence (5' – 3')	Source of Primer Sequence	Expected amplicon size (bp)
sitA-F *sitA*-R	SitABCD system	Forward primer: CGCAGGGGGCACAACTGAT Reverse primer: CCCTGTACCAGCGTACTGG	Sabri et al. (2008)	661
sitD-F *sitD*-R	SitABCD system	Forward primer: CTGTGCGCTGCTGTCGGTC Reverse primer: GCGTTGTGTCAGGAGTAC	Sabri et al. (2008)	571
ompT-FP *ompT*-RP	Episomal outer membrane protease	Forward primer: TCATCCCGGAAGCCTCCCTCACTACTAT Reverse primer: TAGCGTTTGCTGCACTGGCTTCTGATAC	Johnson et al. (2008)	496
fimH-FP *fimH*-RP	Type 1 fimbrial adhesin fimH	Forward primer: GGATAAGCCGTGGCCGGTGG Reverse primer: CTGCGGTTGTGCCGGAGAGG	Van der Westhuizen and Bragg (2012)	331
frz-FP *frz*-RP	frz operon	Forward primer: GAGTCCTGGCTTGCGCCGTT Reverse primer: CCGCTCCATCGCAGCCTGAA	Van der Westhuizen and Bragg (2012)	843
hlyF-FP *hlyF*-RP	Putative avian haemolysin	Forward primer: GGCCACAGTCGTTTAGGGTGCTTACC Reverse primer: GGCGGTTTAGGCATTCCGATACTCAG	Johnson et al. (2008)	450
iutA-FP *iutA*-RP	Aerobactinsiderophore receptor	Forward primer: GGCTGGACATCATGGGAACTGG Reverse primer: CGTCGGGAACGGGTAGAATCG	Johnson et al. (2008)	302
kpsM-FP *kpsM*-RP	Capsule-protein transport of polysaccharides	Forward primer: CAGCCTCGCGGCTTAGCTCC Reverse primer: TGCACGCGCACTGCTTGAGA	Van der Westhuizen and Bragg (2012)	335
vat-FP *vat*-RP	Vacuolating autotransporter toxin	Forward primer: CGCTTCAGGTGCGCTGACCA Reverse primer: AAGGGAGACGATGCCCGCCT	Van der Westhuizen and Bragg (2012)	498
pstB-FP *pstB*-RP	PstSCAB system	Forward primer: CGCGCTCGTCCATGTCAGCA Reverse primer: CGGAACAGCGTGCGGAAGGT	Van der Westhuizen and Bragg (2012)	198
uvrY-FP *uvrY*-RP	APEC virulence regulator	Forward primer: TGAGTGCGATTCGTTCTGTC Reverse primer: TCTCCGCATTACACAGACCA	Herren et al. (2006)	286